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Final Report on the Safety Assessment of Glycyrrhetinic Acid, Potassium Glycyrrhetinate, Disodium Succinoyl Glycyrrhetinate, Glyceryl Glycyrrhetinate, Glycyrrhetinyl Stearate, Stearyl Glycyrrhetinate, Glycyrrhizic Acid, Ammonium Glycyrrhizate, Dipotassium Glycyrrhizate, Disodium Glycyrrhizate, Trisodium Glycyrrhizate, Methyl Glycyrrhizate, and Potassium Glycyrrhizinate[1]

Glycyrrhetinic Acid and its salts and esters and Glycyrrhizic Acid and its salts and esters are cosmetic ingredients that function as flavoring agents or skin-conditioning agents—miscellaneous or both. These chemicals may be isolated from licorice plants. Glycyrrhetinc Acid is described as at least 98% pure, with 0.6% 24-OH-Glycyrrhetinic Acid, not more than 20 μg/g of heavy metals and not more than 2 μg/g of arsenic. Ammonium Glycyrrhizate has been found to be at least 98% pure and Dipotassium Glycyrrhizate has been found to be at least 95% pure. Glycyrrhetinic Acid is used in cosmetics at concentrations of up to 2%; Stearyl Glycyrrhetinate, up to 1%; Glycyrrhizic Acid, up to 0.1%; Ammonium Glycyrrhizate, up to 5%; Dipotassium Glycyrrhizate, up to 1%; and Potassium Glycyrretinate, up to 1%. Although Glycyrrhizic Acid is poorly absorbed by the intestinal tract, it may be hydrolyzed to Glycyrrhetinic Acid by a β-glucuronidase produced by intestinal bacteria. Glycyrrhetinic Acid and Glycyrrhizic Acid bind to rat and human albumin, but do not absorb well into tissues. Glycyrrhetinic Acid and Glycyrrhizic Acid and metabolites are mostly excreted in the bile, with very little excreted in urine. Dipotassium Glycyrrhizate was undetectable in the receptor chamber when tested for transepidermal permeation through pig skin. Glycyrrhizic Acid increased the dermal penetration of diclofenac sodium in rat skin. Dipotassium Glycyrrhizate increased the intestinal absorption of calcitonin in rats. In humans, Glycyrrhetinic Acid potentiated the effects of hydrocortisone in the skin. Moderate chronic or high acute exposure to Glycyrrhizic Acid, Ammonium Glycyrrhizate, and their metabolites have been demonstrated to cause transient systemic alterations, including increased potassium excretion, sodium and water retention, body weight gain, alkalosis, suppression of the renin-angiotensis-aldosterone system, hypertension, and muscular paralysis; possibly through inhibition of 11β-hydroxysteroid dehydrogenase-2 (11β-OHSD2) in the kidney. Glycyrrhetinic Acid and its derivatives block gap junction intracellular communication in a dose-dependent manner in animal and human cells, including epithelial cells, fibroblasts, osteoblasts, hepatocytes, and astrocytes; at high concentrations, it is cytotoxic. Glycyrrhetinic Acid and Glycyrrhizic Acid protect liver tissue from carbon tetrachloride. Glycyrrhizic Acid has been used to treat chronic hepatitis, inhibiting the penetration of the hepatitis A virus into hepatocytes. Glycyrrhetinic Acid and Glycyrrhizic Acid have anti-inflammatory effects in rats and mice. The acute intraperitoneal LD_{50} for Glycyrrhetinic Acid in mice was 308 mg/kg and the oral LD_{50} was >610 mg/kg. The oral LD_{50} in rats was reported to be 610 mg/kg. Higher LD_{50} values were generally reported for salts. Little short-term, subchronic, or chronic toxicity was seen in rats given ammonium, dipotassium, or disodium salts of Glycyrrhizic Acid. Glycyrrhetinic Acid was not irritating to shaved rabbit skin, but was considered slightly irritating in an in vitro test. Glycyrrhetinic Acid inhibited the mutagenic activity of benzo[a]pyrene and inhibited tumor initiation and promotion by other agents in mice. Glycyrrhizic Acid inhibited tumor initiation by another agent, but did not prevent tumor promotion in mice. Glycyrrhizic Acid delayed mortality in mice injected with Erlich ascites tumor cells, but did not reduce the mortality rate. Ammonium Glycyrrhizate was not genotoxic in in vivo and in vitro cytogenetics assays, the dominant lethal assay, an Ames assay, and heritable translocation tests, except for possible increase in dominant lethal mutations in rats given 2000 mg/kg day^{-1} in their diet. Disodium Glycyrrhizate was not carcinogenic in mice in a drinking water study at exposure levels up to 12.2 mg/kg day^{-1} for 96 weeks. Glycyrrhizate salts produced no reproductive or developmental toxicity in rats, mice, golden hamsters, or Dutch-belted rabbits, except for a dose-dependent increase (at 238.8 and 679.9 mg/kg day^{-1}) in sternebral variants in a study using rats. Sedation, hypnosis, hypothermia, and respiratory depression were seen in mice given 1250 mg/kg Glycyrrhetinic Acid intraperitoneally. Rats fed a powdered diet containing up to 4% Ammonium Glycyrrhizate had no treatment related effects in motor function tests, but active avoidance was facilitated at 4%, unaffected at 3%, and depressed at 2%. In a study of 39 healthy volunteers, a no effect level of 2 mg/kg/day was determined for Glycyrrhizic Acid given orally for 8 weeks. Clinical tests in seven normal individuals given oral Ammonium Glycyrrhizate at 6 g/day for 3 days revealed reduced renal and thermal sweat excretion of Na^+ and K^+, but carbohydrate and protein metabolism were not affected. Glycyrrhetinic Acid at concentrations up to 6% was not a skin irritant or a sensitizer in clinical tests. Neither Glycyrrhizic Acid, Ammonium Glycyrrhizate,

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nor Dipotassium Glycyrrhizate at 5% were phototoxic agents or photosensitizers. Birth weight and maternal blood pressure were unrelated to the level of consumption of Glycyrrhizic Acid in 1049 Finnish women with infants, but babies whose mother consumed >500 mg/wk were more likely to be born before 38 weeks. The Cosmetic Ingredient Review (CIR) Expert Panel noted that the ingredients in this safety assessment are not plant extracts, powders, or juices, but rather are specific chemical species that may be isolated from the licorice plant. Because these chemicals may be isolated from plant sources, however, steps should be taken to assure that pesticide and toxic metal residues are below acceptable levels. The Panel advised the industry that total polychlorobiphenyl (PCB)/pesticide contamination should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue, and that toxic metal levels must not contain more than 3 mg/kg of arsenic (as As), not more than 0.002% heavy metals, and not more than 1 mg/kg of lead (as Pb). Although the Panel noted that Glycyrrhizic Acid is cytotoxic at high doses and ingestion can have physiological effects, there is little acute, short-term, subchronic, or chronic toxicity and it is expected that these ingredients would be poorly absorbed through the skin. These ingredients are not considered to be irritants, sensitizers, phototoxic agents, or photosensitizers at the current maximum concentration of use. Accordingly, the CIR Expert Panel concluded that these ingredients are safe in the current practices of use and concentration. The Panel recognizes that certain ingredients in this group are reportedly used in a given product category, but the concentration of use is not available. For other ingredients in this group, information regarding use concentration for specific product categories is provided, but the number of such products is not known. In still other cases, an ingredient is not in current use, but may be used in the future. Although there are gaps in knowledge about product use, the overall information available on the types of products in which these ingredients are used and at what concentration indicate a pattern of use. Within this overall pattern of use, the Expert Panel considers all ingredients in this group to be safe.

INTRODUCTION

Glycyrrhetinic Acid, its salts and esters, and Glycyrrhizic Acid, and its salts and esters are used as cosmetic ingredients. This review presents information relevant to the safety of these ingredients as considered by the Cosmetic Ingredient Review (CIR) Expert Panel.

These ingredients are not plant extracts, powders, or juices, but rather are specific chemical species that may be isolated from the licorice plant. Plant-derived extracts, powders, juices, etc. [e.g., Glycyrrhiza Glabra (Licorice) Leaf Extract] also used as cosmetic ingredients, which may contain these organic compounds, but likely will include other chemicals, will be reviewed in another report.

The ingredients in this safety assessment are:

- Glycyrrhetinic Acid,
- Potassium Glycyrrhetinate,
- Disodium Succinoyl Glycyrrhetinate,
- Glyceryl Glycyrrhetinate,
- Glycyrrhetinyl Stearate,
- Stearyl Glycyrrhetinate,
- Glycyrrhizic Acid,
- Ammonium Glycyrrhizate,
- Dipotassium Glycyrrhizate,
- Disodium Glycyrrhizate,
- Trisodium Glycyrrhizate,
- Methyl Glycyrrhizate, and
- Potassium Glycyrrhizinate.

This review presents information relevant to the safety of these cosmetic ingredients as considered by the CIR Expert Panel.

No published information was identified for Disodium Succinoyl Glycyrrhetinate, Glyceryl Glycyrrhetinate, Glycyrrhetinyl Stearate, Methyl Glycyrrhizate, Potassium Glycyrrhetinate, Potassium Glycyrrhizinate, or Trisodium Glycyrrhizate, but they are considered to be sufficiently structurally related to the rest of the ingredients that the data may be extrapolated to address their safety.

CHEMISTRY

Definition and Structure

Glycyrrhetinic Acid (CAS no. 471-53-4) is an organic compound derived from Glycyrrhizic Acid or shredded licorice roots. It conforms generally to the structure presented in Figure 1 (Gottschalck and McEwen 2004).

Synonyms for Glycyrrhetinic Acid include Glycyrrhetic Acid; Olean-12-En-29-Oic-Acid, 3-Hydroxy-11-Oxo-, (3β, 20β)-; and (3β, 20β)-3-Hydroxy-11-Oxo- Olean-12-En-29-Oic-Acid. All 13 ingredients reviewed in this report have in common the basic core structure of Glycyrrhetinic Acid (Gottschalck and McEwen 2004). There are two stereoisomers of Glycyrrhetinic Acid described in the literature, 18α- and 18β-Glycyrrhetinic Acid. It is unclear which stereoisomer is used in cosmetics or if it is a mixture.

Glycyrrhetinic Acid has the empirical formula of $C_{30}H_{46}O_4$ and conforms to the formula presented in Figure 1, with both R_1 and R_2 being OH. Table 1 lists its derivatives and the R groups corresponding to each one.

Potassium Glycyrrhetinate (CAS no. 85985-61-1) is the potassium salt of Glycyrrhetinic Acid. Potassium Glycyrrhetinate is also known as Olean-12-En-29-Oic Acid, 3-Hydroxy-1, 1-Oxo-, Monopotassium Salt (Gottschalck and McEwen 2004).

Disodium Succinoyl Glycyrrhetinate (no CAS no. listed) is the disodium salt of the ester of succinic alcohol and Glycyrrhetinic Acid that conforms to the structure shown in Figure 1, with R groups given in Table 1 (Gottschalck and McEwen 2004).

Glyceryl Glycyrrhetinate (CAS no. 108916-85-4) is the monoester of glycerine and Glycyrrhetinic Acid. Synonyms for Glyceryl Glycyrrhetinate include Glycyrrhetinic Acid, Glyceryl Ester; Glyceryl Glycyrrhetate; and Olean-12-En-29-Oic Acid, 3-Hydroxy-11-Oxo-, Monoester with 1,2,3 Propanetriol, (3β, 20β) (Gottschalck and McEwen 2004).

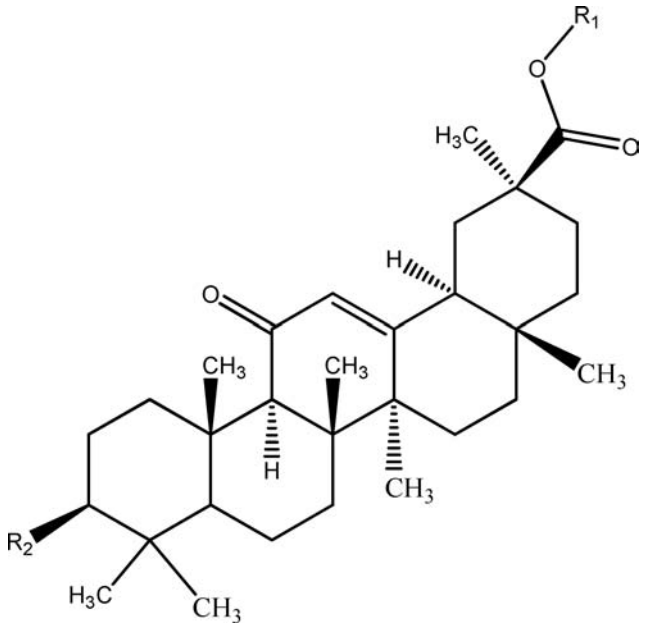

FIGURE 1
Glycyrrhetinic Acid. R_1 and R_2 are OH groups (Gottschalck and McEwen 2004).

Glycyrrhetinyl Stearate (CAS no. 4827-59-2) is the stearic acid ester of Glycyrrhetinic Acid that conforms generally to the structure displayed in Figure 1, with R groups given in Table 1. It is also known as 3-Stearoyloxy Glycyrrhetinic Acid (Gottschalck and McEwen 2004).

Stearyl Glycyrrhetinate (CAS no. 13832-70-7) is the ester of stearyl alcohol and Glycyrrhetinic Acid which conforms to the structure displayed in Figure 1, with R groups given in Table 1. Synonyms include Octadecyl Glycyrrhetinate; Octadecyl 3-Hydroxy-11-Oxoolean-12-En-29-Oate; and Olean-12-En-29-Oic Acid, 3-Hydroxy-11-Oxo-, Octadecyl Ester (Gottschalck and McEwen 2004).

Glycyrrhizic Acid (CAS no. 1405-86-3) is a natural material extracted from the plant *Glycyrrhiza Glabra* (Gottschalck and McEwen 2004). Glycyrrhizic Acid is a conjugate of Glycyrrhetinic Acid and two molecules of glucuronic acid. It conforms generally to the structure in Figure 1, with R groups given in Table 1. Glycyrrhizic Acid is also known as (3β, 20β)-20-Carboxy-11-Oxo-30-Norlean-12-En-3-Yl-2-*O*-β-17-Glucopyranuronosyl-α-D-Glucopyranosiduronic Acid; α-D-Glucopyranosiduronic Acid, (3β, 20β)-20-Carboxy-11-Oxo-30-Norlean-12-En-3-Yl-2-*O*-β-17-Glucopyranuronosyl-; Glycyrrhetinic Acid Glycoside; Glycyrrhizin; and Glycyrrhizinic Acid (Gottschalck and McEwen 2004).

Ammonium Glycyrrhizate (CAS no. 53956-04-0) is the ammonium salt of Glycyrrhizic Acid (q.v.). Synonyms include Ammonium Glycyrrhizinate; α-D-Glucopyranosiduronic Acid, (3β, 20β)-20-Carboxy-11-Oxo-30-Norlean-12-En-3-Yl-2-*O*-β-D-Glucopyranuronosyl-, Ammoniate; Glycyrrhizin Ammonium Salt; Glycyrrhizic Acid Ammonium Salt; Monoammonium Glycyrrhizinate; and Monoammonium α-Glycyrrhizinate (Gottschalck and McEwen 2004).

Dipotassium Glycyrrhizate (CAS no. 68797-35-3) is the dipotassium salt of Glycyrrhizic Acid (q.v.). Synonyms for Dipotassium Glycyrrhizate include Dipotassium Glycyrrhizinate; α-D-Glucopyranosiduronic Acid, (3β, 20β)-20-Carboxy-11-Oxo-30-Norlean-12-En-3-Yl-2-*O*-β-D-Glucopyranuronosyl-, Dipotassium Salt; and 30-Noroleanane, α-D-Glucopyranosiduronic Acid Derivative (Gottschalck and McEwen 2004).

Disodium Glycyrrhizate (CAS no. 71277-79-7) is the disodium salt of Glycyrrhizic Acid. Synonyms for Disodium Glycyrrhizate include Disodium Glycyrrhizinate; α-D-Glucopyranosiduronic Acid, (3β, 20β)-20-Carboxy-11-Oxo-30-Norlean-12-En-3-Yl-2-*O*-β-D-Glucopyranuronosyl-, Disodium Salt; and 30-Noroleanane, α-D-Glucopyranosiduronic Acid Derivative (Gottschalck and McEwen 2004).

Trisodium Glycyrrhizate (no CAS no. available) is the trisodium salt of Glycyrrhizic Acid (q.v.) (Gottschalck and McEwen 2004).

Methyl Glycyrrhizate (no CAS no. available) is the ester of methyl alcohol and Glycyrrhizic Acid (q.v.). It is also known

TABLE 1
R groups in Figure 1 for derivatives of Glycyrrhetinic Acid (Gottschalck and McEwen 2004).

Compound	R_1	R_2
Potassium Glycyrrhetinate	K	OH
Disodium Succinoyl Glycyrrhetinate	Na	O-C(O)-CH_2-CH_2-C(O)-ONa
Glyceryl Glycyrrhetinate	CH_2-CH(OH)-CH_3	OH
Glycyrrhetinyl Stearate	H	O-C(O)-$(CH_2)_{17}$-CH_3
Stearyl Glycyrrhetinate	$(CH_2)_{17}$-CH_3	OH
Glycyrrhizic Acid	H	(β-Glucuronylglucuronic acid)
Ammonium Glycyrrhizate	NH_3	(β-Glucuronylglucuronic acid)
Dipotassium Glycyrrhizate	K	(β-Glucuronylglucuronic acid·K)
Disodium Glycyrrhizate	Na	(β-Glucuronylglucuronic acid·Na)
Methyl Glycyrrhizate	CH_3	(β-Glucuronylglucuronic acid)

TABLE 2
Physical and chemical properties of Glycyrrhetinic Acid and Glycyrrhizic Acid and their derivatives (Lide 1993; CTFA 2004; Cognis 2002).

Compound	Molecular Weight	Boiling point	Melting point	Soluble in:	UV absorbance hrulefill Max.	UV absorbance hrulefill $E^{1\%}_{1cm}$
Glycyrrhetinic Acid	470.69	296°C	—	Ethanol	249	237.6
Disodium Succinoyl Glycyrrhetinate	—	—	—	Water	258.5	186.7
Glycyrrhetinyl Stearate	—	—	—	Ethanol	249	157.1
Stearyl Glycyrrhetinate	—	—	—	Ethanol	248.5	156.3
Glycyrrhizic Acid	882.94	—	220°C	Water	—	—
Ammonium Glycyrrhizate	—	—	—	Ethanol	251.5	134.6
Dipotassium Glycyrrhizate	—	—	—	Water	257.5	119.6

as Glycyrrhizic Acid, Methyl Ester (Gottschalck and McEwen 2004).

Potassium Glycyrrhizinate is an enzymatically produced mixture of potassium salts of Glycyrrhizic Acid (q.v.) and Glycyrrhetinic Acid monoglycoside. It is also known as Glycopyranosiduronic Acid, 20-Carboxy-11-Oxo-30-Norolean-12-En-3-Ly and 2-Glycopyranuronosyl-, Potassium Salt (Gottschalck and McEwen 2004).

Physical and Chemical Properties

The physical and chemical properties of Glycyrrhetinic Acid and Glycyrrhizic Acid are summarized in Table 2. Data were not available for the other ingredients.

Method of Manufacture

Glycyrrhetinic Acid is derived from Glycyrrhizic Acid or isolated from shredded licorice roots (Gottschalck and McEwen 2004). 18β-Glycyrrhetinic Acid is obtained from the licorice root (*Glycyrrhiza glabra L.*) by hydroalcoholic extraction, hydrolysis and crystallization methods (CTFA 2004).

Glycyrrhizin (Glycyrrhizic Acid) and Ammonium Glycyrrhizate are isolated from water-soluble extracts from the dried rhizome and roots of *Glycyrrhiza glabra* or of other *Glycyrrhiza* species yielding a yellow and sweet wood (Informatics, Inc. 1972). Glycyrrhizin and Ammonium Glycyrrhizate may also be isolated from extracts of the roots of *Abrus precatorius*, *Periandra dulcis*, *Periandra mediteranea*, and from the bark of the trees *Lucuma glycophylla*, *Achras sapota*, and *Sideroxylon richarhii* (Informatics, Inc. 1972).

Ammoniated Glycyrrhizin (also known as Ammonium Glycyrrhizate) is prepared from the water extract of licorice root by acid precipitation followed by neutralization with dilute ammonia (21CFR148.1408). It is also reported that Ammonium Glycyrrhizate is obtained from the licorice root (*Glycyrrhiza glabra L.*) by hydroalcoholic extraction, hydrolysis and crystallization methods (CTFA 2004).

Dipotassium Glycyrrhizate is obtained from the licorice root (*Glycyrrhiza glabra L.*) by hydroalcoholic extraction, hydrolysis and crystallization methods (CTFA 2004).

Analytical Methods

Spinks and Fenwick (1990) used high-performance liquid chromatography (HPLC) to determine the content of Glycyrrhizic Acid in licorice-containing confectionary and health products. Okamura et al. (2001) used semi-micro-HPLC to simultaneously detect Glycyrrhizic Acid, Glycyrrhetinic Acid, and glycyrrhetinic acid mono-glucuronide in a combination of licorice root and peony root with rat feces. Liu et al. (2001) used mass spectrometry and nuclear magnetic resonance spectra to isolate and identify Glycyrrhizic Acid, Glycyrrhetinic Acid, and other constituents in a licorice extract.

Impurities

Cognis (2002) reports that its products Plantactiv® GLA18 (Glycyrrhetinic Acid), Plantactiv® AGL (Ammonium Glycyrrhizate), and Plantactiv® PGL (Dipotassium Glycyrrhizate) are a minimum 98%, 98%, and 95% pure, respectively.

Maruzen Pharaceuticals Company, Ltd. (2004) analyzed Glycyrrhetinic Acid. They found 99.4% 18β-Glycyrrhetinic Acid and 0.6% 24-OH-Glycyrrhetinic Acid by the HPLC method. They also found not more than 20 μg/g of heavy metals and not more than 2 μg/g of arsenic. Hill Laboratories (2004) conducted a multiresidue Pesticide Analysis on Glycyrrhetic Acid. There was no residue detected by ethyl acetate extraction, gel permeation chromatography cleanup, gas chromatography-electron capture detector/nitrogen phosphorus detector, and gas chromatography–mass spectrometry (GC-MS).

Cognis reports that Dipotassium Glycyrrhizate, 18β-Glycyrrhetinic Acid, and Ammonium Glycyrrhizate samples are absent of allergens except for *d*-Limonene at <1 ppm (CTFA 2004).

USE

Cosmetic Use

As given in the *International Cosmetic Ingredient Dictionary and Handbook*, **Glycyrrhetinic Acid** is a skin conditioning agent-miscellaneous (Gottschalck and McEwen 2004) and is reportedly used in 65 cosmetic formulations (FDA 2002) at a maximum reported concentration of use of 2% (CTFA 2003).

The frequencies of use and use concentrations of Glycyrrhetinic Acid in specific product categories are listed in Table 3.

Potassium Glycyrrhetinate functions as a flavoring agent and skin-conditioning agent—miscellaneous in cosmetic products (Gottschalck and McEwen 2004). However, there were no reports to FDA (2002) of its use in any cosmetic product categories and no concentration of use data were available.

Disodium Succinoyl Glycyrrhetinate is used in four permanent wave products (FDA 2002) as shown in Table 3. No concentration of use data were available for this compound. The function of this ingredient is not given in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004).

Glyceryl Glycyrrhetinate functions as a flavoring agent in cosmetic products (Gottschalck and McEwen 2004). However, there were no reports to FDA (2002) of its use in any cosmetic product categories and no concentration of use data were available.

Glycyrrhetinyl Stearate functions as a flavoring agent in cosmetic products (Gottschalck and McEwen 2004). However, there were no reports to FDA (2002) of its use in any cosmetic product categories and no concentration of use data were available.

Stearyl Glycyrrhetinate functions in cosmetics as a flavoring agent (Gottschalck and McEwen 2004) and is reportedly used in 35 cosmetic products (FDA 2002) at use concentrations up to 0.1% (CTFA 2003) as shown in Table 3.

Glycyrrhizic Acid functions as a flavoring agent and skin-conditioning agent—miscellaneous (Gottschalck and McEwen 2004). The use of Glycyrrhizic Acid in specific product categories (FDA 2002) are listed in Table 3. Glycyrrhizic Acid is reportedly used at 0.1% in many cosmetic product categories (CTFA 2003).

Ammonium Glycyrrhizate is a flavoring agent and skin-conditioning agent—miscellaneous (Gottschalck and McEwen 2004) and is used in 72 cosmetic formulations (FDA 2002) at a maximum concentration of use of 5% (CTFA 2003) as shown in Table 3.

Dipotassium Glycyrrhizate functions as a flavoring agent and skin-conditioning agent—miscellaneous (Gottschalck and McEwen 2004) and is used in 29 cosmetic formulations (FDA 2002) at a maximum concentration of 1% (CTFA 2003). The frequencies of use and use concentrations of Dipotassium Glycyrrhizate in specific product categories are listed in Table 3.

Disodium Glycyrrhizate is a flavoring agent and skin-conditioning agent—miscellaneous in cosmetic products (Gottschalck and McEwen 2004). However, there were no reports to FDA (2002) of its use in any cosmetic product categories. No concentration of use data were available.

Trisodium Glycyrrhizate is a flavoring agent and skin-conditioning agent—miscellaneous (Gottschalck and McEwen 2004). FDA (2002) reports that it is used in one hair conditioner and in one other noncoloring hair preparation as shown in Table 3. No concentration of use data were available.

Methyl Glycyrrhizate is a flavoring agent in cosmetic products (Gottschalck and McEwen 2004). However, there were no reports to FDA (2002) of its use in any cosmetic product categories. No concentration of use data were available.

Potassium Glycyrrhizinate is a skin-conditioning agent—miscellaneous in cosmetic products (Gottschalck and McEwen 2004). Although there were no reports to FDA (2002) of its use in any cosmetic product categories, a concentration of use of 1.0% was given in an industry survey and is shown in Table 3 (CTFA 2003).

Noncosmetic Use

Bombardelli (1991) demonstrated that 18β-Glycyrrhetinic Acid can be used as a vehicle to increase the transdermal absorption of some topical medications.

Although licorice and licorice derivatives are generally recognized as safe (GRAS) as food ingredients, the Food and Drug Administration (FDA) has established the following maximum restrictions on the levels of Glycyrrhizic Acid in certain types of foods: 0.05% in baked goods; 0.1% in alcoholic beverages; 0.15% in nonalcoholic beverages; 1.1% in chewing gum; 16.0% in hard candy; 0.15% in herbs and seasonings and in plant protein products; 3.1% in soft candy; 0.5% in vitamins and dietary supplements; and 0.1% in all other food except sugar substitutes. Glycyrrhizic Acid may not be used as a non-nutritive sweetener in sugar substitutes (12CFR184.1408).

Touitou et al. (1988) described the use of a Glycyrrhizic Acid gel as a vehicle for an idoxuridine topical preparation. The Glycyrrhizic Acid gel facilitated the idoxuridine absorption through hairless mouse skin.

Glycyrrhizic Acid is used in the treatment of chronic hepatitis B and C (Chitturi and Farrell 2001).

The GRAS status of Ammonium Glycyrrhizate was supported by a review performed by the Life Science Research Office (LSRO 1974).

Dipotassium Glycyrrhizate is an anti-inflammatory agent that is used to treat chronic dermatitis (Trotta et al. 2002).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Overview

Ploeger et al. (2001a) published an extensive review of the pharmacokinetics of Glycyrrhizic Acid in rodents and humans. Glycyrrhizic Acid is poorly absorbed by the gastrointestinal tract. However, Glycyrrhizic Acid is hydrolyzed by a specialized β-glucuronidase produced by intestinal bacteria to make Glycyrrhetinic Acid. The plasma of germ-free rats showed no Glycyrrhetinic Acid after oral administration of Glycyrrhizic Acid. After hydrolysis by bacteria, Glycyrrhetinic Acid is then absorbed into the circulatory system. However, this absorption is saturable at doses above 25 mg/kg in both rats

TABLE 3
Frequency of use and concentration of Ammonium Glycyrrhizate, Dipotassium Glycyrrhizate, Disodium Succinoyl Glycyrrhetinate, Glycyrrhetinic Acid, Glycyrrhizic Acid, and Stearyl Glycyrrhetinate.

Product category (total in category) (FDA 2002)	Frequency of use (FDA 2002)	Concentration of use (%) (CTFA 2003)
	Glycyrrhetinic Acid	
Baby products		
Baby Lotions, Oils, Powders, and Creams (60)	—	0.1–1
Eye Makeup		
Eye Shadow (576)	1	—
Eye Lotion (25)	1	0.2
Other Eye Makeup Preparations (152)	1	—
Fragrance Products		
Other Fragrance Preparations (173)	—	0.1
Noncoloring Hair Products		
Hair Conditioners (651)	—	0.1
Shampoos (noncoloring) (884)	—	0.2
Tonics, Dressings, and Other Hair-Grooming Aids (598)	3	0.05–0.2
Other Hair Preparations (277)	—	0.2
Makeup		
Blushers (All Types) (245)	2	—
Face Powders (305)	4	1
Foundations (324)	3	—
Other Makeup Preparations (201)	1	0.1–1
Personal Hygiene Products		
Underarm Deodorants (247)	1	0.2
Shaving Products		
Aftershave Lotion (231)	2	—
Skin Care Products		
Cleansing Creams, Lotions, and Sprays (775)	2	0.05
Face and Neck Creams, Lotions, and Sprays (301)	6	0.01–2
Body and Hand Creams, Lotions, and Sprays (840)	7	0.001–2
Moisturizers (905)	10	0.01 – 1
Night Creams, Lotions, and Sprays (200)	7	0.3
Skin Fresheners (184)	—	0.01
Paste Masks (Mud Packs) (271)	6	—
Other Skin Care Preparations (725)	3	0.1–2
Suntan Products		
Suntan Gels, Creams, and Liquids (131)	2	0.1
Other Suntan Preparations (38)	3	—
Total	**65**	**0.001–2**
	Disodium Succinoyl Glycyrrhetinate	
Noncoloring Hair Products		
Permanent Waves (207)	4	—
Total	*4*	—
	Stearyl Glycyrrhetinate	
Bath Products		
Other Bath Preparations (196)	—	0.1
Eye Makeup		
Eyeliner (548)	—	0.1
Eye Lotion (25)	1	—
Mascara (195)	—	0.05

(Continued on next page)

TABLE 3
Frequency of use and concentration of Ammonium Glycyrrhizate, Dipotassium Glycyrrhizate, Disodium Succinoyl Glycyrrhetinate, Glycyrrhetinic Acid, Glycyrrhizic Acid, and Stearyl Glycyrrhetinate. *(Continued)*

Product category (total in category) (FDA 2002)	Frequency of use (FDA 2002)	Concentration of use (%) (CTFA 2003)
Fragrance Products		
Other Fragrance Preparations (173)	2	—
Noncoloring Hair Products		
Permanent Waves (207)	1	—
Makeup		
Blushers (245)	—	0.007–0.02
Face Powders (305)	—	0.01
Foundations (324)	—	0.02–0.1
Lipstick (962)	2	0.05–0.2
Makeup Bases (141)	—	0.1
Makeup Fixatives (20)	—	0.04
Other Makeup Preparations (201)	2	—
Nail Care Products		
Basecoats and Undercoats (44)	1	—
Nail Polish and Enamel (123)	4	0.02
Skin Care Products		
Cleansing Creams, Lotions, and Sprays (775)	3	0.05–0.1
Face and Neck Creams, Lotions, and Sprays (310)	1	0.05–0.1
Body and Hand Creams, Lotions, and Sprays (840)	—	0.05–0.1
Moisturizers (905)	13	0.01–0.1
Night Creams, Lotions, and Sprays (200)	1	0.01–0.1
Paste Masks (Mud Packs) (271)	—	0.1–0.2
Other Skin Care Preparations (725)	3	0.05–1
Suntan Products		
Suntan Gels, Creams, and Liquids (131)	—	0.05–0.2
Other Suntan Preparations (38)	—	0.1–0.2
Total	**34**	**0.007–1**
Glycyrrhizic Acid		
Bath Products		
Other Bath Preparations (196)	—	0.1
Bath Soaps and Detergents (421)	—	0.1
Eye Makeup		
Eye Lotion (25)	1	0.1
Noncoloring Hair Products		
Tonics, Dressings, and Other Hair-Grooming Aids (598)	—	0.1
Makeup		
Face Powders (305)	—	0.1
Makeup Fixatives (20)	1	—
Skin Care Products		
Cleansing Creams, Lotions, and Sprays (775)	—	0.1
Face and Neck Creams, Lotions, and Sprays (263)	—	0.1
Night Creams, Lotions, and Sprays (188)	—	0.1
Total	**2**	**0.1**
Ammonium Glycyrrhizate		
Baby Products		
Other Baby Products (34)	—	1
Bath Products		

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TABLE 3
Frequency of use and concentration of Ammonium Glycyrrhizate, Dipotassium Glycyrrhizate, Disodium Succinoyl Glycyrrhetinate, Glycyrrhetinic Acid, Glycyrrhizic Acid, and Stearyl Glycyrrhetinate. *(Continued)*

Product category (total in category) (FDA 2002)	Frequency of use (FDA 2002)	Concentration of use (%) (CTFA 2003)
Bath Oils, Tablets and Salts (143)	—	0.01
Bath Soaps and Detergents (421)	—	0.1
Eye Makeup		
Eye Shadow (576)	1	—
Eye Lotion (25)	—	0.5
Noncoloring Hair Products		
Hair Conditioners (651)	—	0.01
Hair Sprays (275)	—	0.0007
Shampoos (884)	—	0.001–0.1
Tonics, Dressings, and Other Hair-Grooming Aids (598)	3	0.05–0.2
Hair-Coloring Products		
Hair Dyes and Colors (1690)	—	0.2
Makeup		
Lipstick (962)	51	1–5
Other Makeup Preparations (201)	2	0.1
Nail Care Products		
Nail Polish and Enamel Removers (36)	—	0.01
Oral Hygiene Products		
Dentifrices (40)	1	0.02–0.3
Mouthwashes and Breath Fresheners (46)	—	0.05–1
Other Oral Hygiene Products (6)	—	0.07
Personal Hygiene Products		
Other Personal Hygiene Products (308)	—	0.2
Shaving Products		
Aftershave Lotion (231)	—	0.02–0.05
Other Shaving Preparation Products (63)	1	—
Skin Care Products		
Cleansing Creams, Lotions, and Sprays (775)	4	0.05–0.3
Face and Neck Creams, Lotions, and Sprays (310)	1	0.1
Body and Hand Creams, Lotions, Powders and Sprays (840)	—	0.05–1
Moisturizing Creams, Lotions, and Sprays (905)	2	0.05–0.1
Night Creams, Lotions, and Sprays (200)	1	0.05–0.1
Paste Masks (Mud Packs) (271)	3	0.05
Other Skin Care Preparations (725)	1	0.1
Suntan Products		
Suntan Gels, Creams and Liquids (131)	—	0.01–0.1
Indoor Tanning Preparations (71)	1	0.01
Other Suntan Products (38)	—	0.1
Total	**72**	**0.0007–5**
Dipotassium Glycyrrhizate		
Bath Products		
Bath Oils, Tablets, and Salts (143)	—	0.1
Bath Soaps and Detergents (421)	—	0.1–0.2
Other Bath Preparations (196)	—	0.1
Eye Makeup		
Eye Lotion (25)	—	0.1
Mascara (195)	—	0.05

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TABLE 3
Frequency of use and concentration of Ammonium Glycyrrhizate, Dipotassium Glycyrrhizate, Disodium Succinoyl Glycyrrhetinate, Glycyrrhetinic Acid, Glycyrrhizic Acid, and Stearyl Glycyrrhetinate. *(Continued)*

Product category (total in category) (FDA 2002)	Frequency of use (FDA 2002)	Concentration of use (%) (CTFA 2003)
Eye Makeup Remover (100)	2	—
Noncoloring Hair Products		
Hair Conditioners (651)	1	0.0007–0.1
Rinses (Noncoloring) (42)	—	0.1
Shampoos (Noncoloring) (884)	—	0.0007–0.1
Tonics, Dressings, and Other Hair-Grooming Aids (598)	—	0.00007–0.01
Other Hair Preparations (277)	—	0.01–0.2
Fragrance Products		
Colognes and Toilet Waters (684)	—	0.01
Powders (273)	3	—
Oral Hygiene Products		
Dentrifices (40)	—	0.05
Shaving Products		
Aftershave Lotions (231)	—	0.1
Preshave Lotion (14)	—	0.05
Shaving Cream (134)	—	0.1–0.05
Other Makeup Preparations (201)	—	0.05
Makeup		
Foundations (324)	5	0.01–0.2
Lipstick (962)	—	0.05
Makeup Bases (141)	—	0.05
Other Shaving Preparations (63)	—	0.05
Skin Care Products		
Cleansing Creams, Lotions, and Sprays (775)	3	0.01–1
Depilatories (34)	—	0.05
Face and Neck Creams, Lotions, and Sprays (310)	1	0.05–0.5
Body and Hand Creams, Lotions, Powders and Sprays (840)	—	0.05–0.2
Foot Powders and Sprays (35)	—	0.01
Moisturizing Creams, Lotions, and Sprays (905)	8	0.05–0.2
Night Creams, Lotions, and Sprays (200)	—	0.05–0.2
Paste Masks (Mud Packs) (271)	1	0.05
Skin Fresheners (184)	—	0.01–0.1
Other Skin Care Preparations (725)	5	0.1–0.5
Suntan Products		
Suntan Gels, Creams and Liquids (131)	—	0.02
Other Suntan Preparations (38)	—	0.1
Total	**29**	**0.00007–1**
Potassium Glycyrrhetinate		
Skin Care Products		
Cleansing Creams, Lotions, and Sprays (775)	—	1
Face and Neck Creams, Lotions, and Sprays (310)	—	1
Night Creams, Lotions, and Sprays (200)	—	1
Total	—	**1**

and humans. Both Glycyrrhetinic Acid and Glycyrrhizic Acid bind extensively to rat and human plasma albumin.

This binding is saturable at high plasma concentrations. These compounds do not absorb well into tissues, as the tissue-to-blood partition coefficients for all rat tissues tested are less than 1.

Hepatic metabolism of Glycyrrhetinic Acid produces the conjugates 18-β-glycyrrhetyl-3-*O*-monoglucuronide, 18-β-glycyrrhetyl-3-*O*-hydrogen sulfate, and 18-β-glycyrrhetyl-3-*O*-monoglucuronide. Glycyrrhetinic Acid and Glycyrrhizic Acid and metabolites are mostly excreted in the bile. Once in the gastrointestinal tract, bacterial enzymes can remove the glucuronide and sulfate conjugates to restore absorbable Glycyrrhetinic Acid. Thus, Glycyrrhetinic Acid and its metabolites are subject to enterohepatic cycling in rats and, presumably, in humans. Very little is excreted in the urine (Ploeger et al. 2001a).

Imai et al. (1999) used in vivo (Sprague-Dawley rats) and in vitro (Caco-2 cells) models to evaluate the enhancing effects of Dipotassium Glycyrrizate on the intestinal absorption of drugs. The authors found that it is not Dipotassium Glycyrrizate but its metabolite Glycyrrhetinic Acid that enhances drug absorption in the intestine. Salmon calcitonin was the drug used to demonstrate this effect.

Trotta et al. (2002) studied the ability of elastic liposomes to increase the transepidermal permeation of Dipotassium Glycyrrhizate through pig skin. Full-thickness pig ear skin was prepared and fixed in a Franz-type apparatus for the measurement of transdermal permeation. The diffusion area was 2.05 cm^2, and the receptor chamber was filled with 6 ml of 0.002% aqueous sodium azide. The application side was dry when 100 μg of test material was applied. The test materials were 0.25% Dipotassium Glycyrrhizate in water solution, 1% Dipotassium Glycyrrhizate in a water micelle solution, liposomes of soya lethicin or hydrogenated soya lethicin each with 0.25% Dipotassium Glycyrrhizate, or oil/water emulsions containing 0.25% Dipotassium Glycyrrhizate. Test material was left on the skin surface for 12 h, and samples of fluid from the receptor chamber were collected at regular intervals for ultraviolet (UV)-HPLC analysis. All treatments showed negligible flux of Dipotassium Glycyrrhizate across the pig skin, as concentrations of Dipotassium Glycyrrhizate in the receptor chamber fluid were below the UV-HPLC detection limit.

After the 12-h treatment period, the treatment surface was washed five times with 50% ethanol and then water. The skin was removed from the apparatus and homogenized, and the Dipotassium Glycyrrhizate content of the skin was determined by HPLC. Dipotassium Glycyrrhizate was found in the skin at 12 ± 3 $\mu g/cm^2$ from the 0.25% water solution, 19 ± 5 $\mu g/cm^2$ from the 1% water micelle solution, and 13 ± 4 $\mu g/cm^2$ from the 0.25% oil/water emulsion. The liposomes containing 0.25% Dipotassium Glycyrrhizate increased the skin content from 62 $\pm$ 8 to 71 $\pm$10 $\mu g/cm^2$, approximately four to five times the amount absorbed without liposomes. Thus, although Dipotassium Glycyrrhizate was able to enter the pig skin, it was unable to permeate through all layers of the skin tissue (Trotta et al. 2002).

Animal

Parke et al. (1963) treated six albino rats with a single oral dose of 60 mg/kg of tritium-labeled β-Glycyrrhetinic Acid in aqueous suspension. Two additional rats received a single subdermal injection of 60 mg/kg tritium-labeled β-Glycyrrhetinic Acid in aqueous suspension. Blood, urine, and feces were collected for several time points after the dose was given. Detection of Glycyrrhetinic Acid and its metabolites in the biological samples was accomplished by paper chromatography using different media and scintillation counting of radioactivity.

In rats receiving 60 mg/kg orally, an average of 86% of the radioactivity administered was recovered in 1 to 3 days with 83% in the feces, 1% in the urine, and 4% remained in the liver. In rats given 60 mg/kg subdermally, 74% of the administered radioactivity was recovered with 73% in the feces and 1% in the urine. Of the radioactivity recovered in the feces, 7.4% was unchanged β-Glycyrrhetinic Acid in the orally dosed rats, and 5.2% in subdermally dosed rats. The remaining radioactivity in the feces was attributed to α-Glycyrrhetinic Acid, 3-keto- β-glycyrrhetinic acid, 3-aceto- β-glycyrrhetinic acid, Ammonium Glycyrrhizate, and other metabolites (Parke et al. 1963).

Ishida et al. (1989) investigated the pharmacokinetics of Glycyrrhetinic Acid. Male Wistar rats were given a bolus intravenous injection of 2, 5, or 12 mg/kg Glycyrrhetinic Acid. The sample size per group was not reported. Samples of blood, bile, and lymph were collected by cannulae. The investigators found that Glycyrrhetinic Acid in rats adhered to a two-compartment pharmacokinetic model.

The first-order partition rate from the central compartment to the peripheral compartment was 0.0797 min^{-1}, and from the peripheral back to the central compartment was 0.0817 min^{-1}. The volume of distribution of the central compartment was 72.293 ml/kg. The Michaelis constant and maximum velocity of elimination from the central compartment were 27.970 μg/ml and 15.329 μg/ml/min/kg, respectively. The plasma-to-blood concentration ratio was constant at all doses (1.787), indicating that Glycyrrhetinic Acid was not well absorbed into erythrocytes. Tissue-to-plasma concentration ratios were all less than 1 (maximum was 0.121 in kidney), indicating poor tissue distribution. The tissue-to-plasma concentration ratio of skin was 0.089 ± 0.006. The steady state volume of distribution was statistically similar among the three dose groups. However, the total body clearance decreased in a dose-dependent manner with increasing doses: 8.194, 6.047, and 3.694 ml/min/kg for the 2, 5, and 12 mg/kg groups, respectively. The authors concluded that the elimination of Glycyrrhetinic Acid was saturable (Ishida et al. 1989).

Cantelli-Forti et al. (1997) studied the biliary excretion of Glycyrrhizic Acid and Glycyrrhetinic Acid after oral or intravenous administration of licorice extract or Glycyrrhizic Acid. Male Sprague-Dawley rats received 480 mg/kg Glycyrrhizic

Acid or 6278 mg/kg licorice extract by oral gavage ($n = 6$ rats per treatment group). The Glycyrrhizic Acid dose was selected to give the same amount of Glycyrrhizic Acid found in the licorice extract dose. Six control rats were given 10 ml/kg distilled water. After dosing, animals were anesthetized, and their abdomens were opened. The common bile duct of each animal was tied and cannulated with a polyethylene catheter, through which bile was steadily collected every 120 min for 16 h.

Bile samples were analyzed by HPLC to detect Glycyrrhizic Acid and Glycyrrhetinic Acid. After oral gavage of Glycyrrhizic Acid, the excretion of Glycyrrhizic Acid via bile peaked (18.02 μg/min·kg) at 8 to 10 h and then decreased sharply. After oral gavage with licorice extract (containing the same amount of Glycyrrhizic Acid), the biliary excretion of Glycyrrhizic Acid was significantly reduced and peaked (3.43 μg/min·kg) at 6 to 8 h. Area under the curve analysis indicated a significant sevenfold increase in biliary excretion of Glycyrrhizic Acid after oral Glycyrrhizic Acid, compared to licorice extract. Thus, excretion of Glycyrrhizic Acid is greater after oral Glycyrrhizic Acid than oral licorice extract. Levels of Glycyrrhetinic Acid in the bile were below the detection limits in all bile samples.

In another experiment, rats were anesthetized and their bile ducts were cannulated for bile collection. Baseline bile was collected for 1 h and then 32.7 mg/kg licorice extract or 2.5 mg/kg Glycyrrhizic Acid was injected intravenously over a period of 1 h by means of a peristaltic pump. The Glycyrrhizic Acid dose was selected to give the same amount of Glycyrrhizic Acid found in the licorice extract dose. Bile samples were collected hourly for 6 h. After intravenous administration of Glycyrrhizic Acid, bile flow was increased after extract injection, compared to Glycyrrhizic Acid injection. The authors did not compare the biliary Glycyrrhizic Acid concentrations after intravenous injection of licorice extract and Glycyrrhizic Acid (Cantelli-Forti et al. 1997).

Ichikawa et al. (1986) demonstrated that Glycyrrhizic Acid is excreted in the bile and undergoes enterohepatic cycling in rats. One group of rats was surgically fitted with biliary fistulas, whereas control rats were left intact. The rats received a single intravenous dose of 100 mg/kg Glycyrrhizic Acid. Plasma concentrations of Glycyrrhizic Acid were measured from blood samples taken at several time points after the dose was given. Plasma decay in control rats was biphasic. The area under the curve (AUC) of plasma concentrations for 12 h after dosing was significantly increased ($p < 0.01$) in control rats than in rats with biliary fistulization. Total body clearance of Glycyrrhizic Acid was 0.372 ml/min/kg in control rats and 0.936 ml/min/kg in rats with biliary fistulization, a significant difference ($p < 0.01$). The total cumulative excretion of Glycyrrhizic Acid in rats for 48 h was as follows: 80.6% of administered dose in bile in both control and fistulated rats; 9.8% in urine of control rats and 4.5% in urine of rats with bile fistulas; 5.5% in feces of control rats; and no Glycyrrhizic Acid was detected in the feces of rats with bile fistulas. These findings were attributed to the elimination of Glycyrrhizic Acid primarily in the bile.

Ishida et al. (1992) studied the pharmacokinetics of Glycyrrhizic Acid in rats. Male Wistar rats received 5, 10, 20, or 50 mg/kg Glycyrrhizic Acid via intravenous administration in the femoral vein. Sample size per group was not reported. Blood, bile, and urine were collected by cannulae at several time points up to 48 h. The decrease in mean plasma concentration was biphasic.

The steady-state volume of distribution (Vd_{ss}) for the 20 and 50 mg/kg groups (86.4 and 115.0 ml/kg, respectively) was significantly higher ($p < 0.05$) than in the 5 and 10 mg/kg groups (58.2 and 59.8 ml/kg, respectively). Total body clearance (Cl_{tot}) for the 20 and 50 mg/kg groups (1.54 and 1.20 ml/min/kg, respectively) was significantly lower ($p < 0.05$) than in the 5 and 10 mg/kg groups (1.91 and 1.98 ml/min/kg, respectively). Biliary excretion for 8 h after dosing was similar between all dose levels, ranging from 85.9% to 95.7% of the administered dose, suggesting a high potential of enterohepatic cycling. Calculations of plasma protein concentration and binding suggested that plasma protein binding may play a role in the changes in pharmacokinetic parameters at different dose levels (Ishida et al. 1992).

Yamamura et al. (1995) compared the phamacokinetic behavior of Glycyrrhizic Acid as a function of route of administration. Male Wistar rats were exposed to 2, 10, or 50 mg/kg Glycyrrhizic Acid either by intravenous (i.v.), intraperitoneal (i.p.), or oral (p.o.) routes. The number of rats per group was not reported. Cannulae were surgically placed and used to collect blood from the femoral artery and urine from the bladder. Blood and urine were collected at regular intervals before and after dosing.

After each i.v. administration, the Glycyrrhizic Acid plasma concentration decreased biexponentially, resulting in a biological half-life of 2.1 to 2.7 h. The steady-state volume of distribution was 98.6 to 166.0 ml/kg [p = n.s. (nonsignificant) between doses], and the total body clearance was 77.3 to 93.0 ml/h·kg (p = n.s. between doses). Urinary excretion of Glycyrrhizic Acid over the 24 h after 2, 10, and 50 mg/kg amounted to 1.3%, 1.9%, and 3.2% of the administered dose, respectively. The metabolite Glycyrrhetinic Acid appeared in the urine at 0.03%, 0.04%, and 0.01% of the administered dose of Glycyrrhizic Acid, respectively, to the above doses.

After i.p. injection, Glycyrrhizic Acid appeared rapidly in the plasma and reached maximum concentration within 30 min. The mean maximum concentration for the 2, 10, and 50 mg/kg dose groups was 4.7, 33.0, and 238.9 μg/ml, respectively. The biological half-life ranged from 2.5 to 2.9 h. The respective apparent clearance values for the three doses were 137.3, 108.62, and 85.6 ml/kg·h.

Rats receiving 2 or 10 mg/kg doses orally had Glycyrrhizic Acid plasma concentrations of 0.2 or 0.4 μg/ml, respectively. Glycyrrhizic Acid was eliminated by 2 h after dosing, whereas, it was detected at a concentration of 1.3 μg/ml in rats of the 50 mg/kg p.o. group, and Glycyrrhizic Acid remained in the plasma for over 8 h.

These investigators also studied the intestinal absorption of Glycyrrhizic Acid in an in situ experiment. A closed intestinal

loop of ileum was prepared, and the mesenteric vein was cannulated. A dose of 10 or 50 mg/kg Glycyrrhizic Acid was injected into the ileum, and mesenteric blood was collected for up to 100 min. Only 1.2% of the 10 mg/kg dose and 1.9% of the 50 mg/kg dose was absorbed as Glycyrrhizic Acid by the ileum. From 0.2% to 0.4% of the administered dose was collected in the blood as Glycyrrhetinic Acid. The intestinal contents contained 4.8% of the administered dose as Glycyrrhizic Acid. Two unspecified metabolites of Glycyrrhizic Acid were in the intestinal contents, amounting to 83.8% and 0.45% of the administered dose. Glycyrrhetinic Acid was not detected in the intestinal contents. In another experiment, Glycyrrhizic Acid was stable in gastric juices at 37°C and pH 1.4 for 3 h.

In summary, the pharmacokinetic profiles of i.v.- and i.p.-administered Glycyrrhizic Acid were similar, whereas the oral route had low bioavailability, possibly due to poor absorption from the intestinal tract (Yamamura et al. 1995).

Imai et al. (1999) and Akao (1994, 1997) demonstrated that Dipotassium Glycyrrhizate and Glycyrrhizic Acid, respectively, are metabolized by intestinal microflora into Glycyrrhetinic Acid before absorption can occur.

Wang et al. (1994) found that, in rats, Glycyrrhizic Acid is hydrolyzed to Glycyrrhetinic Acid in the stomach and large intestine, and that most of the Glycyrrhetinic Acid formed was absorbed from the large intestine.

Cantelli-Forti et al. (1994) and Wang et al. (1995) reported that more Glycyrrhizic Acid is absorbed in the intestine after oral administration of pure Glycyrrhizic Acid than after an oral administration of licorice extract with equal Glycyrrhizic Acid content. The authors suggested that other constituents of licorice extract inhibit the intestinal absorption of Glycyrrhizic Acid.

Human

Terasawa et al. (1986) measured the disposition of Glycyrrhetinic Acid in healthy human subjects. Five healthy male subjects (aged 20 to 24 years) each ingested 5 g of licorice dissolved in 100 ml water. The dose was determined by HPLC to contain 133 mg Glycyrrhizic Acid and no detectible Glycyrrhetinic Acid. Volunteers were prohibited from consuming alcohol but were not otherwise restricted in normal diet and activities. Blood was collected daily for 7 days after administration. Urine was collected every 10 h over the 7-day post-dose period. Glycyrrhetinic Acid and Glycyrrhizic Acid concentrations in the serum were measured using an enzyme immunoantibody assay. Urinary levels were measured by HPLC.

The maximum serum Glycyrrhizic Acid occurred at 4 h and levels rapidly decreased afterwards. By the 96th h, Glycyrrhizic Acid was undetectable in the sera of all subjects. Likewise, urinary Glycyrrhizic Acid peaked at the first 10-h collection after dose administration and then rapidly decreased. Serum Glycyrrhetinic Acid concentrations peaked at 30 ng/ml about 24 h after dosing and then declined. Four subjects had low but detectable serum Glycyrrhetinic Acid at 48 h, and two at 96 h. Glycyrrhetinic Acid appeared in the urine at the 20- and 30-h time points and decreased at a rate consistent with serum levels. Total urinary excretion of Glycyrrhetinic Acid was about 2% of the administered dose (Terasawa et al. 1986).

Yamamura et al. (1992) investigated the pharmacokinetics of Glycyrrhetinic Acid in human subjects. Three healthy volunteers received a single oral dose of 100 mg Glycyrrhizic Acid in tablet form. Demographics (sex, age, etc.) of the subjects were not reported. Blood was drawn from a cannula in the forearm 1 h before and 0.5, 1, 2, 4, 6, 8, 10, and 12 h after dosing. Urine was collected for a 24-h period after dosing. Diet and beverage were controlled during the study duration. Glycyrrhizic Acid was not detected in plasma after oral administration. Glycyrrhetinic Acid was detected at $<$200 ng/ml. Total urinary excretion of Glycyrrhizic Acid and its metabolites over the 24-h post-dose period for each of the subjects was 0.8%, 0.3%, and 0.4% of the administered dose.

The stability of Glycyrrhizic Acid in gastric juices at 37°C and pH 1.78 was examined. After 5 h, the hydrolysis products glycyrrhetinic acid 3-*O*-glucuronide and Glycyrrhetinic Acid were not detected. Thus, Glycyrrhizic Acid does not appear to be hydrolyzed in the stomach and is absorbed as intact Glycyrrhizic Acid.

Three healthy volunteers received either 40, 80, or 120 mg Glycyrrhizic Acid by intravenous infusion in the right forearm. Demographics (sex, age, etc.) of the subjects were not reported. Blood was drawn from a cannula in the forearm 1 h before administration and 5, 15, and 30 min, and 1, 2, 4, 6, 8, 10, and 12 h after dosing. Urine was collected for a 24-h period after dosing. The plasma concentration of Glycyrrhizic Acid decreased in a biphasic manner. The half-life of intravenously administered Glycyrrhizic Acid at the three dose levels was 3.8 to 4.8 h and total body clearance was 16 to 25 ml/kg/h. The steady-state volume of distribution was 78 to 98 ml/kg. Urinary excretion of Glycyrrhizic Acid over 24 h amounted to 1.1% to 2.5% of the administered dose. The metabolites, glycyrrhetininc acid 3-*O*-glucuronide and Glycyrrhetinic Acid, were not detected in plasma or urine (Yamamura et al. 1992).

Ploeger (2001b) compared the pharmacokinetics of Glycyrrhetinic Acid after a single dose and after multiple doses in humans. Twelve healthy male volunteers, aged 19 to 29 years with a mean body weight of 70 $\pm$ 6.6 kg, ingested 130 mg/day Glycyrrhetinic Acid in a propylene glycol and water vehicle for 5 days. Blood samples were collected prior to dosing and at several time points after dosing on the 1st and 5th days and again 5 days after the last dose. Urine was also collected throughout the study. After the first dose, the maximum plasma concentration (C_{max}) of Glycyrrhetinic Acid was achieved within 4 h post-dose, C_{max} was 1.11 $\pm$ 0.94 mg/L. After the fifth dose, the C_{max} was 1.80 $\pm$ 0.95 mg/L. Time to C_{max} after the fifth dose was not reported. The half-life of Glycyrrhetinic Acid was 23.6 h. Glycyrrhetinic Acid (0.028 $\pm$ 0.044 mg/L) was still detectable in the subjects' plasma 219 h after the last dose.

Tanaka et al. (1993) studied the phamacokinetics of Glycyrrhizic Acid in patients with chronic hepatitis. Hepatitis patients, aged 38 to 69, received an intravenous injection of 120 mg Glycyrrhizic Acid. Plasma concentrations of Glycyrrhizic Acid were monitored for 10 h after dosing and declined in a monophasic manner. The mean biological half-life of Glycyrrhizic Acid was 6.0 ± 2.1 h, the mean total clearance was 7.9 ± 3.0 ml/h/kg, and the mean volume of distribution was 61.8 ± 18.3 ml/kg.

GENERAL BIOLOGY

Systemic Effects Overview

Moderate chronic or high acute exposure to Glycyrrhizic Acid, Ammonium Glycyrrhizate, and their metabolites have been demonstrated to cause several transient systemic alterations, including increased potassium excretion, sodium and water retention, body weight gain, alkalosis, suppression of the renin-angiotensis-aldosterone system, hypertension, and muscular paralysis. Most of these effects are the consequence of the Glycyrrhizic Acid inhibition of the enzyme 11β-hydroxysteroid dehydrogenase-2 (11β-OHSD2) in the kidney. This enzyme protects mineralocorticoid (aldosterone) receptors from being stimulated by cortisol. Stimulation of mineralocorticoid receptors in the distal renal tubules causes the excretion of potassium and hydrogen ions in the urine and the reabsorption of sodium bicarbonate and water. Retention of sodium and water causes hypertension. Reabsorption of bicarbonate causes alkalosis. Hypokalemia resulted in myoglobinuria, which caused muscular weakness and paralysis. Ataxia has been observed after a very high acute exposure to Glycyrrhizic Acid. These effects dissipate when exposure to Glycyrrhizic Acid is ceased, and these effects can be blocked by the mineralocorticoid receptor antagonists spironolactone and eplerenone (Olukoga and Donaldson 2000; Davis and Morris 1991; Conn et al. 1968).

Animal

Finney et al. (1958) observed a marked antidiuretic effect in rats within 30 min after a single i.p. dose of 125 mg/kg Glycyrrhetinic Acid. Sodium retention and increased urinary excretion of potassium were also observed.

Rossi et al. (1994) compared the pharmacological effects of Glycyrrhizic Acid and 18β-Glycyrrhetinic Acid in rats. Normotensive Sprague-Dawley rats received 30 mg/kg/day Glycyrrhizic Acid or 15 mg/kg/day 18β-Glycyrrhetinic Acid by oral intubation for 30 days (exact sample size not reported, $n \geq 10$ per dose group). A control group of unspecified size received a comparable volume of water on the same dosing schedule. Systolic blood pressure was measured prior to the first dose and on the 7th, 15th, and 30th day of treatment. In order to reduce stress-induced effects while measurements were made during the study, all animals were acclimated to the blood pressure measuring procedure prior to study initiation. The day prior to the first dose and on days 6, 14, and 29 of dosing, rats were placed in metabolism cages, and 24-h urine samples were collected. The urine volume was recorded and the urine was analyzed for electrolyte content. Additional systolic blood pressure measurements and urine samples were collected on day 60 (30 days after the last dose).

Systolic blood pressure was significantly increased at 15 days in the Glycyrrhizic Acid and the 18β-Glycyrrhetinic Acid groups ($p < 0.05$) and at 30 days in the 18β-Glycyrrhetinic Acid group only ($p < 0.01$). Urine volume was decreased and urine Ca^{2+} concentration was increased in the 18β-Glycyrrhetinic Acid group at 15 and 30 days. All parameters returned to normal by day 60. The authors concluded that 18β-Glycyrrhetinic Acid is an active agent in the hypertensive effects seen in excessive licorice consumption, and that these effects are reversible (Rossi et al. 1994).

Rossi et al. (1999) studied the effects of Glycyrrhizic Acid and the 18α- and 18β- isomers of Glycyrrhetinic Acid on heart function, cardiac tissues, and apoptosis. This study had two parts, acute treatments and subchronic treatments. In the acute treatment, Sprague-Dawley rats were given a single intraperitoneal injection of 0.5, 1.0, or 1.5 mg/kg of Glycyrrhizic Acid, 18α-Glycyrrhetinic Acid, or 18β-Glycyrrhetinic Acid ($n = 4$ rats per dose per test material). An additional group of four rats received 2.0 mg/kg 18α-Glycyrrhetinic Acid, of which all died within 140 min of dosing due to atrioventricular block, as detected by electrocardiogram (ECG). The ECG profiles of all other animals were similar to controls. Microscopic examination showed no abnormalities in the surviving rats. However, edema in the brain, cerebellum, and lungs, and hematic stasis in the kidneys occurred in the 2.0 mg/kg 18α-Glycyrrhetinic Acid group. Calculi of calcium salt occurred in the renal tubules of two of the dead rats. Focal changes in the papillary muscles of the heart included edema, myolysis, and cell distortion. Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) analysis of the papillary muscles of the heart indicated evidence of apoptosis in the 18α-Glycyrrhetinic Acid group.

In the subchronic treatment, Sprague-Dawley rats were assigned to four treatment groups and received daily oral doses of 30 mg/kg Glycyrrhizic Acid, 15 mg/kg 18β-Glycyrrhetinic Acid, 15 mg/kg 18α-Glycyrrhetinic Acid, or an equal volume of water (control) for 30 days ($n = 40$ rats per group on day 0 of dosing). Ten rats per group were killed prior to the first dose. Ten rats per group were killed on day 15 of dosing and another 10 on day 30 of dosing. The remaining 10 animals per group were kept alive an additional 30 days following the treatment period, at which time they were killed. Twenty-four hours prior to scheduled deaths, animals were placed in metabolism cages for urine collection. Blood was collected at death, and tissues and organs were examined microscopically. Certain tissues were set aside for apoptosis analysis by the TUNEL assay.

At 15 days urine electrolyte analysis had potassium (K^+) increased in the Glycyrrhizic Acid group and sodium (Na^+), and calcium (Ca^{2+}) increased in the 18α-Glycyrrhetinic Acid group; at 30 days, K^+ and Na^+ in the 18α-Glycyrrhetinic Acid group, and K^+ and Ca^{2+} increased in the 18β-Glycyrrhetinic Acid

group; and at 60 days (30 days after last dose), Na^+ increased in the Glycyrrhizic Acid group, and K^+ and Ca^{2+} increased in the 18α-Glycyrrhetinic Acid group (all urine electrolyte differences reported were significant at $p < 0.05$). Na^+ levels were elevated in serum of all three treatments at 15 and 30 days but returned to normal by day 60. Myolysis of the papillary muscles appeared in the Glycyrrhizic Acid and 18α-Glycyrrhetinic Acid group at day 30 and did not regress over the 30-day recovery period. Animals in these two treatment groups also had tubular caliculi in the bronchus-associated lymphoid tissue at day 15 and thereafter. Renal calculi occurred in concert with increased urinary Ca^{2+} (see above). In this study 18α-Glycyrrhetinic Acid was the most potent cardiotoxin of the three compounds studied, and while some effects occurred and ceased with exposure, other damage (e.g., myolysis) persisted after exposure had ended (Rossi et al. 1999).

Human

Ploeger et al. (2001b) studied the inhibitory effect of Glycyrrhetinic Acid on 11β-hydroxysteroid dehydrogenase-2 (11β-OHSD2) by measuring the cortisol cortisone ratio in urine before and after human exposure to Glycyrrhetinic Acid. Twelve healthy male volunteers, aged 19 to 29 years with a mean body weight of 70 $\pm$ 6.6 kg, refrained from eating any licorice or Glycyrrhetinic Acid-containing foods for 72 h prior to and throughout the study. The subjects did not eat or drink anything except water for 10 h before dose administration. Urine was collected for 24 h before the first dose was given. Subjects ingested 130 mg/day Glycyrrhetinic Acid in a propylene glycol and water vehicle for 5 days. Blood samples were collected prior to dosing and at several time points after dosing on the 1st and 5th days and again 5 days after the last dose. Urine was also collected throughout the study.

The ratio of cortisol/cortisone was significantly increased on days 2, 3, 4, and 5 of dosing, compared to baseline data. Also, the ratio of cortisol/cortisone increased with each successive day of dosing. Because 11β-OHSD2 is the enzyme responsible for converting active cortisol to inactive cortisone ("active" here is in reference to activity as an aldosterone receptor antagonist), the inhibition of 11β-OHSD2 by Glycyrrhetinic Acid (or its metabolite Glycyrrhizic Acid) caused the changes in cortisol/cortisone ratio. The median concentration of inhibition (IC_{50}) of 11β-OHSD2 was between 325 and 394 μg/L Glycyrrhetinic Acid in plasma (Ploeger et al. 2001b).

Molhuysen et al. (1950) stated that licorice extract was found to be an effective treatment for gastric ulcers during World War II, but edema occurred in about 20% of the patients receiving this treatment. To investigate possible causes of edema, these authors administered a licorice extract (succus liquiritiæ) containing 15% Glycyrrhizic Acid to seven patients who had persistent gastric ulcers and to three patients who did not. Subjects received 20 to 45 g of the licorice extract, administered in eight equal parts (2-h intervals) throughout the day and night (duration of treatment was not specified, but seemed to vary with each patient). Urine was collected at each dose interval. Subjects were on a strictly controlled diet during the experiment.

Urine output volume decreased within the first 3 days of treatment, as did chloride (Cl^-) excretion. Excretion of urea plus ammonia was not affected, nor was urea clearance. There was no albumin or abnormal urinary sediment detected in the urine. Blood hemoglobin level decreased with dosing. Venous blood pressure, pulse pressure, and systolic pressure all increased significantly. Patients also gained several kilograms in body weight due to edema. The authors attributed this combination of effects to "stimulation of the renal tubules to an excessive reabsorption of water and chlorides, and probably also of sodium." Another patient received an unspecified amount of pharmaceutical Ammonium Glycyrrhizate, and the effects were similar to those receiving the licorice extract (Molhuysen et al. 1950).

Louis and Conn (1956) administered seven normal human subjects 6 g/day Ammonium Glycyrrhizate for three days. Compared to baseline data before dosing, sodium (Na^+) and chloride (Cl^-) excretion in the urine was significantly decreased while potassium (K^+) excretion was slightly increased during the dosing period. Withdrawal of the Ammonium Glycyrrhizate caused an immediate above-normal increase in renal excretion of Na^+ and Cl^- and retention of K^+. During the exposure period, four subjects gained weight attributed to water retention. Body weights returned to normal 5 to 7 days after withdrawal of Ammonium Glycyrrhizate treatment. Details of other effects are described in Clinical Experimental Studies.

Hormonal Effects

Quaschning et al. (2001) demonstrated that inhibiting the aldosterone (mineralocorticoid) receptor prevents the hypertension normally induced by Glycyrrhizic Acid in rats. Glycyrrhizic Acid (3 g/L) was added to the drinking water of Wistar-Kyoto rats for 21 days. From days 8 to 21, 5.8 mg/kg/day spironolactone, 182 mg/kg/day eplerenone, or a placebo was added to the diet ($n = 7$ animals/group). Glycyrrhizic Acid increased systolic blood pressure from 142 to 185 mm Hg. Spironolactone and eplerenone, known antagonists of the aldosterone receptor, normalized blood pressure in animals also given Glycyrrhizic Acid.

Takii et al. (2001) demonstrated an anti-diabetic effect of Glycyrrhizic Acid in genetically diabetic mice. Male 4-week-old KK-A^y/Ta Jcl mice with genetic type II non–insulin-dependent diabetes were given control diet, diet containing 0.27% Glycyrrhizic Acid, or diet containing 0.41% Glycyrrhizic Acid for 9 weeks ($n = 8$ mice/group, matched for body weight and blood glucose concentration). Animals were fasted for 5 h, and blood was collected for analysis before, weekly during, and after the treatment period. Animals were killed for necropsy after the treatment period.

Food consumption and body weight gain did not differ between treatment groups. Water consumption was decreased at week 7 and thereafter. Five-hour fasting blood glucose

levels were similar between groups until week 7 of treatment, when the control group had increased blood glucose levels, whereas Glycyrrhizic Acid–treated mice maintained steady blood glucose levels. At week 8, the blood glucose of the 0.27% Glycyrrhizic Acid group increased to match that of the control group. At weeks 8 and 9, the blood glucose of the 0.41% Glycyrrhizic Acid group was significantly less than that of the other two groups. At week 9, the blood insulin level of the 0.41% Glycyrrhizic Acid group was significantly less ($p < 0.05$) than that of the other two groups.

After week 9, an oral glucose tolerance test was performed in which the mice received an oral dose of 200 μl 20% glucose solution (1 g/kg glucose) after an 18-h fast. Blood samples were taken before the glucose dose and 30, 60, and 120 min after. Blood glucose levels of the 0.41% Glycyrrhizic Acid group at 60 and 120 min were significantly less ($p < 0.05$) than the blood glucose levels of the control group and 0.27% Glycyrrhizic Acid group, which were similar. Necropsy did not reveal any remarkable effects of Glycyrrhizic Acid treatment. The authors proposed that Glycyrrhizic Acid inhibits the Na^+-glucose active transport system in the small intestine, but further studies would be required to investigate the mechanism (Takii et al. 2001).

Cardiac Effects

Glycyrrhizic Acid and related compounds can increase blood pressure by promoting the retention of sodium and water from the kidneys and suppression of the aldosterone-renin-angiotensin system as described above.

Kilgore et al. (1998) discovered that treatment with Glycyrrhizic Acid but not Glycyrrhetinic Acid can reduce the size of myocardial infarcts in rabbits. New Zealand white rabbits were anesthetized, and the left jugular vein was cannulated for drug administration and blood sampling. The left carotid artery was cannulated and connected to a transducer to monitor systolic blood pressure. Thoracotomy and pericardiotomy were performed on each rabbit. A silk suture was placed around the left coronary artery to occlude blood flow for 30 min. Ischemia was confirmed by cyanosis distal to the site of occlusion. At the end of the occlusion period, the suture was released to allow reperfusion of the cardiac tissue. Animals received an i.v. bolus infusion of 10 mg/kg/h Glycyrrhizic Acid or 10 mg/kg/h Glycyrrhetinic Acid immediately before reperfusion and every hour for 5 h ($n = 6$ rabbits/group). Eleven control animals received saline infusions on the same schedule. Blood was collected at hourly intervals. After the 5-h reperfusion and dosing period the infarcted area was determined by perfusion of the heart with dyes that are metabolized by healthy tissue. The dyes differentiated between tissue unaffected by the occlusion of the left coronary artery (red), noninfarcted tissues that became ischemic during the occlusion (purple-black), and infarcted tissue (pale yellow).

There was no difference between treatment groups in the area affected by the occlusion. However, 59.3% of the occlusion-affected area was infarcted in the control group, whereas that percentage was only 30.3% in the Glycyrrhizic Acid group, a statistically significant difference ($p < 0.05$). When expressed as percent of the left ventricle, 27.5% was infarcted in control rabbits while 13.0% was infarcted in the Glycyrrhizic Acid group, also a significant difference ($p < 0.05$). The infarcted area of the Glycyrrhetinic Acid group was similar to that of control animals. The myeloperoxidase activity in left ventricle tissue of the Glycyrrhizic Acid group was lower than that of control animals. The authors suggest that Glycyrrhizic Acid is effective in reducing the degree of myocardial injury after an acute period of ischemia and reperfusion (Kilgore et al. 1998).

Effects on Enzyme Activities

Whitehouse et al. (1967) found that Glycyrrhetinic Acid at 100 mM is a potent inhibitor of oxidative phosphorylation coupled to succinate oxidation in rat liver mitochondria.

O'Brian et al. (1990) demonstrated that Glycyrrhetinic Acid inhibits the calcium- and phospholipid-dependent phosphotransferase activity of protein kinase C (PKC). The IC_{50} of PKC inhibition was approximately 450 μM Glycyrrhetinic Acid. Glycyrrhizic Acid was a much weaker inhibitor of PKC activity.

Jung et al. (2001) exposed B16/F10 murine melanoma cells to Glycyrrhizic Acid or Glycyrrhetinic Acid for three days and then determined the melanin contents and tyrosinase activities in the cells. Cellular melanin content and tyrosinase activity were dose dependently increased by Glycyrrhizic Acid. However, the rise in melanin content reached a plateau of 160% of control at 0.50 mM Glycyrrhizic Acid, whereas tyrosinase activity continued to rise 220% of control at the highest concentration tested, 1.00 mM Glycyrrhizic Acid. Additionally, the intracellular levels of tyrosinase mRNA and expression of tyrosine-related protein-2 (TRP-2), but not TRP-1, were also dose dependently increased by Glycyrrhizic Acid. These effects were not seen when the cells were treated with Glycyrrhetinic Acid. Rather, Glycyrrhetinic Acid caused decreases in tyrosinase activity and melanin content. These data indicate that the glycoside structure is important in the Glycyrrhizic Acid–induced stimulation of melanogenesis in this cell line.

Noda (1964) found that 0.13 to 4 mmol/L Glycyrrhizic Acid inhibited the enzyme activity of proteinase in an in vitro system. This inhibition was greater at higher Glycyrrhizic Acid concentrations.

Paolini et al. (1998) studied the effects Glycyrrhizate on the cytochrome P450 monooxygenase activities in Swiss Albino CD1 mice. The mice received daily oral doses of 480 mg/kg Glycyrrhizic Acid for 1, 4, or 10 days. Controls received vehicle only. Animals were fasted for 16 h after the last dose and then killed. The livers were rapidly removed and prepared for enzymatic assays, electrophoresis, Western immunoblot, RNA isolation, and Northern hybridization. The single dose of Glycyrrhizic Acid did not induce any enzymes. However, hepatic cytochrome P450 (CYP) 3A-, 2B1-, and 1A2-dependent

microsomal monooxygenase were induced by the multiple doses of Glycyrrhizic Acid. The following testosterone hydroxylase (TH) enzymes in the liver were also induced by multiple doses of the two test materials: 6β-TH, 2α-TH, 6α-TH, 7α-TH, and 16β-TH. The authors suggested that these results indicate that the induction of cytochrome P450-dependent activities by the prolonged intake of Glycyrrhizic Acid at high doses may cause accelerated metabolism of coadministered drugs, and that the adverse effects associated with cytochrome P450 changes may also have clinical consequences.

Sakamoto et al. (2001) found that Glycyrrhizic Acid binds to high mobility group proteins 1 and 2 (HMGP1/2), causing a conformational change in the proteins, and thus inhibiting the phosphorylation of HMPG1/2 by protein kinases. Glycyrrhetinic Acid had 10 times the binding affinity of Glycyrrhizic Acid to HMPG1/2. HMPG1/2 requires phosphorylation for its DNA-binding affinity; however, the physiological significance of this DNA-binding was unclear. Blockade of HMPG1/2 by Glycyrrhizic Acid or Glycyrrhetinic Acid may be involved in the anti-inflammatory activity of these compounds.

Gap Junction Inhibition

As described by Rozental et al. (2001), gap junctions are pores connecting the cytoplasm of adjacent cells and are important for intercellular communication and normal function in several tissue types. Glycyrrhetinic Acid and its derivatives reversibly block gap junction communication. 18α-Glycyrrhetinic Acid and 18β-Glycyrrhetinic Acid mediate a concentration-dependent inhibition of junctional conductance by 60%. Complete blockade of the gap junction channels does not occur even at concentrations as high as 100 μM. Concentrations above 75 μM Glycyrrhetinic Acid become cytotoxic and irreversible damage occurred. The mechanism for action is unknown. The inhibitory effect of Glycyrrhetinic Acid and its derivatives is used as a tool in electrophysiology experiments and other areas of cellular research.

Davidson and Baumgarten (1988) studied the structure-activity relationship of Glycyrrhetinic Acid derivatives as gap junction inhibitors in human fibroblasts. The authors reported the relative potencies of the test compounds in terms of median concentration of inhibition of gap junction channels (IC_{50}) and median toxic concentration (TC_{50}). For 18α-Glycyrrhetinic Acid, the IC_{50} was 1.5 μM, and the TC_{50} was $>$100 μM. For 18β-Glycyrrhetinic Acid, the IC_{50} was 2 μM, and the TC_{50} was 25 μM.

Gou et al. (1999) found that 18α-Glycyrrhetinic Acid inhibits communication between alveolar epithelial cells at concentrations as low as 5 μM. Extended exposure of alveolar epithelial cells to higher concentrations of 18α-Glycyrrhetinic Acid caused inhibition of gap junction intracellular communication and time- and concentration-dependent reductions in Cx43 protein and mRNA expression. Cx43 is one of the proteins responsible for gap junction formation.

Schiller et al. (2001) found that gap junction intercellular communication between cultured MC3T3-E1 osteoblastic cells was concentration-dependently inhibited by 100 μM 18α-Glycyrrhetinic Acid. Likewise, the blockade of gap junctions by 18α-Glycyrrhetinic Acid interfered with the maturation of the osteoblastic cells, possibly by affecting signal regulating the expression of genes involved in maturation and/or differentiation.

Bou-Flores and Berger (2001) used 50 μM 18α- and 18β-Glycyrrhetinic Acid in neuronal slice preparations from neonatal Swiss Webster mice to demonstrate the importance of gap junction coupling in interneuronal communication in the central respiratory rhythm-generating system.

Böhmer et al. (2001) measured the effects of 18β-Glycyrrhetinic Acid on electrolyte transmission between confluent primary cultures of rat hepatocytes. 18β-Glycyrrhetinic Acid at 40 μM did not affect Na^+ and K^+ conductance, but it did block the conductance of Cl^-.

Tabernero et al. (2001) found that gap junction communication is important in the metabolic syncytium of sharing energetic intermediates among astrocytes. Inhibition of gap junctions by 100 μM 18α-Glycyrrhetinic Acid caused increased glucose uptake in individual astrocyte cells and induced astrocyte proliferation.

Cytotoxicity

Babich et al. (1993) studied the cytotoxicities of the 18α- and 18β- stereoisomers of Glycyrrhetinic Acid among a list of dietary non-nutrients with chemopreventive properties. Cytotoxicity was measured with the neutral red assay, using BALB/c mouse 3T3 fibroblasts as indicators. The neutral red assay quantifies the number of viable, uninjured cells after incubation with a test compound and is based on the uptake and lysosomal accumulation of neutral red dye. Relative toxicity was reported as the concentration at which the count of dead cells was 50% of control cells (NR_{50}). The range of concentrations used was not reported. The NR_{50} values for 18α-Glycyrrhetinic Acid and 18β-Glycyrrhetinic Acid were 0.26 mM and 0.13 mM, respectively. For comparison, 18α-Glycyrrhetinic Acid and 18β-Glycyrrhetinic Acid were more cytotoxic than caffeic acid (NR_{50} = 1.1 mM), vanillin (8.0 mM), and D-saccharic acid 1,4-lactone (18.6 mM), and less toxic than benzyl isothiocyanate (0.019 mM) and tamoxifen (0.016 mM).

Hepatotoxin Protection

Kiso et al. (1984), noting that Glycyrrhizic Acid and Glycyrrhetinic Acid have been shown to protect liver tissue from known hepatotoxins such as carbon tetrachloride (CCl_4) and galactosamine, exposed rat hepatocytes in primary culture to medium containing 10 mM CCl_4 or 0.5 mM D-galactosamine with 0.01, 0.1, or 1.0 mg/ml Glycyrrhizic Acid or 0.01, 0.1, or 1.0 mg/ml Glycyrrhetinic Acid. The concentration of glutamic-pyruvic transaminase (GPT) in the medium was used as a measure of cytotoxicity. Glycyrrhizic Acid and Glycyrrhetinic Acid

at the 0.1 and 1.0 mg/ml concentrations each showed a dose dependent decrease in the GPT levels, compared to hepatotoxin treatment without Glycyrrhizic Acid or Glycyrrhetinic Acid. The 0.01 mg/ml concentrations of Glycyrrhizic Acid or Glycyrrhetinic Acid did not protect the cells from cytotoxicity. Glycyrrhetinic Acid at 1.0 mM also decreased the generation of free radicals induced by CCl_4 in rat liver microsomes. Glycyrrhetinic Acid at 0.01, 0.1, and 1.0 mg/ml reduced the lipid peroxidation induced by ADP/Fe^{3+} or ascorbate/Fe^{3+} in rat liver microsomes. The authors suggested that Glycyrrhizic Acid or Glycyrrhetinic Acid may be hepatoprotective by preventing lipid peroxidation and free radical generation.

Shibayama (1989) treated male Wistar rats with 200 mg/kg Glycyrrhizic Acid in saline intraperitoneally (i.p.) or by injection into the tail vein. Two or 20 h after the Glycyrrhizic Acid injection, the rats received a hepatotoxin: 0.15 ml/kg CCl_4 in olive oil injected i.p.; 0.1 ml/kg allyl formate in saline injected i.p.; or 3 mg/kg endotoxin in saline injected i.p. or via the tail vein ($n = 3$ rats/treatment condition). Appropriate vehicle controls were used for each combination of Glycyrrhizic Acid and hepatotoxin. The rats were anesthetized 24 h after the hepatotoxin injection, and blood was collected from the inferior vena cava for liver function tests. The livers were fixed and stained for microscopic examinations.

All groups of rats receiving hepatotoxin without Glycyrrhizic Acid had elevated serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels, an indicator of hepatotoxicity. Pretreatment with Glycyrrhizic Acid for 2 h had no effect on the serum GOT and GPT levels given hepatotoxins. Pretreatment with Glycyrrhizic Acid for 24 h significantly reduced ($p < 0.001$) the serum GOT and GPT levels in rats given CCl_4 or allyl formate. Glycyrrhizic Acid did not affect the rise in serum GOT or GPT in rats given endotoxin. There were close relationships between the serum GPT and GOT level and the degree of cellular necrosis seen in the microscopic examinations. Thus, pretreatment with Glycyrrhizic Acid protected the liver from CCl_4 and allyl formate toxicity, but apparently did not affect the hepatotoxicity of endotoxin (lipopolysaccharide B, derived from *Escherichia coli*) (Shibayama 1989).

Nakamura et al. (1985) exposed primary cultures of rat hepatocytes to 0, 1, 2, 3, 4, or 5 mM CCl_4 for 24 h to demonstrate hepatoxocity as measured by the cellular leakage of the cytosolic enzymes lactic dehydrogenase (LDH), GOT, and GPT. CCl_4 at lower doses caused concentration dependent leakage of the enzymes without cytolysis, but cytolysis was seen with 5 mM CCl_4. When the hepatocytes were exposed to 5 mM CCl_4 with 25, 50, 100, or 200 μg/ml Glycyrrhizic Acid, there was a concentration-dependent decrease in the LDH and GOT leakage from the cells, and cytolysis was reduced. These data support the ability of Glycyrrhizic Acid to protect the liver from hepatotoxic injury.

Nose et al. (1994) compared the in vivo and in vitro antihepatotoxic activities of Glycyrrhizic Acid and Glycyrrhetinic Acid. Male Wistar rats received 100 or 300 mg/kg of 18β-Glycyrrhetinic Acid or Glycyrrhizic Acid orally 1, 24, and 48 h (three injections) prior to an i.p. dose of 500 mg/kg D-galactosamine. Additional groups of rats received 18β-Glycyrrhetinic Acid or Glycyrrhizic Acid i.p. 1 h prior to an i.p. dose of 500 mg/kg D-galactosamine. Blood was collected from the hearts of the rats 24 h after the D-galactosamine dose. Serum GOT and GPT activities were determined using transaminase kits. Rats pretreated with 100 or 300 mg/kg 18β-Glycyrrhetinic Acid had significantly decreased ($p < 0.05$) serum GOT and GPT activities than rats given D-galactosamine without the pretreatment. Glycyrrhizic Acid at 100 and 300 mg/kg resulted in a slight but nonsignificant reduction in the enzyme activities.

Rat parenchymal cells in primary culture monolayers were treated with 5, 10 or 50 μg/ml 18β-Glycyrrhetinic Acid or 5, 50, or 1000 μg/ml Glycyrrhizic Acid for 24 h. The cells were then washed and exposed to medium containing 5 mM CCl_4 for 6 h, when transaminase activities in the medium were assayed. All three concentrations of 18β-Glycyrrhetinic Acid reduced the GOT and GPT activities, compared to cultures exposed only to 5 mM CCl_4. Glycyrrhizic Acid only reduced transaminase activities at the 1000 μg/ml concentration.

The authors concluded that these studies demonstrated that 18β-Glycyrrhetinic Acid is a more potent protector against hepatotoxicity than Glycyrrhizic Acid. The authors suggested that the difference in antihepatotoxicity of these two compounds may lie in the transformation rate of Glycyrrhizic Acid to Glycyrrhetinic Acid (Nose et al. 1994).

Lin et al. (1999) used an in vivo study design similar those described above to demonstrate that pretreatment with 200 mg/kg/day Glycyrrhizic Acid or 10 mg/kg/day Glycyrrhetinic Acid i.p. for 3 days protected rats from hepatotoxicity induced by 35 mg/kg i.p. of retrorsine. A single pretreatment with 200 mg/kg/day Glycyrrhizic Acid or 10 mg/kg/day Glycyrrhetinic Acid i.p. did not protect against retrorsine-induced hepatotoxicity.

Hu et al. (2001) demonstrated that 50 μM Glycyrrhizic Acid improved the viability of V79 cells also exposed to 5 mM acetaldehyde or 10 μM cadmium. Glycyrrhizic Acid at up to 200 μM, however, did not prevent cadmium-induced lipid peroxidation in these cells.

Antihepatitis Activity

Crance et al. (1994) demonstrated that Glycyrrhizic Acid at 1000 and 2000 μg/ml concentration dependently inhibited the replication of hepatitis A virus in PLC/PRF/5 cells in vitro in a dose-dependant manner. Glycyrrhizic Acid was not virucidal, but it did inhibit an early stage of replication of the virus. Glycyrrhizic Acid inhibited the penetration of hepatitis A virus through the plasma membranes of the PLC/PRF/5 cells.

In a summary of presentations at a symposium on chronic active liver disease, Fujisawa and Tandon (1994) reported that Glycyrrhizic Acid has been known as a treatment for chronic hepatitis in Japan for over 20 years. The author suggested that Glycyrrhizic Acid appears to inhibit the penetration of hepatitis

virus into hepatocytes and it may also work as a free radical scavenge and modulate the immune system through interferon mechanisms and T cells (Crance et al. 1994).

Iino et al. (2001) randomly selected 100 subjects from a pool of 178 chronic hepatitis patients whose liver function did not improve with 2 weeks of 40 ml/day stronger neo-minophagen C (SNMC), a hepatitis treatment containing 0.2% Glycyrrhizic Acid, 0.1% cysteine, and 20% aminoacetic acid as active ingredients. The 100 patients received 40 ml/day or 100 ml/day SNMC for 3 weeks ($n = 50$/group). Hepatic enzyme function was recorded 4 and 8 weeks prior to the first dose, weekly during the dosing period and weekly for 3 weeks after the last dose. Serum alanine transaminase (ALT) activity was used as a measure to rate improvement in liver function. Six patients in the 100 ml/day SNMC group were unable to complete the experiment, but of those 44 remaining, 23 (52.3%) had improved liver function. Of the 46 patients in the 40 ml/day group that completed the trials, 12 (26.1%) had improvement in liver function. Thus, the 100 ml/day group had significantly more improvement ($p = 0.017$) in liver function than patients in the 40 ml/day group. The authors proposed that patients who do not respond to moderate doses of SNMC might respond better to a higher dose.

van Rossum et al. (2001) gave Glycyrrhizic Acid to patients with chronic hepatitis C who did not respond to interferon therapy. Forty-one patients received 80, 160, or 240 mg Glycyrrhizic Acid by drip infusion three times per week for 4 weeks (number of patients per dose was not reported). Thirteen patients received a placebo three times per week for 4 weeks. In another experiment, 15 patients received 200 mg Glycyrrhizic Acid by intravenous injection six times per week for 4 weeks. Hematological and biochemical assessments were performed weekly during the study. Virological assessments by enzyme-linked immunosorbent assay (ELISA) were done prior to and after treatment. Serum ALT was used to measure hepatic health before and after treatments and was reported in terms of upper limit of normal (ULN), which is 41 IU/L for men and 31 IU/L for women.

Serum ALT levels after placebo treatments were similar to those before the study. In the placebo group, 92% of patients had ALT levels $>1.5\times$ ULN and 8% $<1.5\times$ ULN but higher than normal. Ten percent of the patients that received Glycyrrhizic Acid three times per week had normal ALT levels, whereas 27% and 63% of the patients in that group had ALT levels $<1.5\times$ ULN and $>1.5\times$ ULN, respectively. Of the patients receiving 200 mg Glycyrrhizic Acid six times per week, 20% had normal ALT, 27% had ALT levels $<1.5\times$ ULN, and 53% had ALT levels $>1.5\times$ ULN. Serum ALT levels increased in both Glycyrrhizic Acid groups after treatment ended, and by 4 weeks after the end of treatment, the ALT levels in the dosed groups were similar to the placebo group.

The levels of hepatitis C virus RNA after treatment with Glycyrrhizic Acid were similar to those before treatment. Thus, in chronic hepatitis C patients who do not respond to interferon, hepatic function as measured by serum ALT improves with Glycyrrhizic Acid treament, but the virus appears to be unaffected (van Rossum et al. 2001).

Other Antiviral Activities

Pompei et al. (1979) demonstrated that Glycyrrhizic Acid inhibits the growth of several virus types. Human aneuploid HEp2 cells were prepared into monolayers for 24 h and then infected with vaccinia, herpes simplex 1, Newcastle disease, vesicular stomatitis, or polio type 1 for 1 h. Cells were then washed, and 1, 2, 4, or 8 mM Glycyrrhizic Acid or control medium was applied to the cells for 18 h, when the cells were stained for microscopic examination. Glycyrrhizic Acid at 8 mM completely inhibited the growth of and cell damage caused by vaccinia, herpes simplex 1, Newcastle disease, and vesicular stomatitis. The 2 and 4 mM Glycyrrhizic Acid treatments caused concentration dependent reductions in virus load. Glycyrrhizic Acid at 1 mM did not inhibit any virus growth. The growth of polio type 1 virus was unaffected by any of the Glycyrrhizic Acid concentrations tested.

Badam (1997) tested the anti-viral activities of Glycyrrhizic Acid and Ammonium Glycyrrhizate on the Japanese encephalitis virus (JEV). Porcine stable kidney cells in culture were infected with JEV strains Nakayama, P-20778, or 821564 XY48. One hour after infection, the cells were exposed to various (unspecified) concentrations of Glycyrrhizic Acid, licorice root, or Ammonium Glycyrrhizate for 96 h. Infected control cells received no treatment with licorice-related compounds. The cells were then stained with Amido black, rinsed, dried, and the plaques were counted. The experiments were performed in triplicate. Glycyrrhizic Acid at 500 μg/ml completely inhibited plaque formation in all strains, whereas this effect was seen with Ammonium Glycyrrhizate at 1000 μg/ml. No signs of cytotoxicty were observed at these concentrations.

Additional groups of cells infected with the three strains of JEV were exposed to 500, 1000, or 2000 μg/ml Glycyrrhizic Acid for only 2 h. Cells were stained, and plaques were counted. After 2 h of exposure to Glycyrrhizic Acid, only the 2000 μg/ml treatment caused inhibition of plaque formation, and the inhibition was complete. The two lower concentrations had no effect on plaque formation.

Pretreatment of the cells with 1000 μg/ml Glycyrrhizic Acid for 24 h prior to JEV infection did not affect subsequent plaque formation (Badam 1997).

Utsunomiya et al. (1997) infected 8-week-old BALB/c mice with the mouse-adapted Kumamoto strain of influenza virus A_2 (H_2 N_2) by inhalation with a nebulizer. Viral doses were 1, 10, 20, and 100 times the median lethal dose (LD_{50}) of virus, as determined in preliminary tests. Mice received doses of 1.25 to 80 mg/kg Glycyrrhizic Acid i.p. the day before viral infection and 1 and 4 days after infection. Mice receiving 1, 10, and 20 times the viral LD_{50} and 10 mg/kg or higher doses of Glycyrrhizic Acid had greater survival rate (10 to 20 mice/dose; 100% survival), compared to similarly infected control mice given saline (15 mice; 50% survival). Mean survival time in Glycyrrhizic

Acid–treated mice was greater than that of infected control mice. The grade of pulmonary consolidations and the virus titers in lung tissues were less in treated mice than in infected control mice. Glycyrrhizic Acid had no protective impact on the mice given 100 times the LD_{50} of the virus.

In additional experiments, healthy mice were treated with 0 or 10 mg/kg/day Glycyrrhizic Acid i.p. 1, 3, and 5 days before their spleens were removed. The spleens were processed into single-cell suspensions of intact cells. Whole spleen cells (WSCs) or macrophages, T cells, or B cells derived from WSCs were then injected i.v. into recipient mice at 5×10^6 cells per mouse. Two hours later, the mice were infected with 10 times the LD_{50} of influenza virus. Infected mice that had been given WSCs or T cells from Glycyrrhizic Acid–treated donor mice had a 100% survival rate 21 days after infection. Infected mice given B cells or macrophages from Glycyrrhizic Acid–treated donor mice all died by day 15 after infection. When the donor mice had been given saline and no Glycyrrhizic Acid, the infected recipient mice all died by day 15, regardless of the donor cell type. The authors suggested that Glycyrrhizic Acid protected the mice from the virus through a function of T cells, but not through B cells or macrophages (Utsunomiya et al. 1997).

Sekizawa et al. (2001) applied herpes simplex virus type 1 (HSV1) to the abraded corneas of 66 young female SJL mice to infect the mice with herpetic encephalitis. Fifty mice were given i.p. injections of 50 μg/g/day Glycyrrhizic Acid on post-infection days 3, 4, and 5. Fifty control mice received the vehicle physiological saline without Glycyrrhizic Acid. Mice were monitored until post-infection day 14. The survival rates at post-infection day 14 were 81.8% for the mice given Glycyrrhizic Acid and 37.5% in infected control mice. The brains from 26 Glycyrrhizic Acid–treated mice and 25 control mice were removed on post-infection day 6 and processed for a plaque assay to determine propagation of the HSV1 virus in the mouse brains. The brains of mice treated with Glycyrrhizic Acid had 45.6% of the number of plaque forming units (PFU) found in the control mice. Thus, the survivability of HSV1-infected mice was improved with Glycyrrhizic Acid treatment, which also decreased the propagation of the virus in the brain.

Anti-Inflammatory Effects

Tangri et al. (1965) induced arthritis in albino rats by injection of formalin into the ankle joints. Normal and arthritic rats received 0 or 2 mg/100 g/day Glycyrrhetinic Acid i.p. for 10 days. The degree of inflammation was determined by the anteroposterior diameter of the feet. The inflammation was significantly decreased ($p < 0.01$) in the ankles of rats treated with Glycyrrhetinic Acid. Without Glycyrrhetinic Acid treatment, the serum ALT and aspartate aminotransferase (AST) of arthritic rats was 57.4% and 33.2% higher, respectively, than of normal rats. Glycyrrhetinic Acid reduced the serum ALT and AST in arthritic rats by 37.4% and 25.4%, respectively. Glycyrrhetinic Acid did not affect the serum AST or ALT level in normal rats.

Amagaya et al. (1984) found that Glycyrrhetinic Acid (3 to 30 mg/kg p.o.) inhibits edema caused by an injection of λ-carrageenan in the hind feet of mice. Glycyrrhetinic Acid (30 mg/kg p.o.) also reduced the granuloma formation on dorsally implanted cotton pellets in mice. The 18α-isomer of Glycyrrhetinic Acid was more potent in these effects than the 18β-isomer.

Rui (1997) found that Glycyrrhetinic Acid inhibited ear edema induced by croton oil in mice. No other details of this study were given.

Gujral et al. (1961) induced arthritis in bilaterally adrenalectomized albino rats by injecting a fomaldehyde solution into the ankle joints daily for five days. In one experiment, the rats received 250 μg/100 g of deoxycorticosterone acetate (DOCA) subcutaneously, 250 μg/100 g DOCA plus 0.5 mg/100 g hydrocortisone orally, or 250 μg/100 g DOCA plus 20 mg/100 g Glycyrrhizic Acid orally. Doses were administered daily for 10 days after the adrenalectomy surgery. Inflammation of the ankle joint was measured by a micrometer screw gauge to determine the linear cross section of the ankle joint. The DOCA-plus-Glycyrrhizic Acid group showed an anti-arthritic effect by having a significantly smaller ($p < 0.01$) ankle diameter than the other groups.

In a second experiment using the same arthritis model, rats received 0.5 mg/100 g hydrocortisone orally or 20 mg/100 g Glycyrrhizic Acid plus 0.5 mg/100 g hydrocortisone orally. Doses were administered daily for 10 days after the adrenalectomy surgery. Ankle diameter was measured with a micrometer screw gauge as above. The Glycyrrhizic Acid with hydrocortisone group had significantly smaller diameter ankles ($p < 0.005$) than the group that only received hydrocortisone (Gajral et al. 1961).

Ohuchi et al. (1981) found that in rats Glycyrrhizic Acid inhibits prostaglandin E_2 in activated peritoneal macrophages. Sprague-Dawley rats received 5 ml/kg i.p. injections of 5% bacto peptone and 5% soluble starch in solution in order to stimulate macrophage migration. The rats were killed and peritoneal macrophages were isolated and seeded in culture at 6×10^6 cells/60 mm dish and incubated for 2 h. Non-adherent cells were washed away, and the replacement medium contained 0.1, 1, 10, 100, or 1000 μg/ml Glycyrrhizic Acid or Glycyrrhetinic Acid in ethanol for 20 h. Control plates contained only medium with the ethanol vehicle. Sheep red blood cells were added to the medium, and 95% of them had been ingested within 2 h. Prostaglandin E_2 in the medium was determined by radioimmunoassay. Glycyrrhizic Acid and Glycyrrhetinic Acid each concentration dependently inhibited prostaglandin synthesis at 100 and 1000 μg/ml. However, the Glycyrrhetinic Acid caused detachment of 30% of the cells after just 8 h of exposure, which was seen as a cytotoxic effect. In an additional experiment, 3000 μg/ml Glycyrrhizic Acid also inhibited the release of arachidonic acid from macrophages. The authors proposed that the known anti-inflammatory effect of Glycyrrhizic Acid may be partially due to inhibition of prostaglandin E_2.

OTHER CELLULAR EFFECTS

Horigome et al. (2001) found that Glycyrrhetinic Acid caused apoptosis in splenocytes and thymocytes in C57BL/6 mice that had received an i.p. injection of 2.5 mg/animal. Additional in vitro studies indicated that the apoptosis of splenocytes was a result of the inhibition of 11β-hydroxysteroid dehydrogenase by Glycyrrhetinic Acid, which increased the levels of corticosterone.

Glycyrrhizic Acid has also been reported to enhance the production of interleukin-12, interleukin-2, and interferon (Dai et al. 2001; Utsunomiya et al. 2001; Abe et al. 1982; Zhang et al. 1993).

Interactions with Other Chemicals

Hydrocortisone

Teelucksingh et al. (1990) conducted several experiments to study the ability of Glycyrrhetinic Acid to potentiate the activity of hydrocortisone in the skin. Healthy female and male volunteers, aged 21 to 50 years, were treated with test materials on a 7 $\times$ 7-mm site on the flexor side of the forearm. The treatments included 0.1, 0.3, 1.0, 3.0, and 10 mg/ml hydrocortisone acetate, 20 mg/ml Glycyrrhetinic Acid only, and 20 mg/ml Glycyrrhetinic Acid with each of the hydrocortisone concentrations listed above (four to six subjects per treatment). A positive-control group received 0.1, 0.3, 1.0, 3.0, or 10 mg/ml beclomethasone diproprionate. Solutions were prepared in 95% ethanol and made within 24 h before application. After the applied solution had dried, the test area was covered with polyester film for 16 to 18 h. The dose sites were scored for degree of blanching 1, 2, 3, and 6 h after removal of the occlusive dressing. Scoring was performed by observers unaware of the doses applied, and the scale was 0 = none, 1 = mild, 2 = definite, and 3 = intense blanching.

The degree of blanching was presumed to be associated with cutaneous vasoconstriction. Hydrocortisone acetate alone and Glycyrrhetinic Acid alone each had no effect on degree of blanching (vasoconstriction). However, hydrocortisone and Glycyrrhetinic Acid combined produced significant dose-dependent increases ($p < 0.01$–0.001) in the degree of blanching of the skin at the application site, compared to the vehicle control 95% ethanol. Beclomethasone diproprionate, the positive control, produced the expected dose-dependent increase in degree of blanching. The authors concluded that Glycyrrhetinic Acid appeared to potentiate the activity of hydrocortisone in the skin.

Dorsal skin from freshly killed nude mice was removed, homogenized, and proteins were separated and measured. The proteins on suspension were diluted to match the protein content per weight in the skin. The protein suspension was incubated in Krebs-Ringer with 12 nmol/L tritiated corticosterone (82 Ci/mmol) added. This preparation was treated with 0, 0.01, 1.0, or 100 μmol/L Glycyrrhetinic Acid (duration of treatment was not reported). After centrifugation, corticosteroids were extracted from the supernatant, and ^{3}H-11-dehydrocorticosterone was separated by thin-layer chromatography. The percent of corticosterone converted into ^{3}H-11-dehydrocorticosterone was determined.

Glycyrrhetinic Acid significantly inhibited the production of ^{3}H-11-dehydrocorticosterone in a concentration dependent manner ($p < 0.0001$–0.05), thus verifying the inhibition of 11β-OHSD in the skin by Glycyrrhetinic Acid.

Samples of normal, psoriatic, and eczematous human skin were collected from patients. In immunolabeling assays, 11β-OHSD was found in the epidermis with the exception of the basal layer. Distribution of 11β-OHSD was similar in all three skin types, but the activity of the enzyme seemed higher in the psoriatic and eczamatous skin samples.

Given the results of these studies, the authors suggested that by inhibiting the metabolism of hydrocortisone by 11β-OHSD in the dermis, Glycyrrhetinic Acid allowed greater access of hydrocortisone to glucocorticoid receptors (Teelucksingh et al. 1990).

In a commentary in response to the Teelucksingh et al. (1990) study described above, Greaves (1990) suggested that the potentiation of hydrocortisone by Glycyrrhetinic Acid could be due to the ability of Glycyrrhetinic Acid to increase the percutaneous absorption of hydrocortisone.

Diclofenac Sodium

Nokhodchi (2002) applied formulations of 1% diclofenac sodium (nonsteroidal anti-inflammatory) with or without 0.1% to 0.5% Glycyrrhizic Acid to excised abdominal rat skin in Franz-type diffusion cells. Flux of diclofenac sodium into the receptor compartment was measured. The formulation containing 0.1% Glycyrrhizic Acid produced a 10-fold increase in the flux of diclofenac sodium across the rat skin. The formulation containing 0.5% Glycyrrhizic Acid increased the flux of diclofenac sodium by twofold. The author concluded that Glycyrrhizic Acid enhanced the absorption of diclofenac sodium across rat skin.

ANIMAL TOXICOLOGY

Acute Toxicity

Glycyrrhetinic Acid

The median acute lethal dose (LD_{50}) of intraperitoneal (i.p.) injection of Glycyrrhetinic Acid in mice is 308 mg/kg (Informatics, Inc. 1972).

Finney et al. (1958) found that no albino mice died after acute oral or subcutaneous doses of up to 610 mg/kg Glycyrrhetinic Acid. The LD_{50} of Glycyrrhetinic Acid via intraperitoneal route was 308 mg/kg. Of the animals that died, most did so on the second day after dose administration.

Cognis (2002) reported an oral LD_{50} of 610 mg/kg for Glycyrrhetinic Acid in rats.

Ammonium Glycyrrhizate

The LD_{50} of crude Ammonium Glycyrrhizate in mice via oral (p.o.) and i.p. routes are 12,700 and 1050 mg/kg, respectively.

The i.p. LD_{50} of Monoammonium Glycyrrhizate in mice is 1070 mg/kg. Diammonium Glycyrrhizate has p.o. and i.p. LD_{50} values in mice of 9600 and 1250 mg/kg, respectively (Informatics, Inc. 1972).

Food and Drug Research Laboratories (FDRL) conducted acute oral toxicity studies of Ammonium Glycyrrhizate in several species. The LD_{50} values in rats, mice, hamsters, and rabbits were 7.1, 8.6, 8.8 and 10.0 g/kg, respectively (FDRL 1971a).

Inverni della Beffa (1970) studied the acute oral toxicity of Ammonium Glycyrrhizate (also known as Glycamil) in the mouse, rat, and guinea pig. The range of doses used was not reported, but the LD_{50} values for rats and mice were each >5000 mg/kg. The LD_{50} for guinea pigs was >3000 mg/kg.

Fujimura (no date) reported an LD_{50} of 1050 mg/kg for crude Ammonium Glycyrrhizate i.p. in mice. The oral LD_{50} was 12,700 mg/kg. The LD_{50} of refined Ammonium Glycyrrhizate was 1070 mg/kg i.p. The oral LD_{50} of refined Ammonium Glycyrrhizate was >10,000 mg/kg.

Potassium Glycyrrhizinate

The LD_{50} values of Potassium Glycyrrhizinate via p.o., subcutaneous (s.c.), intravenous (i.v.), and intramuscular (i.m.) routes in mice are 1220, 697, 412, and 695 mg/kg, respectively (Informatics, Inc. 1972).

Fujimura (no date) reported an LD_{50} of 1260 mg/kg for crude Potassium Glycyrrhizinate i.p. in mice. The oral LD_{50} was 12,400 mg/kg.

Short-Term Toxicity

Finney et al. (1958) administered intramuscular injections of 10 or 20 mg/kg Glycyrrhetinic Acid to young rats three times a week for four weeks. Rats were killed for examination at the end of the dosing period. There were no clinical effects observed while the animals were alive. At necropsy all tissues were normal except for a slight thinning of the lipid in the zona glomerulosa of the adrenal glands.

Inverni della Beffa (1970) treated eight Wistar rats with 700 mg/kg/day Ammonium Glycyrrizate via oral gavage for 8 weeks. The author described this dose as 150 times greater than the dose used in man as a sweetening agent with respect to body weight. Eight non-treated rats served as a control group.

All animals survived to the end of the treatment period, and there were no indications of intoxication. Weight gain of treated animals was similar to controls. At the end of treatment, examinations of glycemia, hepatic function, diuresis, and urinary excretion of Na^+, K^+, and Cl^- in treated rats were similar to those of control rats. Systolic blood pressure measured in the tail was similar between treated and untreated rats at weeks 4 and 8 of treatment. Hematology and blood chemistry parameters were similar between the control rats and those treated with 700 mg/kg/day.

There were no appreciable differences between treated and control rats in the histological examination of the heart, liver, kidneys, adrenal glands, spleen, and gonads. Of these organs only the liver had a reduced organ weight in treated rats. In this study, reduced liver weight was the only clinical effect noted after 8 weeks of oral treatment with 700 mg/kg/day Ammonium Glycyrrizate (Inverni della Beffa 1970).

In an unpublished report described by Informatics, Inc. (1972), Fujimura and Okamoto fed Dipotassium Glycyrrhizate or Diammonium Glycyrrhizinate to Wistar rats for 90 days. Doses were 0%, 0.1%, and 0.5% test material mixed in feed ($n = 5$ rats/sex/dose). After the 90-day exposure period, the rats were killed and examined. Male rats in the 0.5% groups for each test material had smaller increases in body weight than control rats; females were not affected. No other findings were reported.

Subchronic Toxicity

Inverni della Beffa (1970) tested male mice with 0, 18, or 90 mg/kg/day Ammonium Glycyrrhizate by oral gavage six days a week for 16 weeks ($n = 10$ mice per group). All mice survived the dosing period, and there were no clinical signs of toxicity. The body weight gain of treated mice was similar to control mice. There were no remarkable observations during necropsy examinations.

Rats were exposed using the same protocol. All rats survived the dosing period, and there were no clinical signs of toxicity.

Male rats of the 90 mg/kg/day group had reduced body weights at weeks 9, 10, and 11, but after this period, body weights for all groups were not statistically significant. Female body weights were similar for all groups. Hepatic function, as determined by the measurable degradation of a 50 mg/kg i.v. injection of bromsulphalein, was similar between all groups. There were no treatment-related effects on glycemia or blood pressure. By urinalysis males of the 90 mg/kg/day Ammonium Glycyrrhizate group had increased Na^+ and Cl^- excretion and diuresis at week 8 but not at week 16. Males of the lower dose had diuresis only at week 8 but not at week 16. Female rats had a decrease in Cl^- excretion in the urine at week 16 only. No groups had a difference in renal excretion of K^+.

Analysis of the blood showed a decrease in lymphocytes and increases in neutrophilic granulocytes in males of the higher dose group at week 8, and at week 16 there was a decrease in the total white blood cell count. Female rats of the higher dose had decreased hemoglobin and eosinophilic granulocytes at week 16. Females of the lower dose group had increased hemoglobin and lymphocytes and decreased neutrophylis at week 8 and decreased eosinophilis at week 16.

At necropsy, histological and gross examinations and weights of the major organs did not differ between treatment and control groups (Inverni della Beffa 1970).

Chronic Toxicity

Monlux (1974) fed Ammonium Glycyrrhizate to Osborn-Mendel rats for 2 years. All rats were F_1 offspring of rats in a subacute Ammonium Glycyrrhizate feeding study in which

the F_0 generation were exposed to concentrations of Ammonium Glycyrrhizate (0%, 0.5%, 1.0%, 2.0%, and 4.0%) mixed in powdered rat diet during breeding. No results were available for the F_0 generation. The F_1 rats were fed the same dose as their respective parents' group or control diet for 102 weeks ($n = 10$ rats/group). At the end of the treatment period, all rats were killed and necropsied. Tissues were fixed in formalin and examined microscopically for lesions. The feeding of Ammonium Glycyrrhizate over a period of 2 years did not produce any lesions or anatomic alterations in this study.

Dermal Irritation and Sensitization

Finney et al. (1958) applied cotton-wool pellets saturated with 0.5 ml of a suspension of 100 mg/ml Glycyrrhetinic Acid to the shaved back of albino rabbits. Three pellets were fixed with an adhesive plaster and the trunk of the rabbit was wrapped in a plastic film for 24 h. Another rabbit was similarly treated, except the three exposure sites were abraded before the pellets were applied. There was no evidence of edema or erythema on the intact or abraded exposure sites upon removal of the pellets or 2 days later. Glycyrrhetinic Acid had no apparent primary irritant activity on the skin of the albino rabbit.

Ocular Irritation

Cognis (2002) reported that 6% Glycyrrhetinic Acid in water was classified as "slightly irritating" in an in vitro test using the chorioallantoic membrane of a chicken embryo in hen's egg (HET-CAM) assay. In the assay, 0.3 ml of the test material was applied to the test system (details of the assay were not provided). The irritation potential was evaluated as a function of reaction time and reaction intensity of effects such as hemorrhage, lysis of vessels, and protein coagulation. No reaction to the test material was observed.

GENOTOXICITY

Wang et al. (1991) showed that 0.025, 0.1, and 0.5 μg/plate18β-Glycyrrhetinic Acid concentration-dependently inhibited the mutagenic activities of 10 μg/plate benzo[a]-pyrene, 20 μg/plate 2-aminoflourene, and 0.5 μg/plate aflatoxin B_1 in *Salmonella typhimurium* strains T98 and T100. 18α-Glycyrrhetinic Acid also inhibited the mutagenicity of these compounds but was less potent, as the only effective concentration was 0.5 μg/plate.

Litton Bionetics, Inc. (1972) investigated the mutagenicity of Ammonium Glycyrrhizate, using the host-mediated assay, in vivo and in vitro cytogenetics studies, and the dominant lethal assay. In the host-mediated assay, ICR male rats received Ammonium Glycyrrhizate (dose not specified), which was considered a possible mutagen at the dose levels used in the host-mediated assay. Oral doses of up to 5000 mg/kg Ammonium Glycyrrhizate produced no detectable significant aberration of the bone marrow metaphase chromosomes of rats. Ammonium Glycyrrhizate did not produce significant aberration in the anaphase chromosomes of human tissue culture cells when tested at concentrations up to 1000 μg/ml. Ammonium Glycyrrhizate was considered to be non-mutagenic in rats in the dominant lethal assay at oral doses of up to 5000 mg/kg.

Green (1977) reported that Ammonium Glycyrrhizate was not mutagenic in screening assays in human WI-38 embryonic lung cells, *Saccharomyces cerevisiae* strain D-3, and in the *Salmonella typhimurium* strains G46 and TA1530.

Stanford Research Institute (SRI) evaluated the mutagenic potential of Ammonium Glycyrrhizate with the dominant lethal assay in rats (SRI 1977). Male Sprague-Dawley rats were given 0, 4000, 13,333, or 40,000 ppm Ammonium Glycyrrhizate mixed in the diet for 10 weeks ($n = 20$ male rats/group). After the dosing period, each male was then caged with two virgin females for 7 days for a first mating period, and then the 2nd week with a different pair of virgin females for the second mating. Fourteen days after the midweek of mating, each female was killed and the uteri were removed for fetal examinations.

The male rats of the 40,000 ppm group had significantly reduced ($p < .01$) body weight gain over the 10-week dosing period. The two lower doses of Ammonium Glycyrrhizate did not cause consistent mutagenic effects in the fetuses. Offspring of the 40,000 ppm group had significantly higher ($p < 0.05$) number of dead implants and dead implants/total implants. The authors stated that Ammonium Glycyrrhizate may be a mutagen in the rat at 40,000 ppm (2000 mg/kg/day) in the diet (SRI 1977).

Generoso et al. (1983) evaluated the mutagenic potential of Ammonium Glycyrrhizate in male germ cells, using a dominant lethal assay and a heritable translocation test. In the heritable translocation test, 100 male (101 $\times$ CH3)F_1 mice were each caged with three female (SEC $\times$ C57BL)F_1 mice for 1 week prior to exposure to Ammonium Glycyrrhizate. Then the male mice were separated from the females and fed powdered feed containing 0 or 2.25% Ammonium Glycyrrhizate for 8 weeks ($n = 50$ treated and 50 control). After the exposure period, the males were caged with the same females for 1 week. Male offspring from the two mating periods (before and after exposure) were mated to hybrid stock (SEC $\times$ C57BL)F_1 females. The outcome of these pregnancies were used to measure any inherited effects of Ammonium Glycyrrhizate on male fertility.

Based on data from 475 experimental and 507 control male progeny tested, the authors determined that exposure to Ammonium Glycyrrhizate in this study did not produce any heritable effects on male fertility.

In the dominant lethal assay, male mice were fed powdered feed containing 0% or 2.25% Ammonium Glycyrrhizate for 8 weeks ($n = 36$ treated and 36 control). Beginning immediately after the treatment period each male was caged with one (C3H $\times$ C57BL)F_1 and one (SEC $\times$ C57BL)F_1 virgin females. Females were examined daily for the presence of vaginal plugs for 1 week. Mated females were replaced with new virgins. All matings occurred $^1/_2$ to $7^1/_2$ days after the end of treatment. Pregnant mice were killed 12 to 15 days after appearance of the vaginal plugs. The uteri were examined to determine frequency of pre- and

post-implantation loss. In this study, Ammonium Glycyrrhizate did not induce any dominant lethal effects (Generoso et al. 1983).

Sheu et al. (1986) performed dominant lethal assays of Ammonium Glycyrrhizate in rats and mice and a heritable translocation test in mice. Male rats were fed diet containing 0.4%, 1.3%, or 4.0% Ammonium Glycyrrhizate or corn oil for 10 weeks (n = 20 rats/group). The males were mated with virgin females in the 1st and 2nd weeks after treatment ended. Male fertility was not affected by treatment. However, analysis of the fetuses at day 14 of gestation resulted in a statistically significant ($p < 0.05$) increase in dead implants in females mated to males treated with 4.0% Ammonium Glycyrrhizate. The authors stated that the biological significance of this result is unknown.

Male mice were fed diet containing 2.0% or 2.5% Ammonium Glycyrrhizate or corn oil for 8 weeks (n = 36 mice per group). Immediately after the treatment period, males were mated with virgin females. Females were killed for uterine analysis on gestation days 12 to 15. There was no evidence of infertility or dominant lethal effect in mice.

In the heritable translocation test, groups of 50 to 75 untreated male mice were each caged with three females for 1 week. The females were then caged individually through gestation, whereas the males were fed diet containing 2.25% Ammonium Glycyrrhizate or 2% corn oil (n = 50/group). At the end of the treatment period, the males were paired with the same females for another week of mating. There was no reduction in litter size after treatment with Ammonium Glycyrrhizate. There was no evidence of heritable genetic toxicity in this test (Sheu et al. 1986).

Sasaki et al. (1980) evaluated the in vitro mutagenic potential of Trisodium Glycyrrhizate in Chinese hamster Don-6 cells and in human HE-2144 fibroblastic cells. Treatment of the cells with 0.49 to 2.46 mg/ml Trisodium Glycyrrhizate in the nutrient medium was nonmutagenic.

CARCINOGENICITY

Kobuke et al. (1985) studied the tumorigenicity of Disodium Glycyrrhizate in B6C3F$_1$ mice. Male mice received drinking water containing 0.04%, 0.08%, or 0.15% Disodium Glycyrrhizate (n = 50 to 70 mice per group). The respective Disodium Glycyrrhizate exposure levels in the three dose groups were 2.5, 5.8, and 8.0 mg/kg/day. Female mice received drinking water containing 0.08%, 0.15%, or 0.3% Disodium Glycyrrhizate (n = 50 mice per group). Female exposure levels were 3.5, 6.5, and 12.2 mg/kg/day, respectively. Control groups of 60 male and 50 female mice received distilled drinking water only. The exposure period was 96 weeks, and treated drinking water was replenished every 3 days. After the 96-week treatment period, surviving mice received distilled drinking water without Disodium Glycyrrhizate. Animals that died during the study and those that were killed at the end of the study were examined. Any tumors found were weighed and described. There was no difference between treated and control groups in the tumor incidence, in the latency period before tumor appearance, or in the types of tumors found. The authors suggested that this study provided no evidence that chronic exposure to Disodium Glycyrrhizate was tumorigenic.

ANTICANCER AND TUMOR SUPPRESSION

Wang et al. (1991) reported tht Glycyrrhetinic Acid also inhibited the tumor initiation and tumor promotion by 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) in mice. Shaved female SENCAR mice were treated with 10 μmol 18α- or 18β-Glycyrrhetinic Acid in dimethyl sulfoxide (DMSO) or 0.2 ml DMSO vehicle daily for seven days. One hour after the last treatment, 40 nmol DMBA was applied to the same site on all mice. Seven days later, 4 nmol TPA was applied to the same site, and this treatment was continued daily for 16 weeks. In two additional groups, mice were treated with DMSO, DMBA, and TPA as described above, but they were given 10 μmol 18α- or 18β-Glycyrrhetinic Acid topically 30 min before each TPA treatment. Each combination of treatments was performed on 20 mice. Tumors larger than 1 mm that persisted for 2 weeks were counted.

Mice treated with 18α- or 18β-Glycyrrhetinic Acid prior to the tumor initiator DMBA had 20% or 50% fewer tumors, respectively, than control mice. Likewise, mice given 18α- or 18β-Glycyrrhetinic Acid prior to the tumor promotor TPA had 80% and 60% fewer tumors, respectively, than control mice. The authors concluded that topical application of either stereoisomer of Glycyrrhetinic Acid inhibited the binding of radiolabeled DMBA and benzo[a]pyrene (B[a]P) to epidermal DNA. 18α- or 18β-Glycyrrhetinic Acid also inhibited the TPA-induced increases in ornithine decarboxylase activity and lipoxygenase activity in vitro (Wang et al. 1991).

Other studies by Nishino et al. (1984, 1986) and Takasaki et al. (1995) demonstrated that Glycyrrhetinic Acid inhibits the tumor-promoting activity of TPA and teleocidin.

Kitagawa et al. (1986) found that Glycyrrhetinic Acid dose-dependently inhibited the binding of TPA to the TPA receptor in mouse skin. Kinetic analysis suggested that Glycyrrhetinic Acid competitively binds directly to the TPA receptor with an inhibition constant (K_i) of 2.2×10^{-4} M. The concentration required to inhibit 50% of TPA binding was around 500 μM Glycyrrhetinic Acid. The authors proposed that this competitive binding of Glycyrrhetinic Acid to the TPA receptor may play a role in the anti–tumor-promoting effect of Glycyrrhetinic Acid. Glycyrrhizic Acid did not show any binding affinity to the TPA receptor.

Inoue et al. (1989) found that pretreatment of a mouse ear with 1 mg/ear Glycyrrhetinic Acid inhibited TPA-induced ear edema by 81%. When 1 mg/ear Glycyrrhetinic Acid was applied after 2 μg TPA, the ear edema was reduced by 23%, compared to mouse ears that received TPA only.

Rossi et al. (1995) transplanted Erlich ascites tumor cells into healthy female Swiss mice. The mice also received oral doses of 60 mg/kg/day Glycyrrhizic Acid or 30 mg/kg/day Glycyrrhetinic Acid for 10 days before infection, 10 days after infection, or for 10 days before and 10 days after infection with the tumor cells. Control animals received the tumor infection without Glycyrrhizic Acid or Glycyrrhetinic Acid. The rate of mouse deaths was used to determine the extent of protection provided by the test materials. Pretreatment or post-treatment with either test material did not protect the mice from the lethal tumors. However, the combination of pretreatment and post-treatment of Glycyrrhizic Acid delayed the 50% mortality rate from days 15 to 18–19. Glycyrrhetinic Acid on any dose schedule tested did not affect the mortality rate.

Kelloff et al. (1994) and Wang and Nixon (2001), in review articles, state that Glycyrrhizic Acid and related compounds have been used as an herbal remedy to prevent and to treat cancer for centuries and that several animal and in vitro studies suggest that these compounds have antimutagenic, tumor-suppressive, and anticarcinogenic properties.

Agarwal et al. (1991) treated SENCAR mice with 0% or 0.05% Glycyrrhizic Acid in drinking water for 50 days. The mice were then shaved and 40 nmol DMBA was applied to the bare skin. Twenty control group and 20 Glycyrrhizic Acid—treated animals were given untreated drinking water for the remainder of the experiment. Twenty Glycyrrhizic Acid–treated mice continued to receive drinking water with 0.05% Glycyrrhizic Acid. Seven days after the DMBA application, all mice were treated with a topical dose of 4 nmol TPA twice weekly for 16 weeks. Tumors were counted.

The two Glycyrrhizic Acid groups had similar outcomes in the number of tumors produced, which was significantly fewer ($p < 0.05$) than incidence of tumors in control mice. The latent period prior to the onset of tumor appearance was later in the Glycyrrhizic Acid–treated mice than in the control mice. The authors suggested that Glycyrrhizic Acid inhibited the tumor initiation by DMBA, but not by TPA (Agarwal et al. 1991).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

FDRL (1971b) studied the developmental toxicity potential of Ammonium Glycyrrhizate in four species of mammals. Pregnant CD-1 outbred mice received 0, 27, 90, 300, or 1000 mg/kg/day Ammonium Glycyrrhizate by oral gavage on gestation days 6 through 15 ($n = 19$ to 21 pregnant dams/dose group). The fetuses were removed by caesarian section on gestation day 17 and examined. There was no effect on maternal or fetal survival and no remarkable observation upon visceral and skeletal examinations of the fetuses.

Pregnant Wistar rats received 0, 27, 90, 300, or 1000 mg/kg/day Ammonium Glycyrrhizate by oral gavage on gestation days 6 through 15 ($n = 21$ to 22 pregnant dams/dose group). The fetuses were removed by caesarian section on gestation day 20 and examined. There was no effect on maternal or fetal survival and no remarkable observation upon visceral and skeletal examinations of the fetuses.

Pregnant golden hamsters received 0, 27, 90, 300, or 1000 mg/kg/day Ammonium Glycyrrhizate by oral gavage on gestation days 6 through 10 ($n = 21$ to 23 pregnant dams/dose group). The fetuses were removed by caesarian section on gestation day 15 and examined. There was no effect on maternal or fetal survival. Aside from dose-dependent delayed cranial ossification, no remarkable observation upon visceral and skeletal examinations of the fetuses was noted.

Pregnant Dutch-belted rabbits received 0, 27, 90, 300, or 1000 mg/kg/day Ammonium Glycyrrhizate by oral gavage on gestation days 6 through 18 ($n = 10$ to 12 pregnant dams/dose group). The fetuses were removed by caesarian section on gestation day 29 and examined. There was no effect on maternal or fetal survival and no remarkable observation upon visceral and skeletal examinations of the fetuses (FDRL 1971b).

In the previously mentioned Monlux (1974) study where rats were fed Glycyrrhizate, exposure to up to 4% Ammonium Glycyrrhizate in utero did not cause any observed developmental defects.

Mantovani et al. (1988) studied the teratogenicity of Ammonium Glycyrrhizate in Sprague-Dawley rats. Pregnant rats received drinking water containing 0, 10, 100, or 250 mg/ml Ammonium Glycyrrhizate on gestation days 7 through 17. The actual exposure levels, based on water consumption calculations, were 0, 21.33, 238.8, and 679.9 mg/kg/day, respectively. Dams were killed on gestation day 20. Dams and fetuses were examined. Water consumption was increased in dams of the 238.8 and 679.9 mg/kg/day groups. Fetuses exposed to Ammonium Glycyrrhizate did not have changes in the rate of external malformations, body weight, or degree of ossification, compared to controls. The incidence of external hemorrhages was increased in fetuses of the low- (21.33 mg/kg/day) and high- (679.9 mg/kg/day) dose groups ($p < 0.01$), but not in the middle- (238.8 mg/kg/day) dose group. Renal variants and ectopic kidneys were observed in fetuses of the lowest-dose group ($p < 0.05$). High-dose fetuses also had ectopic kidneys. Skeletal examinations revealed a dose-dependent increase in sternebral variants, significant ($p < 0.01$) in the middle- and high-dose groups. The lowest observed effect level in this study was 21.33 mg/kg/day.

Itami et al. (1985) gave pregnant Wistar rats 0%, 0.08%, 0.4%, or 2% Disodium Glycyrrhizate mixed in feed on gestation days 0 through 20 ($n = 8$ to 11 pregnant dams/dose group). There was no treatment-related effect on any of the following parameters: number of corpora lutea, number of implants, number of live fetuses, number of intrauterine dead fetuses per litter, sex ratio, fetal body weights, placental weights, degree of ossification, live birth index, survival rate, and pup body weight gain up to 8 weeks postpartum. The authors concluded that Disodium Glycyrrhizate was not teratogenic in rats under the treatment conditions of this study.

NEUROTOXICITY

Finney et al. (1958) found that 1250 mg/kg Glycyrrhetinic Acid given intraperitoneally to mice caused sedation, hypnosis, hypothermia, and respiratory depression. Glycyrrhetinic Acid given i.p. (1250 mg/kg) or subcutaneously (625 mg/kg) did not stimulate or depress either the sympathetic or parasympathetic branches of the autonomic nervous system of mice. A cat given 125 mg/kg Glycyrrhetinic Acid i.p. did not exhibit any changes in blood pressure and had normal responses to stimulation of sympathetic and parasympathetic nerves.

Sobotka et al. (1981) studied the neurobehavioral toxicity of Ammoniated Glycyrrhizin (Ammonium Glycyrrhizate) in male Sprague-Dawley rats. The rats were fed powdered diet containing 0%, 2%, 3%, or 4% Ammonium Glycyrrhizate for 4 to 6 months ($n = 40$ rats per dose group). Based on food consumption rates, the actual exposure levels were 0, 1.2, 1.9, and 2.6 mg/kg/day, respectively. Parameters evaluated included physiology (blood pressure, heart rate, temperature), motor functions (open-field exploration, Rotorod performance, general motility), and cognitive abilities (passive avoidance, active avoidance, and operant conditioning with incremental shock and incremental fixed-interval food reinforcement).

Hypertension (4% group), increased relative kidney weights (all treatment groups), decreased body weights and weight gain (4% group), bradycardia (4% group), and polydipsia (excessive thirst; 3% and 4% groups) were observed. Rectal temperatures were not affected by Ammonium Glycyrrhizate treatment. There were no treatment-related effects on motor function tests. Performance on active avoidance tests was facilitated in the 4% group, unaffected in the 3% group, and depressed in the 2% group. The authors suggested that the observations in this study are consistent with the reported hormonal effects of Ammonium Glycyrrhizate (Sobotka et al. 1981).

CLINICAL ASSESSMENT OF SAFETY

Clinical Studies

Mori et al. (1989) administered 200 to 800 mg/day Glycyrrhizic Acid to nine human immunodeficiency virus (HIV) patients (asymptomatic carrier) with hemophilia for 8 weeks. Lymphocyte counts increased in all nine patients. OKT4-positive lymphocyte numbers increased in eight patients, and the ratio of OKT4 to OKT8 cells increased in six patients. Four patients who previously had signs of liver dysfunction had clear improvements in hepatic function parameters such as serum GOT and GPT. Serum electrolytes, lipids, and protein, along with kidney function, were not affected by the Glycyrrhizic Acid.

van Gelderen et al. (2000) conducted a study using human subjects in the Netherlands to determine a no-effect level for Glycyrrhizic Acid. Thirty-nine healthy female volunteers aged 19 to 40 years and weighing 60 to 70 kg were selected after medical histories, physical examinations, and blood and urine tests. The volunteers were divided into four Glycyrrhizic Acid dose groups based on a randomized double blind treatment scheme: 0 mg/kg control ($n = 10$), 1 mg/kg/day ($n = 9$), 2 mg/kg/day ($n = 9$), and 4 mg/kg/day ($n = 11$). Glycyrrhizic Acid doses were administered orally in capsules. The experiment lasted 12 weeks: initial 2 weeks without treatment, 8 weeks of dosing with Glycyrrhizic Acid, and 2 weeks of post-treatment observations. During the 12-week period, all volunteers were required to abstain from smoking, using drugs, or using products containing Glycyrrhizic Acid. Parameters included physical examinations (at beginning and end of experimental period), questionnaire on physical condition (daily), body weight (weekly), blood pressure (weekly), edema scoring (weekly), blood potassium (weekly), blood plasma renin and aldosterone (every 2 weeks), dietary questionnaire (every 2 weeks), and blood atrial natriuretic peptide (ANP) (at weeks 8 and 10).

The aldosterone concentration and renin activity in the serum of the 4 mg/kg/day group were significantly lower ($p < 0.001$) than control after 2, 4, 6, and 8 weeks of dosing. Aldosterone levels and renin activities in the 1 and 2 mg/kg/day groups were similar to controls. The concentration of ANP was significantly decreased ($p < 0.001$) in the 4 mg/kg/day group, but not in the 1 and 2 mg/kg/day groups. Systolic and diastolic blood pressure measurements remained the same throughout the study in the 2 and 4 mg/kg/day groups but decreased slightly in the control group. Thus, relative to the control group, the 4 mg/kg/day group had significantly higher ($p = 0.018$) blood pressure than the control group. Body weight was similar between all treatment groups. Volunteers in the 4 mg/kg/day group had a significantly lower ($p < 0.01$) blood potassium concentration than those in the control group at 2 and 4 weeks into the dosing period.

In daily questionnaires of physical conditions, volunteers in the 4 mg/kg/day group described headache, nausea, vomiting, change in defecating pattern, swollen face, and tickling in the arms and legs. However, the number of complaints decreased as the study progressed.

The authors determined that the no effect level of Glycyrrhizic Acid in this study was 2 mg/kg/day. They proposed an acceptable daily intake of 0.2 mg/kg/day, using a safety factor of 10. This corresponds to 6 g of licorice per day would be safe for a 60-kg person, assuming that licorice contains 0.2% Glycyrrhizic Acid. They noted that the Dutch Nutritional Council advises a limit of 200 mg Glycyrrhizic Acid per day (van Gelderen et al. 2000).

Sigurjónsdóttir et al. (2001) demonstrated a linear dose-response relationship between Glycyrrhizic Acid and increases in blood pressure. Healthy Swedish and Icelandic volunteers aged 23 to 37 years ingested licorice amounting to 75, 270, or 540 mg/day Glycyrrhizic Acid for 2 to 4 weeks ($n = 24$, 30, or 10/dose group, respectively). After 2 weeks of dosing, mean systolic blood pressure rose 3.1, 5.2, and 14.4 mm Hg for the low-, middle-, and high-dose groups, respectively, compared to blood pressure measured prior to treatments. All rises in systolic blood pressure were statistically significant at $p$values of .03 to .000 [sic]. Increases in blood pressure were of similar magnitude after four weeks of dosing. Thus, a dose-response was demonstrated,

but a time-response was not observed. There did not seem to be a special demographic group that was especially sensitive to the effects of licorice or Glycyrrhizic Acid.

Louis and Conn (1956) administered seven normal human subjects 6 g/day Ammonium Glycyrrhizate for 3 days. Subjects maintained normal diets during the experiment. During the exposure period, renal excretion of Na^+ and Cl^- was significantly decreased, K^+ excretion was slightly increased, and water retention caused a 1.5- to 2-lb increase in body weight. Treatment also caused a reduction in excretion of Na^+ and Cl^- in thermal sweat. Withdrawal of the Ammonium Glycyrrhizate caused an immediate above-normal increase in renal excretion of Na^+ and Cl^- and retention of K^+. Body weights returned to normal five to seven days after withdrawal of Ammonium Glycyrrhizate treatment. Renal excretion of 17-ketosteroids was decreased during the exposure period, which the authors attributed to inhibited production of ACTH. Parameters of carbohydrate metabolism and protein metabolism were not affected by Ammonium Glycyrrhizate exposure.

Dermal Irritation and Sensitization

Universita' Delgi Studi Di Urbino (1990) conducted a human dermal sensitization study of an ointment containing 3% Glycyrrhetinic Acid. The subjects in this study were six male and nine female volunteers, ages 22 to 41. A 4 cm^2 occlusive pad with an unspecified amount of the ointment was applied to the skin of the arm of each volunteer. The pad remained in place for 3 days. The treatment site was scored following removal of the pad. After 7 days without treatment, the procedure was repeated, and the treatment site was examined after the second 3-day exposure. All 15 subjects had scores of "no reaction" after each treatment. The researchers concluded that the ointment (3% Glycyrrhetinic Acid) did not produce irritation or sensitization in human subjects.

Hilltop Research Inc. (1994) conducted a repeated insult patch test of a moisturizer product containing 0.3% Glycyrrhetinic Acid on 10 male and 98 female human subjects. In the induction phase, 0.15 g of the product was applied to a 2 $\times$ 2-cm area of the lateral surface of the upper arm, under an occlusive patch. Details of the protocol were not reported, but there were nine induction applications, an unspecified rest period, and a challenge patch. None of the 108 subjects had a response during the challenge phase. The authors concluded that, under the conditions of this study, there was no evidence that the test material induced a delayed contact hypersensitivity response.

Consumer Product Testing Company (2002) conducted a repeated insult patch test of Glycyrrhetinic Acid on 112 human subjects. Subjects included males and females, ranging in age from 16 to 79 years. One hundred six subjects completed the study. Those subjects who did not complete the study discontinued for reasons not related to the test material. In the induction phase, 0.2 ml of a formulation of 6% Glycyrrhetinic Acid in glycerine was applied to a 1 $\times$ 1-inch area of the scapular region under a gauze occlusive patch. Patches were applied three times per week for 3 weeks for a total of nine applications. Treatment sites were evaluated for signs of irritation 24 h after each application.

After a 2-week rest period, a challenge patch was applied to an untreated site, following the same formulation and application procedure performed in the induction phase. The challenge site was evaluated 24 and 72 h after application. None of the subjects had any sign of erythema or edema during the induction or challenge phase in this study. The authors concluded that 6% Glycyrrhetinic Acid in glycerine did not indicate a potential for dermal irritation or allergic contact sensitization (Consumer Product Testing Company 2002).

Allergisa Pesquisa Dermato-Cosmética Ltd. (2004a) conducted a human dermal sensitization study of 0.6% Glycyrrhetic Acid (also known as Glycyrrhetinic Acid). The subjects in this study were 51 females and 5 males between the ages of 18 and 66. Samples of 0.05 g/cm^2 were spread on filter paper and attached to the right or left part of the back then covered with semi-permeable hypoallergenic tape. Applications were conducted every other day, totaling 15 applications with each patch being removed after 24 h. The same was done with the saline control. After a 14-day rest period, two sample patches were applied in a fresh area. These patches were removed after 48 h of skin contact. These were scored for reaction after 30 min and 24 h. No adverse reactions were detected in the sites where the product was applied.

Allergisa Pesquisa Dermato-Cosmética Ltd. (2004b) conducted a human dermal irritation study of 0.6% Glycyhrrhetinic Acid. The subjects in this study were 52 females and 4 males between the ages of 18 and 62. Samples of 0.05 g/cm^2 were spread onto filter paper and attached to the back then covered with semipermeable hypoallergenic tape. The same was done with a control of saline. The patches were removed after 48 h and reactions were recorded after 30 min and 24 h of removal. No adverse reactions were detected in the sites where the product was applied.

Uemura (no date) used a patch test method to evaluate the skin irritation potential of 0.5% or 1.5% Stearyl Glycyrrhetinate in olive oil. The treatment sites were examined 24 h after application. There was no sign of skin irritation in any of the 89 subjects tested.

Lachartre Laboratories (1992a) evaluated the cutaneous tolerance to an eye gel containing 0.1% Glycyrrhizic Acid in 20 women. The gel was applied to the face in the area around the eyes with a soft digital massaging once or twice daily after washing for 21 days. Amounts of gel product applied ranged from 0.10 to 0.57 g per day. Each subject maintained her usual habits of makeup and hygiene, except for products similar to the test material. The eyes and the skin around the eyes were examined for signs of irritation on the 1st and 21st days of application. After the first day of application, there were no reactions. Twenty-four hours after the 20th application, two subjects had desquamation patches on the eyelids. The researchers did not think this event was related to the treatment. Eight subjects reported discomfort

sensations, as stretching. No other observations were noted. The authors concluded that repeated use of the eye gel (containing 0.1% Glycyrrhizic Acid) was well tolerated.

Lechartre Laboratories (1992b) conducted a repeated insult patch test with an eye gel containing 0.1% Glycyrrhizic Acid on 232 women, aged 18 to 62 years. In the induction phase, 0.1 ml of the test material was applied to the left shoulder of each subject, under occlusive patch, three times a week, for 3 weeks. After a 2-week period of no application, the challenge phase involved a similar application to each the induction site and a naive site. Three subjects had slight and transient erythema during the induction phase. One subject had well-defined erythema with pruritus after the first application. No other signs of irritation were reported in the induction phase. After the challenge application, one volunteer had a well-defined erythema with pruritus. No other treatment-related observations were reported.

Hilltop Research, Inc. (1995) conducted a human repeated insult patch test of an eye gel containing 0.1% Glycyrrhizic Acid on 57 male and 43 female subjects. In the induction phase, 0.1 ml of the test material was applied to a 2 × 2-cm patch pad. The pad was then applied to either the lateral surface of the upper arm or one side of the back. The pads were secured with hypoallergenic tape. Patch pads were applied for 24 h, three times per week, for 3 weeks. Twelve to 20 days after the last induction patch, a challenge patch was applied to the induction site and a naive site on the body for 24 h. The induction and challenge sites were examined 24 h after each application. The report did not give details of the study results. Six subjects had mild erythema with or without edema at the challenge phase. These observations were diminished 48 to 96 h after the challenge application and were considered irritant in nature. The authors concluded that the test material (eye gel containing 0.1% Glycyrrhizic Acid) did not produce evidence of induced contact hypersensitivity.

Quintiles (1999) conducted a human repeated insult patch test of a moisturizer containing 0.1% Glycyrrhizic Acid on 92 subjects. Induction consisted of application of 0.3 ml of the test material to the outer upper arm under occlusive patch for 24 h, three times per week, for 3 weeks. Eleven to 16 days after the last induction patch, the challenge phase involved a 24-h occluded application of the test material at the induction site and a naive site. Treatment sites were evaluated after each application. During induction, 35 subjects had moderate erythema and 42 subjects had mild erythema. There was no evidence of contact sensitization.

Phototoxicity and Photosensitization

Yamamoto (1976a) evaluated Glycyrrhizic Acid, Ammonium Glycyrrhizate, and Dipotassium Glycyrrhizate in human patch tests. In a double-blind method, the test articles (each at 5%) were applied to the forearms of 21 female volunteers for 48 h. The sites were observed for signs of irritation or inflammation. Forty-eight hours later, the dose sites were exposed to irradiation from a Dermaray Model 1 (BLB; 15 cm, 3 min), and the sites were evaluated again 48 h later. None of the exposure sites showed observable irritation reactions before or after irradiation.

Clinical Science Research International, Ltd. (CSRI) conducted a photosensitization study of an eye gel containing 0.1% Glycyrrhizic Acid in nine human subjects (CSRI 1992a). Induction treatments consisted of 24-h occluded applications of 0.2 ml of the test material, followed by UV exposure (1000-W xenon arc solar simulator) of the bare induction site. Induction treatments were performed six times over a period of 3 weeks. About 10 days after the last induction treatment, the challenge treatment consisted of a 24-h application of the test material at the induction site and two naive sites followed by exposure to 4 J/cm^2 of UVA irradiation. There was no evidence of photosensitization.

CSRI (1992b) conducted a phototoxicity study of an eye gel containing 0.1% Glycyrrhizic Acid in nine human subjects. Approximately 0.2 ml of the test material was applied to each of two circular test sites (4.5 cm^2 each) under occlusive pads for 24 h. After the application period, one of the sites was exposed to 10 J/cm^2 of UVA irradiation. The treatment sites were evaluated for up to 72 h. There was no evidence of phototoxicity to the eye gel.

Yamamoto (1976b) applied 5% Glycyrrhizic Acid, 5% Ammonium Glycyrrhizate, or 5% Dipotassium Glycyrrhizate in distilled water to the skin of 21 healthy female volunteers. The treatment area was irradiated with UV (UV dose not specified), and the irradiated treatment site was examined 48 h later. There was no sign of phototoxicity.

St. Marianna University (1995) repeated the above study (Yamamoto 1976b) and found the exact same results of no phototoxicity to 5% Glycyrrhizic Acid, 5% Ammonium Glycyrrhizate, or 5% Dipotassium Glycyrrhizate in distilled water.

Epidemiology Studies

Strandberg et al. (2001) gave questionnaires on Glycyrrhizic Acid (licorice) consumption to a sample of 1049 Finnish women with young infants. Glycyrrhizic Acid consumption was grouped into three levels: low (< 250 mg/week; $n = 751$), moderate (250–499 mg/week; $n = 145$), and heavy (≥ 500 mg/week; $n = 110$). Hospital birth records were analyzed and compared to Glycyrrhizic Acid exposure. Birth weight and maternal blood pressure were not affected by Glycyrrhizic Acid consumption. Babies with heavy exposure were significantly more likely to be born before 38 weeks ($p < 0.03$).

Case Reports

Donaldson and Duthie (1956) treated 30 patients with eczematous skin lesions with an ointment made of wool fat, liquid paraffin, and white soft paraffin with or without 2% Glycyrrhetinic Acid. There was no significant difference in the degree of healing of the lesions between the two ointment types. In this study, 2% Glycyrrhetinic Acid did not improve the condition of eczema patients.

Colin-Jones (1957) reported the results of Glycyrrhetinic Acid treatments on several cases of different skin disorders.

Treatment with 2% Glycyrrhetinic Acid in a water-miscible base markedly improved or cleared 11/13 cases of infantile eczema, 6/8 cases of flexural eczema, 3/4 cases of nummular eczema, 4/4 cases of traumatic dermatitis, 3/3 cases of dermatitis, 3/4 cases of neurodermatitis of the nape of the neck, 2/2 cases of disseminated neurodermatitis, and 3/4 cases of pruritis vulvæ et ani. Treatment with Glycyrrhetinic Acid (% not given) in 0.5% neomycin sulphate markedly improved or cleared 8/8 cases of impetigo, 2/2 cases of impetigo with associated penicillin sensitivity, 3/4 cases of impetiginized eczema, 3/4 cases of impetiginized seborrhœa, 4/5 cases of pustular psoriasis, and 2/2 cases of acne varioliformis.

Evans et al. (1958) reported the outcomes of 124 cases of various skin disorders (e.g., contact and allergic dermatitis, anogenital pruritus, and several types of eczema) after treatment with Glycyrrhetinic Acid (doses not specified). Of the 124 cases, 91 showed marked improvement or cleared, and 25 cases showed improvement. Eight of the reported cases showed no improvement. The author stated that in many cases Glycyrrhetinic Acid was more effective than hydrocortisone in the treatment of subacute, chronic, and intractable skin conditions.

Watanabe et al. (2001) described a case of a woman with chronic hepatitis C who did not initially respond to interferon therapy. She was treated with Glycyrrhizic Acid (60 ml Stronger Neo-minophagen C) three times per week for 2 years in conjunction with interferon-β therapy. After the treatment period, the patient's aminotransferase levels returned to normal, and the hepatitis virus C RNA level was negative. The authors commented that the interferon therapy was successful only after the liver function had improved with Glycyrrhizic Acid treatment.

Conn et al. (1968) reported a case of a 58-year-old man who was admitted to the University of Michigan Hospital with complaints of severe muscular weakness. Tests revealed that the patient had hypertension, hypokalemic alkalosis, suppressed renin activity, and aldosteronopenia. Chlorothiazide (0.5 g twice daily) and chlorthalidone (100 mg/day) failed to correct the hypertension. A careful history revealed that he had ingested 72 to 108 g of licorice candy daily for 6 to 7 years. The candy's manufacturer indicated that amount to contain about 0.5 g/day Ammonium Glycyrrhizate. The patient was put on a strict licorice-free diet with controlled sodium and supplements to replace lost potassium. The second day after admission, the patient began to regain muscular strength. Diuresis and weight loss began 8 days later. Aldosterone excretion was first measured on the 9th day after admission and was 0.8 μg/day, whereas renin activity was below detectable limits. On day 10, the 24-h secretion rate of aldosterone was 30.8 μg/day, compared to the normal mean rate of 109 μg/day. By 28 days after admission, the patient was recovering, with aldosterone excretion at 5.1 μg/day and renin activity at 222 μg/100 ml, values within normal range, and blood pressure was 120/80 to 130/85 mm Hg.

After recovery, the patient agreed to participate in an investigation to reproduce the clinical effects of Ammonium Glycyrrhizate. The patient was given 0.5 g every 12 h for 2 days, followed by 1 g every 12 h for 3 days, and then 2 g every 12 h for 5 days. There was a prompt increase in body weight. Serum sodium and blood pressure increased, and serum potassium steadily declined. Aldosterone excretion fell from 5.1 μg/day before dosing to 1.7 μg/day by day 4 and to 0.3 μg/day by day 9. Plasma renin activity fell from normal 222 μg/100 ml to undetectable levels by day 8. Ten months of a licorice-free diet after discharge, the patient felt generally healthy, and all aldosterone, renin, electrolyte and blood pressure parameters were normal. The data supported the investigators' suspicions that Ammonium Glycyrrhizate in the licorice candy that the patient had eaten for years caused a pseudoaldosteronism that contributed to electrolyte imbalance and hypertension. The patient's initial muscle weakness was attributed to hypokalemia (Conn et al. 1968).

EUROPEAN COMMISSION EVALUATION

The European Commission's Scientific Committee on Food (SCF) prepared an opinion on the safety of Glycyrrhizic Acid and Ammonium Glycyrrhizate in foods (SCF 2003). The opinion noted that the Committee had previously reviewed Glycyrrhizic Acid in 1991 and concluded that the data were inadequate to derive an acceptable daily intake (ADI), but they did advise that regular daily ingestion from all food products should not exceed an upper use level (UUL) of 100 mg/day.

In 2003, the SCF was asked to review new data on Glycyrrhizic Acid and to consider the safety of Ammonium Glycyrrhizate as a flavoring substance. After reviewing the new data, the SCF concluded that there is a stronger basis for the limit of 100 mg/day, but they still did not feel that the human toxicity data were sufficient to develop an ADI. The committee noted that there are subgroups of people for whom a limit of 100 mg/day may not provide sufficient protection. These groups include people with decreased 11-β-hydroxysteroid dehydrogenase-2 activity, people with prolonged gastrointestinal transit time, and people with hypertension or electrolyte-related or water homeostasis-related medical conditions (SCF 2003).

SUMMARY

This review considers the safety of specific organic compounds that may be isolated from licorice plants—Glycyrrhetinic Acid and its salts and esters, and Glyrrhizic Acid and its salts and esters used in cosmetics as considered by the Cosmetic Ingredient Review (CIR) Expert Panel. It does not address the plant extracts from the licorice plant—those will appear in a separate report.

The ingredients in this safety assessment are Glycyrrhetinic Acid, Potassium Glycyrrhetinate, Disodium Succinoyl Glycyrrhetinate, Glyceryl Glycyrrhetinate, Glycyrrhetinyl Stearate, Stearyl Glycyrrhetinate, Glycyrrhizic Acid, Ammonium Glycyrrhizate, Dipotassium Glycyrrhizate, Disodium Glycyrrhizate, Trisodium Glycyrrhizate, Methyl Glycyrrhizate, and

Potassium Glycyrrhizinate. All these compounds have the basic core structure of Glycyrrhetinic Acid with varying R groups.

These ingredients are ethanol or water soluble. Ultraviolet radiation absorption is in the UVC region. Semi-micro-HPLC, mass spectrometry, and nuclear magnetic resonance spectra have been used to detect these ingredients.

Glycyrrhetinic Acid has been found to be at least 98% pure. It has been found to contain 0.6% 24-OH-Glycyrrhetinic Acid, not more than 20 μg/g of heavy metals and not more than 2 μg/g of arsenic. No residue was detected in Glycyrrhetic Acid. Ammonium Glycyrrhizate has been found to be at least 98% pure and Dipotassium Glycyrrhizate has been found to be at least 95% pure.

Glycyrrhetinic Acid and its relatives function in cosmetics as either a flavoring agent, a skin-conditioning agent—miscellaneous or both. Glycyrrhetinic Acid is used at concentrations of up to 2%; Stearyl Glycyrrhetinate, 1%; Glycyrrhizic Acid, 0.1%; Ammonium Glycyrrhizate, 5%; Dipotassium Glycyrrhizate, 1%; and Potassium Glycyrretinate, 1%.

Glycyrrhizic Acid is poorly absorbed by the intestinal tract. However, it may be hydrolyzed by a specialized β-glucuronidase produced by intestinal bacteria into Glycyrrhetinic Acid. Glycyrrhetinic Acid and Glycyrrhizic Acid bind extensively to rat and human albumin but do not absorb well into tissues. Glycyrrhetinic Acid and Glycyrrhizic Acid and metabolites are mostly excreted in the bile and are subject to enterohepatic cycling in rats, and presumably humans. Very little is excreted in urine.

In rats given radioactive Glycyrrhetinic Acid orally, 86% of the radioactivity was recovered in 1 to 3 days with 83% in the feces, 1% in the urine and 4% remained in the liver. In rats given radioactive Glycyrrhetinic Acid subdermally, 74% of the radioactivity was recovered with 73% in the feces and 1% in the urine. Of the radioactivity recovered in the feces, only 7.4% was unchanged Glycyrretinic Acid. In humans Glycyrrhizic Acid was not detected in blood plasma after oral administration, but Glycyrrhetinic Acid was detected at $<$200 ng/ml. In the urine, 0.3% to 0.8% of the Glycyrrhizic Acid was recovered.

Dipotassium Glycyrrhizate was undetectable in the receptor chamber when tested for transepidermal permeation through pig skin.

Glycyrrhizic Acid increased the dermal penetration of diclofenac sodium in rat skin. Dipotassium Glycyrrhizate increased the intestinal absorption of calcitonin in rats. In humans, Glycyrrhetinic Acid potentiated the effects of hydrocortisone in the skin, possibly by increasing the percutaneous absorption of hydrocortisone.

Moderate chronic or high acute exposure to Glycyrrhizic Acid, Ammonium Glycyrrhizate, and their metabolites have been demonstrated to cause several transient systemic alterations including increased potassium excretion, sodium and water retention, body weight gain, alkalosis, suppression of the renin-angiotensis-aldosterone system, hypertension, and muscular paralysis. Many of these effects result from Glycyrrhizic Acid inhibition of 11β-OHSD2 in the kidney. Other enzyme activities affected include proteinases, cytochrome P450 monooxygenase, protein kinases, and tyrosinase.

Glycyrrhetinic Acid and its derivatives block gap junction intracellular communication in a dose-dependent manner in animal and human cells, including epithelial cells, fibroblasts, osteoblasts, hepatocytes, and astrocytes. At high concentrations, Glycyrrhetinic Acid is cytotoxic.

Glycyrrhetinic Acid and Glycyrrhizic Acid have been shown to protect liver tissue from known hepatotoxins such as carbon tetrachloride.

Glycyrrhizic Acid has been used to treat chronic hepatitis, inhibiting the penetration of the hepatitis A virus into hepatocytes. Other anti-viral activity included influenza, vaccinia, herpes simplex 1, Newcastle disease, and vesicular stomatitis, but not polio type 1.

Glycyrrhetinic Acid and Glycyrrhizic Acid have antiinflammatory effects in rats and mice. Other cellular effects include apoptosis and enhanced interleukin and interferon production.

The acute intraperitoneal LD_{50} for Glycyrrhetinic Acid in mice was 308 mg/kg and the oral LD_{50} was $>$610 mg/kg. The oral LD_{50} in rats was reported to be 610 mg/kg. Higher LD_{50} values were generally reported for salts.

Little short-term, subchronic, or chronic toxicity was seen in rats given ammonium, dipotassium, or disodium salts of Glycyrrhizic Acid.

Glycyrrhetinic Acid at 100 mg/ml was not irritating to shaved rabbit skin. In an in vitro irritation assay, Glycyrrhetinic Acid was considered slightly irritating.

Glycyrrhetinic Acid inhibited the mutagenic activity of benzo[a]pyrene in Ames strains TA-98 and TA-100. Glycyrrhetinic Acid inhibited DMBA and TPA tumor initiation and promotion in mice. Glycyrrhizic Acid inhibited tumor initiation by DMBA but not TPA tumor promotion in mice. Glycyrrhizic Acid delayed mortality in mice injected with Erlich ascites tumor cells, but did not reduce the mortality rate. Ammonium Glycyrrhizate was not genotoxic in in vivo and in vitro cytogenetics assays, the dominant lethal assay, Ames strain TA1530 and *Salmonella* strain G46, and heritable translocation tests, except for possible increase in dominant lethal mutations in rats given 2000 mg/kg day^{-1} in their diet. Disodium Glycyrrhizate was not carcinogenic in a mouse drinking water study at exposure levels up to 12.2 mg/kg day^{-1} for 96 weeks.

Glycyrrhizate salts produced no reproductive or developmental toxicity in rats, mice, golden hamsters, or Dutch-belted rabbits, except for a dose-dependent increase (at 238.8 and 679.9 mg/kg day^{-1}) in sternebral variants in study using rats.

Sedation, hypnosis, hypothermia, and respiratory depression were seen in mice given 1250 mg/kg Glycyrrhetinic Acid intraperitoneally. Rats fed a powdered diet containing up to 4% Ammonium Glycyrrhizate had no treatment related effects in motor function tests, but active avoidance was facilitated at 4%, unaffected at 3%, and depressed at 2%.

In a study of 39 healthy volunteers, a no effect of an oral dosage of 2 mg/kg/day was determined for Glycyrrhizic Acid. Glycyrrhizic Acid increased the lymphocyte count in HIV patients with hemophilia, and increased blood pressure in healthy individuals. Clinical tests in six normal individuals using Ammonium Glycyrrhizate at 6g/day revealed reduced renal and thermal sweat excretion of Na^+ and K^+. Carbohydrate and protein metabolism were not affected.

Glycyrrhetinic Acid at concentrations up to 6% was not an irritant or a sensitizer. Neither Glycyrrhizic Acid, Ammonium Glycyrrhizate, nor Dipotassium Glycyrrhizate at 5% was phototoxic agents or photosensitizers. One case reports linked licorice candy consumption (72 to 108 g/day) with muscular weakness, hypertension, alkalosis, suppressed renin activity, and aldosteronopenia. Various uses of these licorice ingredients in clinical treatment have also been reported.

Birth weight and maternal blood pressure were unrelated to the level of consumption of Glycyrrhizic Acid in 1049 Finnish women with infants, but babies whose mothers consumed >500 mg/wk were more likely to be born before 38 weeks.

A European Commission Scientific Committee on Food opinion did not establish an acceptable daily intake of Glycyrrhizic Acid and Ammonium Glycyrrhizate in foods, but did recommend an upper use level of 100 mg/day and identified decreased 11-β-hydroxysteroid dehydrogenase-2 activity, prolonged gastrointestinal transit time, or hypertension or electrolyte-related or water homeostasis-related medical conditions as conditions for which the upper use level may be too high.

DISCUSSION

The CIR Expert Panel noted that the ingredients in this safety assessment are not plant extracts, powders, or juices, but rather are specific chemical species that may be isolated from the licorice plant. Because these chemicals may be isolated from plant sources, however, steps should be taken to assure that pesticide and toxic metal residues are below acceptable levels. The Panel advised the industry that total polychlorobiphenyl (PCB)/pesticide contamination should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue, and that toxic metal levels must not contain more than 3 mg/kg of arsenic (as As), not more than 0.002% heavy metals, and not more than 1 mg/kg of lead (as Pb).

No published information was identified for Disodium Succinoyl Glycyrrhetinate, Glyceryl Glycyrrhetinate, Glycyrrhetinyl Stearate, Methyl Glycyrrhizate, Potassium Glycyrrhetinate, Potassium Glycyrrhizinate, or Trisodium Glycyrrhizate, but they are considered to be sufficiently structurally related to the rest of the ingredients that the data may be extrapolated to address their safety.

Although Glycyrrhizic Acid is cytotoxic at high doses and ingestion can have physiological effects, there is little acute, short-term, subchronic, or chronic toxicity and Dipotassium Glycyrrhizate is poorly absorbed through the skin. Glycyrrhizate salts were not genotoxic in all but one assay at a high exposure level, were not carcinogenic in animals tests, and inhibited tumor initiation and promotion by known tumor producing chemicals. Glycyrrhizate salts produced no reproductive or developmental toxicity in several animal species, except for one finding of sternebral variants in a rat drinking water study. Because of the poor dermal absorption, this one finding did not suggest a safety concern regarding use in cosmetic formulations.

These ingredients are not considered to be irritants, sensitizers, phototoxic agents, or photosensitizers at the current maximum concentration of use. Accordingly, the CIR Expert Panel concluded that these ingredients are safe in the current practices of use and concentration.

The Panel recognizes that certain ingredients in this group are reportedly used in a given product category, but the concentration of use is not available. For other ingredients in this group, information regarding use concentration for specific product categories is provided, but the number of such products is not known. In still other cases, an ingredient is not in current use, but may be used in the future.

Although there are gaps in knowledge about product use, the overall information available on the types of products in which these ingredients are used and at what concentration indicate a pattern of use. Within this overall pattern of use, the Expert Panel considers all ingredients in this group to be safe.

CONCLUSION

Ammonium Glycyrrhizate, Dipotassium Glycyrrhizate, Disodium Glycyrrhizate, Disodium Succinoyl Glycyrrhetinate, Glyceryl Glycyrrhetinate, Glycyrrhetinic Acid, Glycyrrhetinyl Stearate, Glycyrrhizic Acid, Methyl Glycyrrhizate, Potassium Glycyrrhetinate, Potassium Glycyrrhizinate, Stearyl Glycyrrhetinate, and Trisodium Glycyrrhizate are safe for use in cosmetic formulations in the practices of use and concentration as described in this safety assessment.

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