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Review Article

Assessment of the Genotoxic Risk From Laxative Senna Products

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Laxative senna products and several of their specific components have been submitted to a large number of genetic tests. While most studies gave negative responses, results from some of the studies suggest that components of senna products, particularly emodin and aloe-emodin, have genotoxic activity. Assessment of the genotoxicity profile of these substances, in light of other data from animal and human metabolism or kinetic studies, human clinical trials and rodent carcinogenicity studies do not support concerns that senna laxatives pose a genotoxic risk to humans when consumed under prescribed use conditions. Environ. Mol. Mutagen. 29: 1–9, 1997 © 1997 Wiley-Liss, Inc.

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BACKGROUND

During the late 1970s some anthraquinone derivatives of anthracenes found in plants of the rheum, rhamnus, senna, and aloe groups, particularly the 1-hydroxy- and 1,8-dihydroxy-anthraquinones were reported to be mutagenic in bacteria [Brown, 1980]. The agents induced reverse mutation in strain TA1537 of *Salmonella typhimurium*, a mutant strain which responds to frameshift mutagens. The mutagenic activity was initially observed under direct treatment conditions and suggested that the 3-ring structures, as illustrated in Figure 1, function by a mechanism of intercalation similar to acridine mutagens rather than through direct DNA binding [Bösch et al., 1987]. Glycosides of the anthraquinones were not generally mutagenic unless treated with enzymes normally associated with gut flora suggesting that the glycosidic bond interfered with the mechanism responsible for mutagenesis [Brown and Dietrich, 1979].

Some anthraquinones were also reported to induce DNA damage in the rec-assay. This assay assesses DNA reactivity of chemicals using two isogeneic strains of *Bacillus subtilis* differing in their DNA repair capabilities. Decreased survival in the repair deficient strain as compared to the normal strain is an indication of a positive response [Brown, 1980].

Early attempts to demonstrate genotoxicity for hydroxyanthraquinones in mammalian cell culture systems were consistently negative [Bruggeman and van der Hoeven, 1984; Mori et al., 1984]. The lack of activity in these tests was consistent with the proposed mechanism of intercalation mutagenicity. Nonelectrophilic intercalating agents tend to be less effective genotoxins toward mammalian (eukaryotic) DNA than prokaryotic DNA. In 1986, however, Kawai and colleagues reported positive results in the rat hepatocyte UDS assay for dantron and 1-hydroxyanthraquinone. Three other dihydroxyanthraquinones were evaluated with this test and reported as negative. Morita and associates [1988] reported mutation to 6-thioguanine resistance in the FM3A (C3H mouse mammary carcinoma) cell line with emodin. The tests were conducted without an exogenous activation source and the treatment was extended to 48 hours for optimal effects. Surprisingly, another direct-acting mutagen for bacteria, 2-hydroxyemodin, was not mutagenic in this assay, possibly indicative of different mechanisms of mutation for emodin between prokaryotic and eukaryotic DNA and suggesting the need for confirmation of the emodin response.

The initial results from anthraquinones showing predominantly prokaryote frameshift mutation, were not regarded as particularly relevant to human risk, since pure intercalating agents have not been implicated as animal carcinogens.

During the past several years, however, toxicological interest in the biological activity of anthraquinones, as well as products containing them, has been raised as a consequence of two sources of new information: (1) carcinogenicity studies with some anthraquinone derivatives produced tumors in rodents [Mori et al., 1985, 1990],

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and (2) genetic toxicology studies conducted with certain anthraquinone derivatives in cultured mammalian cells in which induction of genotoxicity has been reported [Westendorf et al., 1988, 1990].

Among the chemicals found in laxative senna products, only a limited number have been reported to be genotoxic. Rhein was reported to be weakly positive for bacteria (only TA1573) but is not active in mammalian systems [Westendorf et al., 1990; Heidemann et al., 1993]. Heidemann and Westendorf also reported emodin and aloe-emodin capable of inducing genotoxic responses in bacteria as well as cultured mammalian cells. Because some genetic tests are believed to be predictive of animal carcinogenesis, questions have been raised concerning the safety of laxative extracts, particularly those containing emodin and aloe-emodin, for example, aloe, cascara, and frangula.

PHARMACOLOGY OF PLANT ANTHRANOIDS

Plant extracts used in the production of laxative products contain a mixture of anthracene compounds including O- and C-glycosides of anthrones and anthraquinones, as well as free anthrones and dianthrones and small amounts of free anthraquinones [Fairbairn and Moss, 1970]. The free anthraquinones are believed to be formed in situ as a result of autooxidation of anthrones [Hattori et al., 1988], as shown in Figure 1. Studies of the purgative activities of the components of laxative mixtures indicates that the anthrone glycosides are most active followed by the free anthrones and dianthrones [Fairbairn and Moss, 1970]. Formation of the glycosides increases the pharmacological activity of the anthranoids by (1) decreasing their absorption from the gut and (2) protecting the chemicals from oxidation in the intestine to less active derivatives. Anthrones and anthraquinones probably act by two independent mechanisms, that is, stimulating colonic peristalsis and opening Cl^- channels at the colonic membrane causing a net reduction of liquid absorption in the colon [Leng-Peschlow, 1993]. Studies in conventional and germ-free animals have established that gut flora (e.g., glycosidases) are responsible for the breakdown of glycosides. The ability to metabolize various anthranoids varies greatly across species depending upon the composition of gut flora [Brunton, 1990]. In humans, anthranoid glycosides ingested orally pass to the colon unmodified. Human gut flora are able to break down O-glycosides easily but only to some extent C-glycosides of most anthranoids [Hattori et al., 1988; Che et al., 1991]. Hydrolysis of O-glycosides can also be produced in vitro using enzymatic extracts of rat cecal bacteria [Brown, 1980]. Subsequent systemic metabolism of the free anthranoids depends upon their absorption and ring constituents [Sendelbach, 1989]. Most of the free anthranoids absorbed systemically in humans are excreted in the urine as rhein or as conjugates [Vyth and Kamp, 1979].

GENOTOXICITY OF SENNA

Sennosides (the main anthranoid glycosides in senna) form a group of anthranoids used in laxative products. Rhein and sennidins are metabolites of sennosides formed by gut flora. In addition to these agents, one also finds heterodianthrone glycosides (sennosides C and D containing rhein and aloe-emodin moieties) and flavonols [van Os, 1976; Duke, 1985] as contaminants. Before 1990, few data were published regarding the genotoxicity of sennosides or their metabolites. Those data that were reported were generally negative [Tikkanen et al., 1983]. Additional genetic toxicology studies were conducted on sennosides A, B, C, D and rhein [reviewed in Heidemann et al., 1993]. A summary of the studies conducted is given in Table I.

A recent publication by Sandnes and coworkers [1992] suggests that (1) senna glycosides may be weakly mutagenic in *S. typhimurium* TA102 and (2) senna extracts are mutagenic in *S. typhimurium* TA98 and TA97. The results shown in their publication for senna glycosides (sennosides A and B) indicated weak dose-related increases in strain TA102, both with and without S9 mix. Responses were less than twice the background but suggested an effect when analyzed using linear regression statistics. None of the other Salmonella tester strains, including TA97a, indicated mutagenicity. Sandnes and coworkers [1992] concluded that isolation of senna glycosides from extracts eliminates their mutagenicity in all Salmonella strains except TA102. The mutagenicity measured by TA102 is of a base-pair substitution type and would not be expected from agents acting through a frameshift mechanism. Because no evidence is given on the purity of the senna glycosides used, it is argued that the weak mutagenic effect reported might have been produced from impurities rather than from sennosides A and B. This argument is supported by the fact that an HPLC analysis of the solutions used in the studies [Sandnes et al., 1992] indicated the presence of emodin (~1 μg/ml), aloe-emodin (~1–5 μg/ml), quercetin and kaempferol in all extract solutions. All four of the contaminants have been reported to produce positive responses in the Ames test [Brown, 1980].

Studies conducted in the Ames test [Heidemann et al., 1993] on senna extracts produced results similar to those reported by Sandnes and colleagues [1992] for strains TA98 and TA100 (Table II). It is not possible to directly compare activity ranges because the extract samples used in the studies reported by Sandnes were given in microliters per plate, and in the studies reported by Heidemann and colleagues the samples were given in micrograms per plate. Overall, the two sets of studies appear consistent. Whether the anthranoid or flavonol contaminants are responsible for the activity observed in senna extracts is not clear but might be considered a plausible explanation.

Some interpretive issues are raised by the bacterial mu-

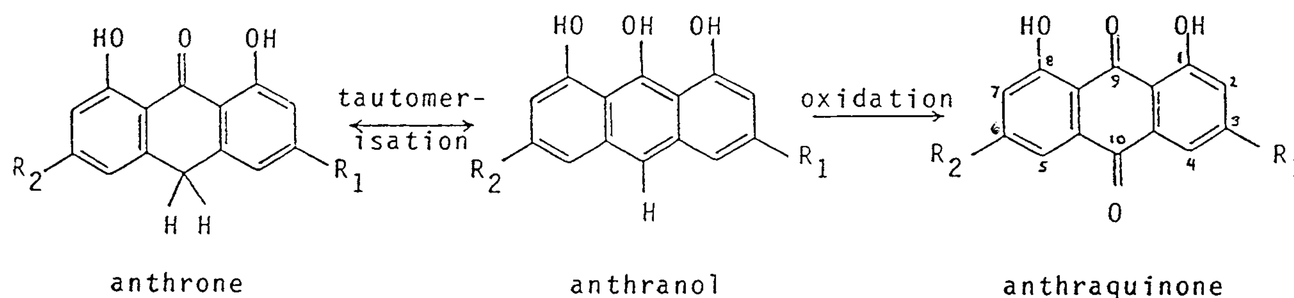

Figure 1. The Interconversion of Aloe Anthranoids

TABLE I. Summary of the Genotoxic Profiles for Sennosides (A, B, C, and D) and Rhein*

Substance tested	Assay[a]	Maximum concentration tested (toxicity observed)[b]	Concentration of potential aleo-emodin[c]	Reported response
Sennosides	Ames (1)	5 mg/plate (N)	67 µg/plate	Negative
	E. coli (1)	5 mg/plate (N)	67 µg/plate	Negative
	Mouse lymphoma (2)	5 mg/ml, −S9 (S)	61 µg/ml	Negative
		1 mg/ml, +S9 (M)	12 µg/ml	Negative
	Mouse lymphoma (1)	3.2 mg/ml, −S9 (M)	43 µg/ml	Negative
		1.6 mg/ml, +S9 (M)	21 µg/ml	Negative
	Chromosome aberrations in CHO cells (1)	9 mg/ml, −S9 (MTD)	121 µg/ml	Negative
		4 mg/ml, +S9 (MTD)	54 µg/ml	Negative
	Mouse micronucleus (1)	2500 mg/kg × 2 days Oral gavage (S)	34 mg/kg	Negative
Rhein	Ames (3)	5 mg/plate (MTD)	7.5 µg/plate	Negative
	Mouse lymphoma (3)	50 µg/ml (S)	0.08 µg/ml	Equivocal
	Chromosome aberrations in CHO cells (4)	50 µg/ml (S–M)	0.13 µg/ml	Negative
	Mouse micronucleus (3)	1500 µg/kg/day Oral gavage (S)	2.25 µg/ml	Negative

*Data based on audited final reports from testing laboratories.
[a]Level of potential aloe-emodin (free and chemically bound) contaminant: (1), 1.34%; (2), 1.21%; (3), 0.15%; (4), 0.26%.
[b]Toxicity observed: (N), None; (M), moderate ≥50%; (S), slight <50%; (MTD), treated up to cytotoxic limit.
[c]Present at the maximum concentration tested.

tagenesis studies of Sandnes and colleagues [1992] and Heidemann and colleagues [1993]. The current database for strain TA102 is very small, and there are no assessments of its predictivity for carcinogens. Little is known regarding the toxicity of chemicals which are not mutagenic in the standard set of tester strains but positive in TA102. Levin and associates [1982] showed that TA102 has A:T bases at the mutant site and reverts by base pair substitution. There are a limited number of chemicals, primarily those acting through active O_2 species, which respond only in TA102. Without additional analytical information, one can only speculate on the significance of the response reported by Sandnes and colleagues [1992]. Wilcox and colleagues [1990] suggested that TA102 and *E. coli* WP_2 *uvr* A strains respond similarly to chemicals. In Table I, it can be seen that sennosides were not mutagenic when tested in an *E. coli* WP_2 *uvr* A reverse mutation study.

Two of the tests employed for the evaluation of senna extracts used cultured mammalian cells (Table II). The first of these was a test for gene mutation at the HGPRT locus in V79 cells, and the second was a test measuring chromosome aberrations in Chinese hamster ovary (CHO) cells [Heidemann et al., 1993]. The results showed that senna extracts were not mutagenic in V79 cells up to a concentration of 5 mg/ml, but were able to induce chromosome aberrations in vitro at concentrations of 4-5 mg/ml in the absence of S9 mix. The addition of S9 mix significantly reduced the clastogenic effects.

In addition to the in vitro studies, three in vivo studies assessing clastogenicity and mutagenicity were conducted with the crude senna drug (*Fructus sennae*). At concentrations of 1 or 1.5 g/kg body weight, all three of the in vivo studies showed no evidence of any genetic effects [Heidemann et al., 1993]. The responses for both the in vitro and in vivo studies with senna preparations are summarized in Table II.

In summary, data suggest that senna extracts contain compounds that produce mutagenic responses in the Ames test, although the exact nature of the chemical is

TABLE II. Summary of the Genotoxic Profiles for Senna Extracts/Crude Senna Drug (*Fructus sennae*)

Assay citation	Maximum concentration tested	Reported response	Aloe-emodin estimated/max blood levels for in vivo studies[a]
In vitro (senna extract)			
Ames [Sandnes et al., 1992]	400 µl/plate	Pos. TA97, TA98, TA100	NA
Ames [Heidemann et al., 1993]	5 mg/plate	Pos. TA1537, TA58, TA100	NA
CHO aberrations [Heidemann et al., 1993]	5 mg/ml	Positive	NA
V79 HGPRT assay [Heidemann et al., 1993]	5 ml/ml	Negative	NA
In vivo (crude senna drug)			
Rat micronucleus [Heidemann et al., 1993]	1.5 g/kg body weight	Negative	~0.012 µg/ml
Rat bone marrow aberration assay [Heidemann et al., 1993]	1 g/kg body weight	Negative	~0.020 µg/ml
Mouse somatic mutation assay [Heidemann et al., 1993]	1 g/kg body weight	Negative	~0.012 µg/ml

[a]Data obtained from laboratory reports. NA, not applicable.

unknown. Plausible candidates are aloe-emodin and/or emodin. Sennosides (senna glycosides) do not appear to be responsible for the activity. Similar to the other anthraquinones, the extracts show mutagenicity in some strains of bacteria but have no effect *in animal tests*, which are essential in defining hazard. In order to understand the potential risks from senna laxative products, an appreciation for the genotoxicity of aloe-emodin and/or emodin is necessary. These agents appear to be the most biologically active contaminants in the laxative products.

GENOTOXICITY OF ALOE-EMODIN AND EMODIN

Aloe-emodin and emodin are found in plant extracts used to make laxative products. Because these two substances appear to be the most biologically active anthraquinones in senna laxatives, a substantial amount of test data have been developed for them.

Ames Test Results

Brown and Dietrich [1979] reported that aloe-emodin was mutagenic in *S. typhimurium* strain TA1537. The mutagenicity was observed in the absence of S9 mix. Westendorf and colleagues [1990] reported that aloe-emodin was not only mutagenic for TA1537 but was also active in strains TA98, TA1538 and TA97 (all frameshift mutant sites). The activity was independent of metabolic activation; in fact, the addition of S9 mix tended to suppress the mutagenicity. Aloe-emodin was evaluated independently [Heidemann et al., 1993] in three independent trials both with and without S9 mix. The results were qualitatively identical with the results reported by Westendorf and colleagues [1990]. Strains TA1537, TA1538, and TA98 all exhibited mutagenic responses in all trials without S9 mix (Fig. 2). Aloe-emodin was active at concentrations approaching 10 µg/plate and higher with no evidence of compound-induced toxicity up to 5 mg/plate. However, in the presence of S9 mix, the mutagenic activity of aloe-emodin for Salmonella was suppressed or eliminated (Fig. 2). Thus, it is clear that aloe-emodin is capable of interacting with Salmonella DNA resulting in the production of frameshift mutations.

Mammalian Cell Mutation Test Results

Westendorf and coworkers [1990] also reported that aloe-emodin was mutagenic at the HGPRT locus of V79 cells; however, a careful analysis of the results raises doubts about this conclusion. The data from this test are shown in Table III. Although the authors report a two- to threefold increase in mutants for one of the trials, the mutation values for aloe-emodin fluctuate across the dose levels and do not exceed the spontaneous background range sufficiently to be considered an unequivocal mutagen. The apparent positive response was based on a very low spontaneous mutant frequency, especially in Experiment 1. Numerous laboratories that use this test, or a similar one with Chinese hamster ovary (CHO) cells, have recognized that the spontaneous background for HGPRT is quite variable (1–12 mutants/million cells) and increases of at least 3- to 5-fold are required in duplicate tests to confirm an effect. New methods using larger sample sizes have apparently solved the problem and could be used to resolve the equivocal results.

An independent assay with aloe-emodin was conducted at the same locus of Chinese hamster V79 cells [Heidemann et al., 1993]. The study conducted used a design similar to that used by Westendorf's group [1990], employing aloe-emodin concentrations up to 350 µg/ml (the limit of solubility) without producing any mutagenic response (Table IV). This study supports the suggestion that the data of Westendorf and coworkers [1990] were overinterpreted as positive.

A test for chromosome aberrations in CHO cells was reported by Heidemann and associates [1993]. Aloe-emo-

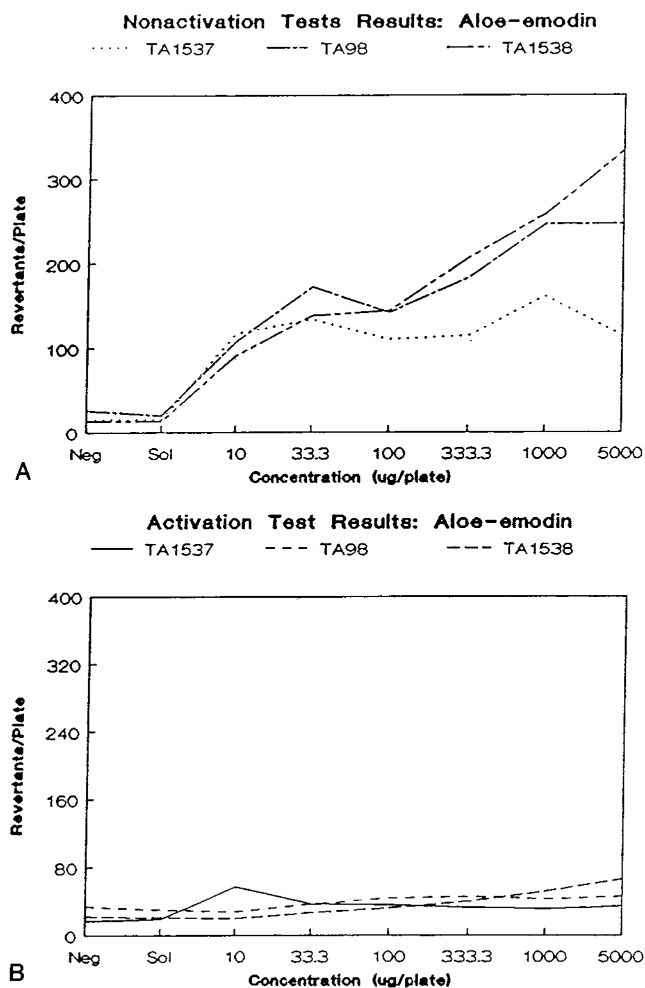

Figure 2. The Results of Aloe-emodin in the Ames Test

din was tested both with and without S9 mix at concentrations ranging from 18.75 μg/ml to 75 μg/ml. All concentrations showed significant increases in cells with aberrations under both treatment conditions. Nonactivation treatment conditions produced the strongest evidence for clastogenicity. In a situation similar to the Ames test, addition of S9 mix reduced the clastogenic effects of aloe-emodin. Toxicity did not appear to play a role in aberration induction, and the results showed definitive evidence of clastogenicity.

In the in vitro UDS assay conducted by Westendorf and colleagues [1990], aloe-emodin was associated with a significant increase in net grains/nucleus. Two trials were reported with concentration ranges from 6.3 μg/ml to 100 μg/ml. At a concentration of 25 μg/ml the net grains per nucleus reached the criteria needed to classify the response positive. This class of chemicals may interact with topoisomerase enzymes in vitro [Westendorf et al., 1988] confounding the evaluation. Consequently, an in vivo study might be a more relevant assessment of the DNA damaging activity of aloe-emodin. An in vivo/in vitro test for UDS was conducted in rats exposed to aloe-emodin (p.o.) at 0.1 g and/or 1 g/kg with 4- and 16-hour sample times [Heidemann et al., 1993]. Three animals were treated per group and evaluated individually and as part of the dose group. No increase in mean net grain counts/nucleus was observed at either dose or treatment time. These results suggest that whatever mechanism might have been responsible for the increased UDS found in isolated hepatocytes treated in vitro it was not expressed after an oral dose of 1 g/kg body weight.

The final assay cited by Westendorf and colleagues [1990], as evidenced for a carcinogenic potential of aloe-emodin, was the morphological transformation of C3H/M2 mouse fibroblast cells. The data were interpreted as demonstrating a compound-induced increase in transformants at the low concentration of 3 μg/ml. Data are shown in Table V.

The reported study does not provide a definitive assessment, as the data shown in Table V are the average of two independent experiments. To make an accurate evaluation of this assay, each experiment must be reported separately to see trial to trial variability. The results, as reported, cannot be evaluated reliably. The ''tumor promotor'' studies [Wölfle et al., 1990] are also of limited value without a reasonable mechanism explaining the effect. This type of assay should be accompanied with data documenting that it is capable of detecting promoting activity with model chemicals.

Three additional in vivo studies evaluating aloe-emodin were reported by Heidemann and associates [1993]. The first was a micronucleus test conducted in mouse bone marrow cells at a concentration of 1.5 g/kg (p.o.). The results of this study showed no evidence of increased micronuclei at any of the sample periods.

A second in vivo assay measuring mouse somatic mutation in fetal melanoblasts was conducted. The test is commonly identified as the Mouse Spot Test and presumably detects single gene mutations in mouse embryonic epithelial cells. NMRI female mice (nonagouti a/a; albino c/c) were mated to DBA males (nonagouti a/a; brown b/b; dilute d/d) resulting in embryos heterozygous for three coat color genes (b/+; c/+; d/+). Pregnant females were exposed to aloe-emodin on day 9 of gestation at 0.2 and 2 g/kg body weight. Mutations induced at any of the three wild-type alleles would result in a coat color spot(s) on one of the embryos following birth. The results of the study showed no evidence for mutation induction in the treatment groups. Ethyl nitrosourea (positive control) induced a significant increase in somatic cell mutation.

The final in vivo study reviewed by Heidemann and colleagues [1993] was a cytogenetic analysis of rat bone marrow cells. Concentrations of aloe-emodin, administered orally to the rats, included 0.2, 0.666, and 2.0 g/kg body weight. Cells were harvested for evaluation at 6, 24, and 48 hours after exposure. The results showed no evidence of compound-induced clastogenicity.

TABLE III. Results of Aloe-emodin in the V79-HGPRT Mutagenicity Assay*

Concentration (μg/ml)	Plating efficiency (%)		8-Azaguanine-resistant mutants/10^5 survivors	
	Exp 1	Exp 2	Exp 1	Exp 2
0	41	55	8	18
5	36	52	16	16
10	36	50	27	24
20	32	54	23	33
30	13	24	13	21
MNNG 1	6	26	460	547

From Westendorf et al. [1990].
*Data for actual mutants recovered per dose level were not given.

TABLE IV. Results of Aloe-emodin in the V79-HGPRT Mutagenicity Assay

Concentration/ml	S9 mix	Cell density % of control[a]		Mutant frequency/10^6 cells[a]	
		Trial 1	Trial 2	Trial 1	Trial 2
Control					
0.0	–	100	100	28.1	28.3
Solvent					
0.0	–	100	100	29.8	24.5
5 μg	–	93.2	(–)	(–)	(–)
10 μg	–	98.2	91.6	(–)	(–)
35 μg	–	102.6	(–)	(–)	(–)
100 μg	–	101.1	100.7	19.6	31.5
200 μg	–	96.1	83.0	15.8	46.2
350 μg	–	95.2	78.9	24.2	26.2
EMS					
0.6 mg	–	70.9	90.1	449.9	485.7
Control					
0.0	+	0.0	100	21.4	27.4
Solvent					
0.0	+	0.0	100	23.9	39.1
5 μg	+	74.7	(–)	(–)	(–)
10 μg	+	85.7	143.0	31.5	22.2
35 μg	+	82.8	(–)	(–)	(–)
100 μg	+	99.2	115.6	42.8	73.3
200 μg	+	66.4	89.5	32.8	34.7
350 μg	+	45.1	39.8	10.2	3.6
DMBA					
3.85 μg	+	30.0	61.7	901.9	1020.7

Unpublished data provided by Madaus AG.
[a](–), cultures not plated.

The in vivo tests are all reliable indicators of mutation, DNA damage, or clastogenicity. The negative result in each test were found at high acute exposure conditions with minimal evidence for toxicity at the target cell site.

As summarized in Table VI, aloe-emodin can only be definitively shown to be mutagenic in the Ames test and to be clastogenic in vitro. Effects in both assays were suppressed by S9 mix suggesting metabolic inactivation. This phenomenon could probably be explained by the findings of Lang [1993], who reported that aloe-emodin can be metabolized by the liver to rhein, a compound that is not mutagenic. In vivo studies with aloe-emodin were uniformly negative.

ANALYSIS OF THE DATA

Senna Extracts and Sennosides

The anthranoid glycosides recovered from senna extracts appear to be free of mutagenic activity in the conventional Ames test. A study conducted with the anthranoid glycosides in strain TA102 did not achieve a twofold increase in revertants at the maximum concentration tested; however, this response was interpreted by the investigators as positive. Base-pair substitution mutation is not consistent with the presumed mutagenic mechanism produced by anthraquinone molecules. Strain TA102 tends to detect reactive agents produced by processes that

TABLE V. Results of Aloe-emodin in the C3H/M2 Cell Transformation Assay*

Concentration (μg/ml)	Plating efficiency (%)	Transformants/dishes treated (ratio)
Control		
0	25	0/9 (0.0)
1	25	0/7 (0.0)
3	34	6/4 (1.5)
5	19	1/8 (0.1)
10	10	13/16 (0.8)
20	1	3/8 (0.4)
30	0	— (—)
MNNG		
0.5	16	14/12 (1.2)
1	8	17/10 (1.7)

From Westendorf et al. [1990].
*The results shown are the average of two independent experiments.

occur in oxidative metabolism (i.e., production of peroxides, oxygen radicals, aldehydes, and ketones), which are not thought to be associated with metabolism of senna glycosides. Therefore, it will be important to extend this observation. Support for the presence of a base-pair substitution mechanism of mutation was not obtained for the glycosides in any of the other tests conducted. The *E. coli* reverse mutation assay in strain WP2, which has been reported to pick up many of the same agents as TA102, did not respond to the glycosides.

Although senna glycosides may not be genotoxic, senna extracts may contain mutagenic and clastogenic compounds. Extracts were shown to produce mutation in several Ames tester strains including TA97, TA98, and possibly TA100. Chemical analysis of the extracts demonstrate the presence of variable quantities of Ames mutagens including aloe-emodin, emodin, quercetin, and kaempferol. It is possible that the mutagenicity reported for the extracts is due to one or more of these molecules. Clastogenic effects from senna extracts were observed in cultured CHO cells. At concentrations between 4 and 5 mg/ml, significant increases in chromosome aberrations were observed in cells treated directly with senna extracts. The addition of S9 mix to the treatment milieu almost entirely eliminated the clastogenicity, suggesting the presence of a relatively reactive molecular species. In mice and rats crude senna drug does not produce somatic mutations or chromosome damage at doses up to 1.5 g/kg body weight.

Based on the available data, senna glycosides per se do not appear to pose a genetic risk to mammalian somatic or germ cell DNA. While it is possible to produce disruption of mammalian chromosomes or DNA by very high dose levels, absorption and distribution of orally administered doses is low, preventing systemic concentrations from approaching those that might have shown a weak effect in a cultured mammalian cell assay. In vitro assays probably represent an overestimate of the possible hazard from exposure.

Aloe-emodin

Aloe-emodin is mutagenic in the Ames test at levels of 10 μg/plate and induces chromosome breakage, in vitro, in CHO cells at 18.75 μg/ml. Other in vitro studies conducted with aloe-emodin must be viewed as equivocal at best, due to technical problems and study design issues (Table VI).

Extrapolation of Genetic Potential

Concerns

Based on the in vitro data, a concern of potential human hazard can be raised with implications for cancer and genetic disease. In order to address this concern, one must assess two additional sets of data. The first consists of metabolism, kinetics, and tissue distribution data from animal and human studies, and the second set of data is the result of genetic studies conducted in animal models.

Experimental Animals

Blood levels of aloe-emodin were measured in mice and rats given the senna drug (Table II). The blood levels of aloe-emodin reported at 3 hours after a 1 g/kg dose orally were very low, averaging about 0.020 μg/ml for rats and 0.012 μg/ml for mice.

Using the blood level values of 0.012 μg/ml for mice and 0.02 μg/ml for rats, one can estimate the liklihood of observing a somatic cell genetic response in vivo from the in vitro lowest effective doses identified in Table VI. Assumptions required for these estimates are based on direct extrapolation of biological effects of the genotoxin in vitro per μg/ml of media to biological effects of the genotoxin in vivo per μg/ml of blood. The results in Table VII suggest that one would not expect responses of senna in vivo (Table II) under the treatment conditions reported.

As shown in Table VI, aloe-emodin gave clearly negative results in four different in vivo assays, although systemic blood concentrations in the animals reached levels in the range of genetically active concentrations in vitro.

One area of animal testing that has not been addressed using genetic screens is the potential for effects in the intestinal tract where (1) the concentrations will be highest and (2) gut flora are able to metabolize some of the molecules to active intermediates [Brown and Dietrich, 1979]. A recent cancer study in rats [Lydén-Sokolowski et al., 1993], using a standardized senna-glycoside extract which contained potential aloe-emodin (sum of free and chemically bound aloe-emodin) as a contaminant (2.29%), showed no evidence of induced tumors in any of the tissues examined.

TABLE VI. Summary of the Genotoxic Profile for Aloe-emodin

Assay (citation)	Maximum conc. tested	Reported response	Estimated max. blood levels for in vivo studies[a]
In vitro			
Ames [Brown and Dietrich, 1979]	100 µg/plate	Pos. in TA1537	NA
Ames [Westendorf, et al., 1990]	1000 µg/plate	Pos. between 1 and 100 µg/plate; activity was found in TA1537, TA1538, TA98	NA
Ames [Heidemann et al., 1993]	5000 µg/plate	Pos. between 1 and 10 µg/plate	NA
V79-HGPRT [Westendorf et al., 1990]	30 µg/ml	Pos. at 10 µg/ml	NA
V79-HGPRT [Heidemann et al., 1993]	350 µg/ml	Neg.	NA
Rat hepatocyte UDS [Westendorf et al., 1990]	100 µg/ml	Pos. at 25 µg/ml	NA
Cell transformation C3H/M2 Cells [Westendorf et al., 1990]	30 µg/ml	Pos. at 3 µg/ml	NA
CHO aberration [Heidemann et al., 1993]	75 µg/ml	Pos. at 18.75 µg/ml	NA
In vivo			
Rat hepatocyte UDS [Heidemann et al., 1993]	1000 mg/kg	Neg. (4- & 16-hr sample times)	NDA
Mouse micronucleus [Heidemann et al., 1993]	1500 mg/kg	Neg. (24-, 48-, & 72-h sample times)	17 µg/ml
Rat bone marrow metaphase [Heidemann et al., 1993]	2000 mg/kg	Neg. (6-, 24-, & 48-h sample times)	13 µg/ml
Mouse somatic cell test for mutation [Heidemann et al., 1993]	2000 mg/kg	Negative	10 µg/ml

[a]Data supplied by Madaus AG. NA, not applicable; NDA, no data available.

TABLE VII. Response Extrapolation Estimates

Genetic effect	In vitro reference (LED)[a]	Fold difference rat[b]	mouse[b]
Mutation	Ames (10 µg/plate)	500	800
	V79-HGPRT (10 µg/ml)	500	800
Aberrations	CHO (18.75 µg/ml)	900	1500
DNA repair	Rat hepatocyte (25 µg/ml)	1250	2000
Cell transformation	C3H/M2 cells (3 µg/ml)	150	250

[a]LED, lowest effective dose of aloe-emodin.

[b] $\frac{\text{LED (µg/ml)}}{\text{Blood level (µg/ml)}}$.

Humans

Human epidemiology data for anthraquinones attempting to demonstrate an association between use of anthraquinone laxatives and colorectal cancer have not demonstrated a linkage [Kune, 1993; Nusko et al., 1993; Loew et al., 1994].

Therapeutic doses of senna laxative products were administered to 10 healthy subjects [Krumbiegel and Schulz, 1993]. Aloe-emodin was not detected in any plasma samples (lower limit of quantification was 0.5 ng/ml). Using the same assumptions for determining effects in rodents would result in human safety margins for somatic genotoxicity in the range of 20,000.

SUMMARY AND CONCLUSIONS

Clearly experimental data for rodent and human metabolism and mutation studies summarized in this report do not indicate that oral consumption of senna laxatives poses systemic risks to somatic cells of humans. Response extrapolation estimates summarized in the previous section lend support to this conclusion.

Human clinical and epidemiological studies do not clearly suggest that use of anthraquinone laxatives represent a risk for colorectal cancer [Kune, 1993; Nusko et al., 1993; Siegers, 1993: Loew et al., 1994]. This is also supported by a 2-year carcinogenicity study with a senna extract given to rats that showed no evidence for tumorigenicity in the intestinal tract [Lydén-Sokolowski et al., 1993].

There is no evidence from studies in animals or from human use to consider that senna laxative preparations containing low levels of aloe-emodin or emodin pose any mutagenic or cancer risk to humans under normal use conditions. Data from the Ames test and from a mammalian cell in vitro test of chromosome breakage indicate that some anthraquinones are genotoxic, but these effects need to be assessed with the perspective of the animal responses. An initial attempt at this perspective suggest margins of safety from genotoxic effects are sufficient

to minimize concern for health effects in this area of toxicology.

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