COMPARISON OF TWO ENTERIC-COATED ACETYLSALICYLIC ACID PREPARATIONS BY MONITORING STEADY-STATE LEVELS OF SALICYLIC ACID AND ITS METABOLITES IN PLASMA AND URINE

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ABSTRACT

In a randomized three-way crossover study, 12 healthy male volunteers were given multiple oral doses, i.e. 1.5 g b.i.d. for 7 days, of two different types of enteric-coated acetylsalicylic acid (ASA) preparations, one being a conventional enteric-coated tablet (ET) and the other enteric-coated granules (EG) in a capsule; conventional ASA tablets were used as a reference. Plasma levels and excretion of salicylic acid and some of its metabolites were investigated under steady-state conditions.

Plasma salicylic acid (SA) and salicyluric acid (SUA) levels were determined using a liquid chromatographic method. Two separate analyses were done to quantitate the metabolites in urine. SA, SUA, and gentisic acid were each assayed by the method used for plasma. Total salicylate was also determined.

There was no significant difference in urinary excretion of total salicylate between the three formulations. A diurnal variation in the excretion of SUA and SA in urine was found. The two enteric-coated formulations provided significantly higher morning plasma concentrations than the conventional aspirin. The AUC was found to be significantly higher for ET than for the other two formulations. EG gave more uniform plasma levels during the studied 12-h intervals and also less inter- and intra-individual variations than ET, indicating that a b.i.d. regimen may be suitable for EG.

KEY WORDS Salicylic acid Absorption Excretion Enteric-coated tablets ASA formulations Diurnal variation

INTRODUCTION

Acetylsalicylic acid (ASA) is still the drug of choice in the treatment of rheumatic and degenerative joint diseases. The therapeutic effect is correlated to plasma concentrations of total salicylate of 1.1–2.2 mmol l⁻¹. Furthermore, a high morning plasma level seems to shorten morning stiffness, but the incidence of side-effects, especially tinnitus and gastrointestinal complaints, also increases with high plasma levels. Careful monitoring of the dose and a reproducible
absorption are necessary to obtain optimal therapeutic results since salicylic acid (SA) has saturable and dose-dependent kinetics.\textsuperscript{4,5}

The choice of ASA formulation is important in drug therapy since the absorption of drug from different kinds of formulations may vary considerably.\textsuperscript{6} Enteric-coated ASA tablets have for instance been shown to be absorbed erratically, especially when given with food.\textsuperscript{7-9} A recently developed acetylsalicylic acid formulation consisting of a large number of enteric-coated granules was found in single-dose studies to interact considerably less with food than enteric-coated tablets.\textsuperscript{8,9}

The aim of the present study was to investigate the influence of different dosage forms on the absorption and excretion of ASA under steady-state conditions by monitoring SA and some of its metabolites in plasma and urine.

MATERIALS AND METHODS

Study design

Twelve healthy male subjects, aged 23–32 (\(\bar{x} = 25.6\)) years, took part in the study after giving written consent. Their weights were between 66 and 97 (\(\bar{x} = 80.3\)) kg and their heights between 171 and 196 (\(\bar{x} = 183.0\)) cm. Each subject took all three test formulations in a three-way crossover trial. The subjects were assigned to the drugs in a randomized order. The drugs, 1.5 g b.i.d. (8 am and 8 pm) for 7 days, were given together with 100 ml of water at room temperature (18–22°C). There were at least 4 days between the standardized recording days, 5, 6, and 7 (see Figure 1). Three consecutive morning plasma samples were drawn on days 5, 6, and 7. Plasma samples were also drawn every second hour between 8 am and 8 pm during a single dosage interval on days 6 and 7. Meals and fluid intake were standardized in time and amount from the evening meal on day 5 until the evening meal on day 7.

Drugs

Conventional acetylsalicylic acid tablets (CA) (Aspirin\textsuperscript{®}—Bayer) were used as reference. Enteric-coated ASA tablets (ET) (Premaspin—Lääke) were obtained from a pharmacy in Sweden and enteric-coated ASA granules (EG) (Reumyl\textsuperscript{®}) from the Department of Pharmaceutics, Hässle. All three formulations were declared to contain 0.5 g ASA.

Plasma samples

An intravenous cannula was inserted into a forearm vein and venous blood, 5 ml, was drawn into a tube with heparin. After at least 1 h at room temperature, the samples were centrifuged. The separated plasma was stored at \(-20^\circ\) until analysed.

Plasma analyses

Salicylic acid and salicyluric acid (SUA) in plasma were determined using a liquid chromatographic method,\textsuperscript{10} which was modified slightly. Proteins were
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precipitated from plasma by addition of an equal volume of acetonitrile; 20 µl of
the supernatant was injected onto a reverse-phase LC column and the
compounds were detected by UV detection.

The liquid chromatograph consisted of an Altex LC pump (Berkeley,
California, U.S.A.; 110A), a Rheodyne injection valve (Berkeley, California; 70–
10) with a 20-µl loop, a Brownlee (Santa Clara, California) precolumn and
separation column packed with LiChrosorb RP 8, 10 µm (30 × 4.5 mm and
100 × 4.5 mm, respectively), and an LDC Spectromonitor III (Riviera Beach,
Florida, U.S.A.) LC detector operated at 314 nm. The mobile phase consisted of
glacial acetic acid, acetonitrile, and water, 4:12:84 (by volume), and was
pumped with a flow rate of 2.4 ml min⁻¹. The minimum determinable concen-
tration (relative standard deviation ≤ 10 per cent) for salicylic acid was
30 µmol l⁻¹ and for salicylic and salicyluric acid 10 µmol l⁻¹.

**Urine collection**

Urine was collected in 12-h cartons from the evening dose on day 5 until the
evening dose on day 7 (see Figure 1). Each subject was instructed to empty his
bladder before changing or ending the program. It was also clearly understood
that all urine was to be collected. From every carton a 10 ml sample was drawn
after stirring and kept frozen (−20 °C) until analysis.

![Figure 1. Time chart for one period in a three-way crossover study](image)

**Urine analyses**

Two different assays were performed. Firstly, a simultaneous analysis of
salicylic, salicyluric, and gentisic acid (GA) was made by direct injection of
centrifuged urine sample onto a reverse-phase LC column using fluorescence
detection. The minimum determinable concentration was about 10 µmol l⁻¹
(of urine). A chromatogram showing the separation of the three acids in a urine
sample is shown in Figure 2. Secondly, the total concentration of salicylates
(including various conjugates) was assayed by treatment of the urine in an
ampoule with an equal volume of concentrated hydrochloric acid for 2 h at 120 °
in an autoclave. One part of the hydrolysed urine was then diluted with 10 parts of NaOH (0·6 mol l$^{-1}$) in phosphate buffer pH 7 (I = 0·10). Twenty millilitres of the solution was injected and the content of salicylic acid measured. The amount of glucuronides (GLU) was then calculated

$$(\text{total salicylate} = \text{SUA} + \text{SA} + \text{GLU}).$$

The liquid chromatographic system was the same as that used for the plasma analyses, except that the detection was performed by a Perkin-Elmer LC1000 fluorescence detector (Norwalk, Connecticut, U.S.A.) operated at 315/420 nm. The mobile phase was an aqueous solution containing tetrabutylammonium (0·01 mol l$^{-1}$), phosphate buffer, pH 7 (I = 0·05), and 22 per cent of acetonitrile. The flow rate was 1 ml min$^{-1}$.

Adverse reactions

Subjects were asked to fill in a special form if they noted any unusual reactions. They were also asked at the end of every period about the incidence of any adverse reaction.

Statistics

Friedmann's analysis of variance was used when drugs/periods/days were compared. In a direct comparison, Wilcoxon's sum of ranks test was used.$^{12}$
RESULTS

Plasma levels

Individual plasma levels of total salicylate calculated as the sum of the salicylic acid (SA) and salicyluric acid (SUA) are given in Figure 3.

The plasma levels varied greatly during the day, the variation being considerably more pronounced for tablets than for granules. The differences between \( C_{\text{pmax}} \) and \( C_{\text{pmin}} \) were significantly greater for both CA and ET in comparison with EG \((2p<0.01)\). See Table 1.

Morning plasma levels of total salicylate (= SA + SUA) were found to be significantly higher with enteric-coated formulations than with conventional Aspirin\(^\circledR\) \((2p<0.01)\). See Table 1.

The AUC, calculated by the trapezoidal rule, for both day 6 and day 7 is significantly greater for the enteric-coated tablets than for the enteric-coated granules and the conventional acetylsalicylic acid formulations \((2p<0.01)\) (Table 1).

The difference in AUC\(_{0-12h}\) values between two consecutive study days was significant for each of the three formulations. ET gave a significantly greater difference between days than both CA \((2p<0.01)\) and EG \((2p<0.05)\). The variance between individuals was also significantly higher \((2p<0.05)\) for ET than the other two formulations indicating a greater variability between individuals in the absorption from ET than CA and EG. CA and EG did not differ significantly from each other.

Recovery in urine

Neither the total recovery of SA and its metabolites in urine for 48 h nor the volume of urine differed significantly between the studied formulations. The excretion of total salicylates at night was significantly lower than during the day \((2p<0.01\) for CA and \(2p<0.001\) for both enteric-coated formulations); the difference between day and night excretion was less for conventional Aspirin\(^\circledR\) than for the enteric-coated formulations (see Table 2 for details).

When the AUC\(_{0-12h}\) was divided by the ratio of the day and night excretion of total salicylate there was no statistical difference between the three formulations, although there was a statistical difference between the two consecutive days for CA and ET \((2p<0.05)\).

There was a diurnal variation in urine volume as well as urine pH, the daytime values being significantly higher than night-time values for all three formulations \((2p<0.001)\). The excretion of salicyluric acid (per cent of total) was less during the day than at night \((2p<0.01)\) but there was no significant difference between formulations in this respect. Salicylic acid (per cent of total) was less at night-time than during the day \((2p<0.001)\) for all formulations. There was no diurnal variation in the excretion of glucuronides for enteric-coated formulations. For conventional aspirin, however, glucuronide excretion in urine during the day was less than during the night.
Figure 3. Individual plasma concentrations of total salicylate, as the sum of salicylic (SA) and salicyluric (SUA) acid, over two consecutive days (day 6—- and day 7—) after administration of three different (ASA) preparations.
Table 1. Steady-state plasma concentration data (days 6 and 7) from 12 subjects given 1.5 g ASA b.i.d. Mean ± S.D. of morning plasma salicylate ( = SA + SUA) (Cₚ), area under the plasma concentration curve (AUC), the difference between Cₚmax and Cₚmin, and the time to reach Cₚmax (Tₘₐₓ).

<table>
<thead>
<tr>
<th></th>
<th>CA</th>
<th>ET</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₚ morning</td>
<td>mean 263.4</td>
<td>568.2</td>
<td>480.0</td>
</tr>
<tr>
<td>(μmol l⁻¹)</td>
<td>± S.D. 172.5</td>
<td>259.3</td>
<td>138.6</td>
</tr>
<tr>
<td>AUC₀₋₁₂</td>
<td>mean 5510.8</td>
<td>7136.3</td>
<td>5718.7</td>
</tr>
<tr>
<td>(μmol l⁻¹ h)</td>
<td>± S.D. 1982.5</td>
<td>2272.7</td>
<td>1444.1</td>
</tr>
<tr>
<td>Cₚmax − Cₚmin</td>
<td>mean 526.9</td>
<td>499.2</td>
<td>230.6</td>
</tr>
<tr>
<td>(μmol l⁻¹)</td>
<td>± S.D. 85.9</td>
<td>163.9</td>
<td>69.4</td>
</tr>
<tr>
<td>Tₘₐₓ (h)</td>
<td>mean 2.5</td>
<td>5.2</td>
<td>8.1</td>
</tr>
<tr>
<td>(± S.D.)</td>
<td>0.9</td>
<td>3.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table 2. Mean ± S.D. of urine volume and pH, total salicylate, and its metabolites in night-time and daytime urine, from 12 subjects given 1.5 g ASA b.i.d. for at least 5 days.

<table>
<thead>
<tr>
<th></th>
<th>CA</th>
<th>ET</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Night</td>
<td>Day</td>
<td>Night</td>
</tr>
<tr>
<td>Urine volume (l)</td>
<td>0.6</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Urine pH</td>
<td>5.7</td>
<td>6.6</td>
<td>5.8</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.2</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Total salicylate</td>
<td>89.9</td>
<td>101.3</td>
<td>72.8</td>
</tr>
<tr>
<td>S.D. (% of given dose)</td>
<td>24.2</td>
<td>12.8</td>
<td>24.6</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>74.6</td>
<td>71.4</td>
<td>75.4</td>
</tr>
<tr>
<td>S.D. (% of total)</td>
<td>10.9</td>
<td>5.1</td>
<td>7.1</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>4.8</td>
<td>13.4</td>
<td>4.0</td>
</tr>
<tr>
<td>S.D. (% of total)</td>
<td>2.0</td>
<td>7.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Glucuronides</td>
<td>19.7</td>
<td>14.0</td>
<td>19.1</td>
</tr>
<tr>
<td>S.D. (% of total)</td>
<td>10.6</td>
<td>8.8</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Adverse reactions
Gastrointestinal complaints, namely diarrhoea, constipation, and stomachache, were reported for all three formulations. No difference was observed between the formulations, the number of subjects being too small. A small number of subjects also reported tinnitus.

DISCUSSION
The present investigation was designed to compare different dosage forms of ASA with regard to inter- and intra-individual variations in plasma salicylate...
levels during two consecutive days of a steady-state treatment period and with respect to the total urinary excretion of salicylate and its metabolites during day and night.

Enteric-coated tablets have been shown to have an unpredictable rate of absorption even at multiple doses. However, it has been demonstrated that a dosage form consisting of a large number of enteric-coated granules, after a single dose, has more reproducible absorption than ET, which also is affected by simultaneous intake of food. The present study confirms these data and shows that the EG formulation gives a more uniform plasma level during the studied 12-h time intervals as well as less inter- and intra-individual variations than ET. Thus the EG preparation seems to be able to give a reproducible and satisfactory control of the plasma salicylate levels when administered b.i.d.

Diurnal variation in the excretion of salicylate and its metabolites was evident regardless of which of the three formulations had been administered. However, in contrast to earlier studies by Reinberg et al., the excretion of salicylate during the day was less than during the night. The relative amount of the given dose excreted as SUA and SA varies also between day and night for all three preparations. For CA even a diurnal difference in the excretion of glucuronides was found. A greater diurnal variation in the amount of SUA and SA is obtained with ET compared to CA and EG. ET also gives a higher AUC \(0-12\) value than the other dosage forms, but this effect seems to be compensated for by a low urinary excretion of total salicylates during the night. If the AUC values are divided by the ratio of the day/night excretion values, there is no significant difference between the dosage forms. Willis et al. showed that, in combination with food, resting in a supine position caused a delayed absorption from enteric-coated tablets, even after multiple doses. During the present study, the subjects were allowed to walk about freely in the laboratory during the plasma sample collection period. However, the second dose of the day was taken after a light, standardized meal, shortly before bedtime, when there is probably a decrease in gastrointestinal emptying. It therefore seems reasonable to speculate that the higher sensitivity of ET to variations in the rate of gastric emptying may explain the observed difference in the steady-state plasma levels of the studied dosage.

The difference in the diurnal variation of excreted SUA and SA may be considered as a logical result of the dose-dependent metabolism of SA, since different amounts of the drug were absorbed during day and night. A relatively high amount of total salicylate seems for instance to be correlated with a relatively higher amount of SA.

**REFERENCES**