METABOLISM OF SALICYLATE DURING CHRONIC ASPIRIN THERAPY

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1. The effects of chronic administration of aspirin in therapeutic doses (3.9 g/day) on plasma and salivary salicylate levels were studied in eight subjects.

2. The urinary excretion profile for free salicylic acid and metabolites of salicylate were examined.

3. Plasma and salivary salicylate levels declined significantly after peak levels were achieved between days 3 and 10.

4. The decline in plasma and salivary salicylate levels may be due to an induction of a metabolic pathway such as salicylate formation (Furst, Gupta & Paulus, 1977). Only the mean fraction of salicylate excreted as salicylate appears to increase with time during the present study, although the change was not significant statistically.

5. The decline in plasma and salivary salicylate levels during chronic therapy may lead to an apparent 'tolerance' of some rheumatoid patients to aspirin.

Introduction

Rheumatoid arthritis is generally treated by the chronic administration of non-steroidal anti-inflammatory agents. Of these agents, aspirin is still considered by most rheumatologists to be the drug of first choice (Brooks, Roberts & Patel, 1978; Hart, Huskisson & Ansell, 1978). In the chronic administration of aspirin, maintenance of steady state plasma salicylate levels above 150 μg/ml (1.1 mmol/l) is considered necessary to achieve a therapeutic response (Graham, Champion, Day & Paul, 1977). The therapeutic range is generally considered to be 150 to 300 μg/ml (1.1 to 2.2 mmol/l) (Gibaldi, 1977; Hart et al., 1978) and above this range toxicity occurs, the optimum plasma levels being only a little below toxic levels (Levy, Tsuchiya & Ansell, 1972).

Few studies have examined steady state plasma salicylate levels during chronic administration of aspirin over a period of weeks. In a study of subjects ingesting soluble aspirin, 60 mg kg⁻¹ day⁻¹ in divided doses for 21 days, Müller, Hundt & de Kock (1975) showed that there was a steady decrease in plasma salicylate levels after peak levels had been achieved and that on day 21 plasma salicylate levels were 48% on average, of peak plasma salicylate levels obtained between days 3 and 7. This suggests that a person whose peak plasma salicylate level was below 300 μg/ml (2.2 mmol/l) could have a plasma level below that required for a therapeutic response by day 21. If the decline continued after 21 days then it is possible that most patients, while ingesting large doses of aspirin, would be achieving subtherapeutic levels after a time of chronic treatment. This decline in steady state levels of salicylate may further compound the difficulties already experienced in attempts to predict steady state salicylate levels after multiple dosing from single dose kinetics (Levy & Tsuchiya, 1972).

In the study of Müller et al. (1975), plasma salicylate levels alone were monitored over 21 days. In the present study aspirin was given for 36 days and the plasma and salivary levels of salicylic acid were monitored. In addition the metabolic profile of salicylate in the urine during this chronic administration was examined.

Method

In the present study, aspirin was administered in a dose and form frequently used clinically in the treatment of rheumatoid arthritis. Nine subjects ingested 3.9 g/day of aspirin in a slow-release formulation (SRA, Boots) in two equal doses (at 08.00 h and 20.00 h). This dosage regime was maintained for 36 days. All subjects were healthy male volunteers aged 21 years and all had normal liver function tests, urea and electrolytes. One subject

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withdrawn due to tinnitus. During the study all subjects followed their normal daily activities and no restriction was placed on their diet or fluid intake.

Blood and mixed saliva samples were collected at midday on designated days. The saliva samples were collected by the method of Graham & Rowland (1972). Blood samples were centrifuged and the plasma was drawn off. Twenty-four hour urine collections were made at weekly intervals starting on day 8, the volumes were measured and an aliquot retained. All samples were frozen until assayed.

Salicylate in the plasma and in the saliva was determined by the method of Rowland & Reigelman (1967) and Graham & Rowland (1972) respectively. Total urinary salicylate was determined by the method of Page, Anderson, Brown & Roberts (1974) and urinary free salicylic acid, salicyluric acid and salicylguanides were determined by the method of Levy & Procknow (1968). Urinary guanidine was determined by a gas-liquid chromatographic technique using flame ionization detection. In this method 1 ml of plasma was acidified to pH 1–2 with 5% w/v potassium bisulphite solution and p-toluic acid was added as an internal standard. The acidified plasma was extracted with ether, the ether layer removed and dried over anhydrous sodium sulphate. The ether extract was then evaporated to dryness and the residue was heated with 30 µl BSTFA at 60°C for 1 h. An aliquot (3 to 5 µl) was injected onto a 3% OV1 on Chromosorb Q (100–200 mesh) column. The operating conditions were as follows: injector and detector oven 250°C; column temperature prograded from 170°C to 230°C at 12°C/min; flow rate of carrier gas (nitrogen), 60 ml/min.

Results

The mean ± s.e. mean plasma salicylate levels as shown in Figure 1. The mean plasma salicylate concentration reached plateau levels within 4 days of commencing chronic aspirin administration and remained relatively constant until day 10. Between the plateau level and day 36 the mean plasma salicylate declined by 25%. Statistical analysis of the data by analysis of variance and the Neuman-Keuls test showed that there is a significant difference between the mean level achieved on day 36 and those achieved on days 3 to 10 inclusive and between the mean level achieved on day 29 and those on days 1 and 10 (P < 0.05). The large standard errors shown in Figure 1 result primarily from individual differences. Table 1 shows the individual plasma salicylate levels at days 8 and 36 and it is observed for all subjects in

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day 8 µg/ml (mmol/l)</th>
<th>Day 36 µg/ml (mmol/l)</th>
<th>Difference µg/ml (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>201 (1.46)</td>
<td>164 (1.19)</td>
<td>37 (0.27)</td>
</tr>
<tr>
<td>2</td>
<td>161 (1.17)</td>
<td>121 (0.88)</td>
<td>40 (0.29)</td>
</tr>
<tr>
<td>3</td>
<td>287 (1.65)</td>
<td>208 (1.51)</td>
<td>19 (0.14)</td>
</tr>
<tr>
<td>4</td>
<td>212 (1.59)</td>
<td>161 (1.17)</td>
<td>50 (0.32)</td>
</tr>
<tr>
<td>5</td>
<td>162 (1.17)</td>
<td>78 (0.57)</td>
<td>44 (0.26)</td>
</tr>
<tr>
<td>6</td>
<td>173 (1.25)</td>
<td>112 (0.81)</td>
<td>61 (0.44)</td>
</tr>
<tr>
<td>7</td>
<td>105 (0.76)</td>
<td>45 (0.33)</td>
<td>60 (0.43)</td>
</tr>
<tr>
<td>8</td>
<td>162 (1.17)</td>
<td>140 (1.01)</td>
<td>22 (0.16)</td>
</tr>
</tbody>
</table>
levels declined. Mean salivary salicylate levels also declined during chronic aspirin administration. The extent of the decline is greater than that observed for the plasma data and resulted in a decrease in the salivary/plasma ratio for salicylate (Figure 2). Statistical analysis (by analysis of variance and Newman-Kuels test) showed the mean level on day 36 differed significantly from mean levels on day 8 and 15 ($P < 0.05$). The decline in salivary-plasma ratio was also found to be significant.

The total amount of salicylate excreted in the urine (as free salicylic acid plus metabolites) did not change significantly over the period of the study (Table 2). This suggests that the fraction absorbed remained constant during the study and subject compliance was good. The mean percentage of the daily dose recovered in a 24 hour urine specimen was only about 70%. The urinary pH of individuals did not alter significantly during the study. There was no significant change in creatinine clearance over the course of the study (Table 2) indicating that renal function had not altered.

Figure 3 shows the fraction of total urinary salicylate excreted as free salicylic acid and individual metabolites. The fraction of salicylate excreted as salicylic acid (SU) appears to progressively increase during the 5 weeks of the study and the fraction of salicylate excreted as glucuronides (SO) appears to decline. The mean fraction of free salicylic acid was found not to change during the course of the study to any noticeable extent. No change in the gentisic acid fraction, with time, is apparent. Statistical analysis of the changes in fractions of individual metabolites and free salicylic acid with time shows no significant change.

Discussion

In this study it has been shown that when a constant dose of aspirin (3.9 g/day) is administered on a chronic basis, plasma salicylate levels decline. Miller et al. (1975) observed a similar effect. There is a large intersubject variation in the magnitude of the decline but for all subjects a decline in the plasma salicylate levels was observed.

Since maintenance of steady-state plasma salicylate levels during chronic dosing is important in the treatment of rheumatoid arthritis patients, this change in plasma salicylate levels is of clinical importance. Although this decline is clearly described in the work of Miller et al. (1975) and in the present study, it may have been observed in other studies but not recognized. Levy, Vogel & Ansell (1969), for instance, reported that for certain subjects receiving 2.4 g/day of aspirin for 8 days then 7.2 g daily for the next 8 days, plasma salicylate levels had declined by day 15. They attributed this effect to poor subject compliance. In other studies where plasma salicylate levels were monitored for longer than 7 days, this decline may not have been observed due to dosage changes during the particular study (Gibson, Zaphiropoulos, Grove, Widdop & Berry, 1975; Paulus, Seigel, Mongan, Okin & Calabro, 1971).

We have observed, in the treatment of certain patients undergoing chronic aspirin therapy, the development of an apparent tolerance to aspirin. This 'tolerance' could be accounted for by a decline in steady-state plasma salicylate levels. Consequently

![Figure 3](image_url)

**Table 2** Total salicylate (expressed as g aspirin) recovered in 24 h urine collections, urine pH and creatinine clearance observed in subjects ingesting aspirin (3.9 g/day) on a chronic basis. The results are expressed as mean ± s.e. mean.

<table>
<thead>
<tr>
<th>Day</th>
<th>Total urinary salicylate (g)</th>
<th>Urinary pH</th>
<th>Creatinine clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3.04 ± 0.32</td>
<td>5.90 ± 0.12</td>
<td>101 ± 8.37</td>
</tr>
<tr>
<td>15</td>
<td>2.67 ± 0.29</td>
<td>5.98 ± 0.14</td>
<td>125 ± 8.73</td>
</tr>
<tr>
<td>22</td>
<td>3.03 ± 0.28</td>
<td>5.97 ± 0.13</td>
<td>143 ± 11.38</td>
</tr>
<tr>
<td>29</td>
<td>2.77 ± 0.36</td>
<td>5.89 ± 0.16</td>
<td>106 ± 5.78</td>
</tr>
<tr>
<td>36</td>
<td>2.65 ± 0.33</td>
<td>5.97 ± 0.15</td>
<td>119 ± 8.52</td>
</tr>
</tbody>
</table>
rather than change to different non-steroidal anti-inflammatory agents, an increase in the dose of aspirin being administered may lead to a return to therapeutic efficacy. The increase in dose required is relatively small due to the capacity-limited pharmacokinetics of salicylate (Levy et al., 1972). Thus plasma salicylate levels of rheumatoid patients should be monitored periodically so that any decline can be detected and plasma levels can be maintained within the therapeutic range. Regular monitoring of plasma salicylate levels will also help avoid toxic levels being reached as it has been found that the old guide of tinnitus as an indicator of toxic levels is not always reliable (Gibaldi, 1977).

Salivary salicylate levels have been used previously as an indicator of plasma salicylate levels (Graham & Rowland, 1972; Brooks et al., 1978) in single dose studies. It has also been suggested by Martin, Wan & Karam (1974) that the saliva/plasma ratio for salicylate may reflect its plasma protein binding. In the present study the saliva levels showed a similar trend to the plasma salicylate levels but the mean saliva/plasma ratio declined significantly during the course of the study. This decline is consistent with the reported concentration dependence of salicylate plasma protein binding. As the plasma salicylate concentration decreases, the fraction of salicylate bound to plasma proteins increases (Levy & Yaffe, 1974; Hart et al., 1978). In the present study, the salivary pH and salivary protein content were not monitored. Alterations in either of these variables over the treatment period could also affect the saliva/plasma ratio. No alteration in plasma protein content of individual subjects was observed during the study.

The decline in salivary and plasma salicylate levels during chronic aspirin administration could possibly be explained by induction of one of the metabolic pathways for salicylate elimination. Although, Fung et al. (1977) have explained an increased salicylate formation rate after 3 days of oral aspirin therapy by an induction of gliocyn synthesis, their results may also partly result from the different dosage regimen used in their study. If the decline in plasma salicylate levels observed in the present work (Figure 1) is due to stimulation of one of the salicylate excretion pathways, then induction of the salicylate pathway would appear to be the most probable (Figure 3). The fractions of free salicylate and other salicylate metabolites excreted in the urine are either unchanged or decline over the 5 weeks of the study. Only the fraction of salicylate excreted as salicylate appears to increase, although the change was not found to be statistically significant, during the present study.

It was observed that, during the course of the study, an average of only about 70% of the daily dose was accounted for in a 24 hour urine specimen. A recovery of 86 to 79% was found when the same dosage form was given in a single dose study (Brooks et al., 1978). This lower urinary recovery could possibly be accounted for by a dose-dependent change in bioavailability or in excretion pattern such as bile excretion. This dose-dependent effect is presently being examined.

References


