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Note

Comparison of the major urinary metabolites of Saddamine and aspirin using thin-layer chromatography

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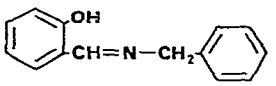
Saddamine [N-(2-hydroxyphenylmethylidine)benzylamine] is a novel Schiff-base[1] with promising anti-inflammatory properties[2]. This compound is at present undergoing clinical trials in Iraq.



Previous work in this laboratory showed that two of the major urinary metabolites of Saddamine, *i.e.* salicyluric and salicylic acids, were the same as those reported[3] for aspirin. In order to study this finding in more detail, the urinary excretion of the major metabolites was monitored over a 48-h period using thin-layer chromatography (TLC), after administration to two male volunteers.

Furthermore, it is known that the benzylamine moiety of Saddamine is almost quantitatively metabolised to benzoic acid and excreted as hippuric acid[4]. Therefore, since both benzoic and salicylic acids are formed from Saddamine, it was thought possible that there might be competition between these two metabolites for glycine conjugation. Indeed, it has been shown that sufficiently large and frequent doses of either benzoic acid[5] or *p*-aminobenzoic acid[6] practically block the formation of salicyluric acid from salicylic acid. In order to study this possibility the ethyl acetate-extractable urinary metabolite patterns of Saddamine and aspirin were compared to that found after corresponding doses of aspirin with sodium benzoate.

EXPERIMENTAL

The following equimolar amounts (5 mmoles) of each of the compounds under investigation were administered orally to two normal male volunteers, following a light breakfast. There were intervals of two weeks between the different doses.

The dose administered was, in week 1 1.055 g Saddamine, in week 3 0.900 g aspirin, and in week 5 0.900 g aspirin + 0.720 g sodium benzoate.

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Thus, assuming complete hydrolysis of Saddamine and oxidation to salicylic and benzoic acids, equivalent amounts of these acids to those formed after Saddamine dosage were administered. Aspirin was thought to be more comparable to Saddamine than salicylic acid since both must first undergo hydrolysis before salicylic acid can be formed. Salicylaldehyde released from Saddamine, however, requires an additional oxidation step to form salicylic acid.

Before administration of each of the three doses a control sample of urine was first obtained. Urine was collected hourly up to 16 h after the dose, then at 23, 24 and 48 h. At each collection the total urine volume and pH were noted and the samples then stored at −20° until analysed.

Extraction and TLC procedure

Representative urine samples (5 ml) were adjusted to pH 3 with 1 *N* HCl and extracted with ethyl acetate (3 × 1 volume) by shaking by hand for 1 min at each extraction. Three extractions were found to extract quantitatively salicyluric, salicylic and gentisic acids from urine. The three extracts were then pooled and evaporated to dryness under nitrogen in a water bath at 80°. The tubes were then cooled and the residue taken up in methanol (1 ml). The aqueous layer was adjusted to pH 5 and incubated with β-glucuronidase (Ketodase, Warner–Chilcott Labs., Morris Plains, N.J., U.S.A.) for 18 h. The hydrolysed samples were further extracted with ethyl acetate as previously described and prepared for TLC.

When TLC was carried out, for each 5 ml of the original total urine volume, 1 μl of the final methanol concentrate was plated. Thus for each urine sample used, 0.1 % of the total metabolites extracted during a 1-h period was plated. Each plate was also run with a control urine sample prepared in the same manner and also with a mixture of reference compounds containing hippuric, salicyluric, gentisic, acetylsalicylic, salicylic and benzoic acids.

A suitable solvent system for the separation of these compounds was found to be benzene–acetic acid (90:30). Plates pre-coated with silica gel F254 (Schleicher and Schüll, Dassel, G.D.R.) were used and the developed plates examined under both long- and short-wavelength ultraviolet (UV) light. Fig. 1 is a photograph taken under UV light of the reference compounds and illustrates the excellent separation obtained.

RESULTS

Saddamine

Fig. 2 is a photograph taken under UV light of a TLC plate showing chromatographed unhydrolysed urine extracts obtained after Saddamine administration. All applications contain 0.1 % of the total extractable metabolites excreted in 1 h and thus are directly comparable. It can be seen from Fig. 2 that salicyluric acid is excreted in small but increasing amounts up to 4 h after Saddamine administration and thereafter at an almost constant rate up to about 14 h. Only a relatively small amount of salicyluric acid is excreted during the 24–25-h period, indicating that little remains in the body.

Gentisic acid is also detectable between 4 and 14 h, reaching a peak in excretion rate at approximately 8 h. Salicylic acid is excreted in very small amounts between 8 and 14 h.

It can be seen in the case of Saddamine, that between 4 and 8 h hippuric acid

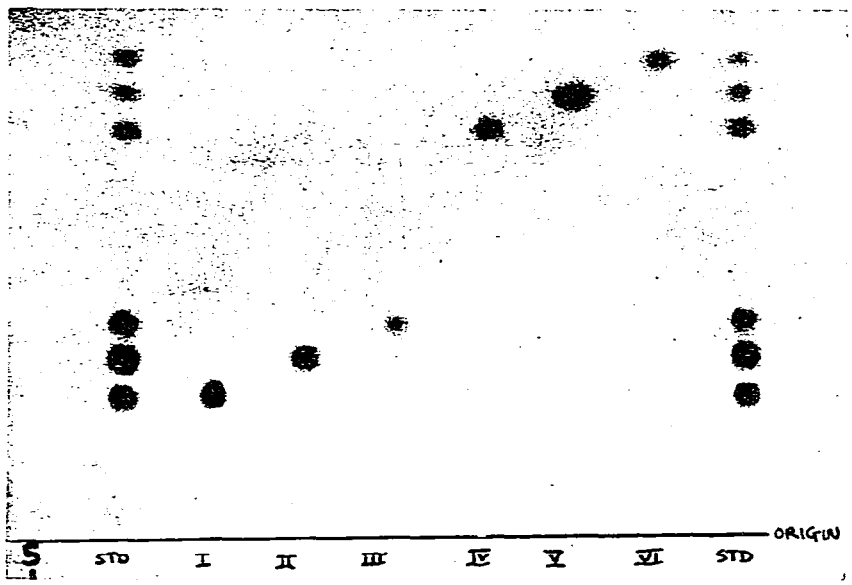


Fig. 1. TLC of reference compounds. I = Hippuric acid, II = salicyluric acid, III = gentisic acid, IV = acetylsalicylic acid, V = salicylic acid, VI = benzoic acid. STD = standard mixture of 20 μg of each of the above compounds.

is excreted in much greater amounts than in either the control or in any other sample and this is probably due to the benzoic acid arising from the benzylamine part of the molecule. Any benzoic acid so formed would be conjugated with glycine and excreted as hippuric acid.

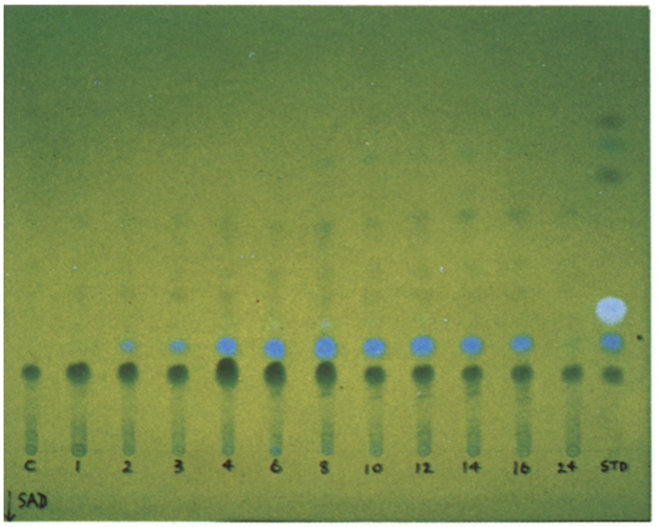
Aspirin

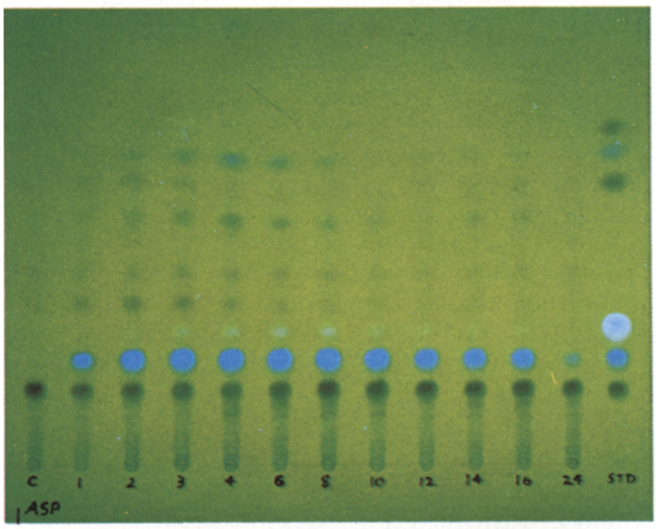
The results from the aspirin study are shown in Fig. 3. A comparison of the metabolites before hydrolysis shown in Figs. 2 and 3 reveals several differences in the rate of metabolite excretion for these two compounds. A major difference is that, unlike Saddamine, aspirin gives urinary salicyluric acid in the first hour after administration. Furthermore, a relatively larger amount of salicyluric acid appears after aspirin administration and at an almost constant rate from 2 to 10 h. A similar constant rate of salicyluric acid excretion has also previously been reported[3].

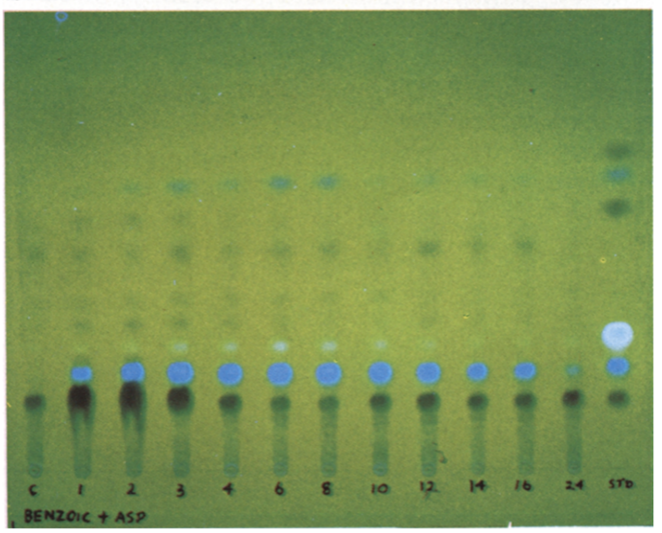
Fig. 2. TLC of unhydrolysed urine after Saddamine administration. STD = standard mixture of 20 μg of reference compounds given in Fig. 1, C = control urine sample. Urine was collected every hour; 1, 2, 3, etc. indicate times of urine collection after the oral dose (1.055 g Saddamine). Each sample plated represents 0.1% of total urine collected each hour.

Fig. 3. TLC of unhydrolysed urine after aspirin administration. Key as for Fig. 2. Oral dose of 0.90 g aspirin.

Fig. 4. TLC of unhydrolysed urine after aspirin plus sodium benzoate administration. Key as for Fig. 2. Oral dose of 0.90 g aspirin plus 0.72 g sodium benzoate.


C 1 2 3 4 6 8 10 12 14 16 24 STD
SAD


C 1 2 3 4 6 8 10 12 14 16 24 STD
ASP


C 1 2 3 4 6 8 10 12 14 16 24 STD
BENZOIC + ASP

Gentisic acid is also excreted in larger amounts and for a longer period than in the case of Saddamine. Likewise, salicylic acid is also present in larger amounts and appears in the urine much earlier than was observed with Saddamine, the peak excretion rate occurring at 4 h.

Aspirin plus sodium benzoate

A representative TLC plate obtained in this study is illustrated in Fig. 4. It would appear that the overall picture of aspirin metabolite excretion is changed very little by co-administration of a single dose of sodium benzoate. The major difference between Fig. 3 and Fig. 4 is the presence of large amounts of hippuric acid in the latter, excreted in the first three hours, the result of a rapid metabolism of the benzoic acid. No unchanged benzoic acid could be detected in the urine. These last observations are in agreement with the finding of Wu and Elliott[7] that there is a very rapid elimination of benzoate as hippurate, and also with that of Bridges *et al.*[8], who showed that in man benzoic acid is entirely excreted as hippuric acid.

Glucuronic acid conjugates

Analysis of the urine samples for glucuronic acid conjugates of salicylic acid indicated that at any given time more of these conjugates were formed from aspirin than from Saddamine. The co-administration of benzoate with aspirin again had no apparent effect on the level of glucuronides formed.

DISCUSSION

The results presented here confirm that the overall pattern of urinary metabolite excretion is similar for Saddamine and aspirin. However, several differences were noted in the rates of Saddamine metabolite excretion, as compared to aspirin, and these may arise from incomplete or delayed absorption of Saddamine or its hydrolysis products. Additionally, differences between the rates of certain metabolic steps affecting Saddamine and aspirin could be involved in producing the delayed metabolite output of Saddamine.

Although the difference between Saddamine and aspirin as regards the relative amounts of metabolites excreted could be explained in terms of different products of metabolism, our earlier studies showed that salicyluric acid is the major Saddamine metabolite and this is also known to be the case for aspirin. Furthermore, the results of the analysis of the urine samples for glucuronic acid conjugates ruled out the possibility of glucuronide formation as a major alternative pathway for salicylate excretion of Saddamine. Large fluctuations in urinary pH are known to affect the rate of salicylate excretion but in all these studies the urinary pH varied only between the limits 5.4 and 6.5.

It would appear from the comparisons of the results of Saddamine administration with those of aspirin and benzoate that competition for glycine conjugation is not a significant factor in controlling the rate of excretion of Saddamine metabolites following a single dose. Thus metabolite competition for glycine conjugation may only become important after chronic administration of Saddamine.

Overall, our findings would indicate that plasma salicylate levels would be lower following Saddamine than after the equivalent salicylate dose and it is proposed to investigate this hypothesis in future studies.

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