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ABSORPTION, METABOLISM AND EXCRETION OF DI(*p*-AMINOPHENYL) SULPHONE (DAPSONE) AND DI(*p*-AMINOPHENYL) SULPHOXIDE IN MAN

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The first large-scale clinical trial of di(*p*-aminophenyl) sulphone (dapsone) against leprosy was reported by Lowe (1952). Since then it has been very widely used for the treatment of leprosy and today is regarded by many workers as the drug of choice. Di(*p*-aminophenyl) sulphoxide has been used on a much more limited scale. Initial reports suggested that the sulphoxide at a dose similar to that employed with dapsone (about 100 mg/day) gave a corresponding clinical and bacteriological improvement and several workers considered it to be somewhat less toxic than dapsone (Buu-Hoi, Khuyen & Xuong, 1955; Davey, Kissaun & Moneta, 1957; Laviron, Lauret, Kerbastard & Jardin, 1957). More recent reports have been much less favourable as considerable nephrotoxicity has been encountered (Browne & Davey, 1961; Buu-Hoi, 1963).

Dapsone is well absorbed in man, 70 to 80% of an oral dose being excreted in the urine as diazotizable compounds (Smith, 1949; Dharmendra, Chatterjee & Bose, 1950). In man blood levels of dapsone take several days to fall to half their maximal amount and the excretion of diazotizable compounds in the urine can be demonstrated for many days after an oral dose (Buttle, Stephenson, Smith, Dewing & Foster, 1937; Smith, 1949; Dharmendra *et al.*, 1950; Chatterjee & Poddar, 1957). Differential solvent extraction methods have shown that not all the diazotizable compounds present in the blood and urine after dosage with dapsone consist of the unchanged drug (Simpson & Molesworth, 1950; Lowe, 1952; Davey, 1956; Bushby & Woiwod, 1955, 1956).

The metabolites of dapsone in man have yet to be isolated and characterized. Simpson & Molesworth (1950) obtained evidence to show that a labile additive compound of dapsone is formed in the body, which breaks down to liberate the free drug in the presence of either dilute acid or alkali. Dapsone is also metabolized by a number of animal species to more polar derivatives, some of which can be hydrolysed to the sulphone by dilute acid (Titus & Bernstein, 1949; Boyer, Troestler, Rist & Tabone, 1950; Bushby & Woiwod, 1955, 1956). Bushby & Woiwod (1955, 1956) demonstrated that the major metabolite of dapsone in the rabbit is the acid-labile *N*-glucuronide. They also showed that the main metabolite of dapsone in man has extremely similar electrophoretic and chromatographic properties to this *N*-glucuronide, but is less acid-labile. Jardin (1958) examined

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the urine of patients treated with dapsone by paper chromatography and demonstrated the presence of four additional Ehrlich-positive spots, one of which was attributed to unchanged dapsone and the others to more polar metabolites.

The antibacterial activity of di(*p*-aminophenyl) sulphoxide *in vitro* is generally considered to be inferior to that of dapsone (Buttle, Dewing, Foster, Gray, Smith & Stephenson, 1938; Youmans & Doub, 1946; Levi & Snow, 1960; Rose, 1962). It should be noted however that some workers consider the sulphoxide to have similar activity *in vitro* to dapsone (Freedlander & French, 1946; Bushby, 1958).

The sulphoxide has definite activity against bacterial infections in the mouse and guinea-pig, which suggests that the sulphoxide may be oxidized in the animal body to dapsone. In order to study this possibility Levi & Snow (1960) devised an analytical method which permits the simultaneous determination of di(*p*-aminophenyl) sulphoxide and dapsone, and used this method to study the fate of di(*p*-aminophenyl) sulphoxide in the rabbit, rat and guinea-pig. Although blood levels were too small to measure, Levi & Snow showed that all three species excreted dapsone as well as the sulphoxide in the urine. In the rat and guinea-pig there was considerable oxidation *in vivo* of di(*p*-aminophenyl) sulphoxide to dapsone, and dapsone made up about 33% of the excretion products detected. In the rabbit however only about 6 to 12% of the excretion products consisted of dapsone, presumably because of the more rapid excretion of the sulphoxide by this animal.

Laviron *et al.* (1957) measured the amounts of diazotizable material in the blood and urine of patients receiving 200 mg of di(*p*-aminophenyl) sulphoxide daily and concluded that the results obtained were very similar to those expected with dapsone. Jardin (1958) examined the urine of patients, receiving 150 mg of sulphoxide each day, by paper chromatographic tecnhiques and detected four additional Ehrlich-positive spots. One of these was attributed to the sulphoxide, one to dapsone and the other two to more polar metabolites, one of which was identical to one of the metabolites of dapsone.

This paper describes the results obtained when the method of Levi & Snow (1960) was used to study the absorption, excretion and metabolism of dapsone and di(*p*-aminophenyl) sulphoxide in man.

METHODS

Dosage of patients and collection of urine

The complete daily excretion of urine was collected from leprosy patients receiving daily doses of 100 mg of dapsone or di(*p*-aminophenyl) sulphoxide. Before these studies were commenced the patients had been stabilized at this dosage for over a month and before that at a dosage of 100 mg 6 days a week for at least 3 months. Each " 24-hr specimen " of urine was diluted to 3 l. before analysis and the completeness of its collection was checked by a determination of its creatinine content (King & Wootton, 1956).

Determination of " total " diazotizable compounds

The method used was a modification of those used by Smith (1949) and Simpson & Molesworth (1950). 0.2 ml. and 0.4 ml. aliquots of each 24-hr specimen were pipetted into test tubes marked at 10 ml., and 3 ml. of 2N-hydrochloric acid were added together with 3 ml. of 12% (w/v) trichloracetic acid and made to the mark with water. Two drops of sodium nitrite solution (0.3%, w/v), prepared immediately before use, were added and the contents of the tube were well mixed. Exactly 3 min later two drops of ammonium sulphamate solution (1.5%, w/v) were added and after a further 2 min, two drops of *N*-1-naphthylethylene-diamine hydrochloride solution (0.1%, w/v). The tubes were placed in the dark for 20 min to allow the

colour to develop. The optical densities of the colours were measured at 550 mμ against a reagent blank and compared with the standard curve (rectilinear) obtained by reacting 4, 8 and 12 μg of dapsone or di(*p*-aminophenyl) sulphoxide respectively in the same way.

Determination of free dapsone and di(p-aminophenyl) sulphoxide and their acid-labile conjugates

The dapsone and di(*p*-aminophenyl) sulphoxide contents of urine samples were measured by the method of Levi & Snow (1960) (*a*) before and (*b*) after hydrolysis with N-hydrochloric acid (0.5 volumes) for 1 hr at 20 to 30° C or (*c*) for 1 hr in a boiling-water bath. Since the concentrations of dapsone and the sulphoxide were often low, after the samples had been neutralized with N-sodium hydroxide, 3 ml. aliquots were taken and mixed with 0.8 M, *p*H 7.0, phosphate buffer (1 ml.) before extraction with methyl isobutyl ketone. Under these conditions both dapsone and the sulphoxide were quantitatively extracted into the organic phase. The sulphoxide was then extracted into 0.1 N-hydrochloric acid, diazotized and determined by coupling with β-sulphatoethyl-*m*-toluidine. Dapsone, which does not extract into 0.1 N-hydrochloric acid, was then extracted into 2N-hydrochloric acid, diazotized and estimated by coupling with N-1-naphthyl-ethylenediamine.

RESULTS

Daily excretion of diazotizable compounds in the urine after daily oral dosage with 100 *mg of dapsone*

The results obtained are summarized in Table 1. Individual determinations of the " total " diazotizable compounds, estimated as dapsone, were made on each 24-hr specimen. In experiment 1 the amounts of sulphoxide, sulphone and their acid-labile conjugates excreted by each patient were determined. In experiments 2 and 3, after the excretion of " total " diazotizable compounds by each patient had been measured, equal volumes of each 24-hr specimen were pooled and the sulphoxide and sulphone contents of the resultant pool determined, before (*a*) and after acid hydrolysis (*b* and *c*), to give the mean values shown for the group.

TABLE 1

DAILY EXCRETION OF DIAZOTIZABLE COMPOUNDS IN THE URINE AFTER DAILY ORAL DOSAGE WITH 100 MG OF DAPSONE

Excretion of compounds is given in mg/day. Values are means and standard deviations. (*a*) (*b*) and (*c*) refer to Methods

Expt. No.	No. of patients	" Total " diazotizable compounds	(*a*) Before acid hydrolysis		(*b*) After acid hydrolysis at 20–30° C		(*c*) After acid hydrolysis at 96° C	
			Sulphoxide	Sulphone	Sulphoxide	Sulphone	Sulphoxide	Sulphone
1	7	72±14	<1	17.5±3.5	<1	42.5±16.5	<1	71.5±22.5
2	6	77±8	<1	13	<1	41	<1	67
3	9	76±12.5	<1	–	<1	40.5	<1	64
Mean		75	<1	15	<1	41	<1	68

About 75% of the dose of dapsone was excreted in the urine as " total " diazotizable compounds, but less than 1% of the dose was excreted as di(*p*-aminophenyl) sulphoxide or its acid-labile conjugates, demonstrating that no significant reduction of dapsone to the sulphoxide occurs in the body. About 15% of the dose of dapsone was excreted as the unchanged drug (*a*); 26% (41 minus 15%) as compounds hydrolysed by dilute acid at room temperature to free dapsone (*b*); and a further 27% (68 minus 41%) as compounds liberating dapsone after boiling the dilute acid (*c*). Kinetic studies showed that the hydrolysis of both these acid-labile metabolites of dapsone is complete in 30 min.

Daily excretion of diazotizable compounds in the urine after daily oral dosage with di(p-aminophenyl) sulphoxide

The results are summarized in Table 2. Individual determinations of the " total " diazotizable compounds, estimated as di(*p*-aminophenyl) sulphoxide, were made on each 24-hr specimen. The amounts of di(*p*-aminophenyl) sulphoxide and sulphone excreted, either free or as acid-labile conjugates, by each patient were also determined in experiment 4. In experiments 5 to 7, after the excretion of " total " diazotizable compounds by each patient had been measured, equal volumes of the 24-hr specimens were pooled and the sulphoxide and sulphone contents of the resultant pool were determined, before and after acid-hydrolysis, to give the mean values shown for the group.

TABLE 2

DAILY EXCRETION OF DIAZOTIZABLE COMPOUNDS IN THE URINE AFTER DAILY ORAL DOSAGE WITH 100 MG OF DI-(*p*-AMINOPHENYL) SULPHOXIDE

Excretion of compounds is given in mg/day. Values are means and standard deviations
(*a*) (*b*) and (*c*) refer to Methods

Expt. No.	No. of patients	" Total " diazotizable compounds	(*a*) Before acid hydrolysis		(*b*) After acid hydrolysis at 20–30° C		(*c*) After acid hydrolysis at 96° C	
			Sulphoxide	Sulphone	Sulphoxide	Sulphone	Sulphoxide	Sulphone
4	7	51 ± 10.5	19.5 ± 6.5	9 ± 4	22 ± 7.5	10 ± 4	29 ± 6.5	4.5 ± 1.5
5	8	59 ± 10	23	8	23	8	23	2.5
6	10	52 ± 8.5	21	13	26	16.5	32	3.5
7	9	56 ± 8	30.5	6.5	38	6	35	3.5
	Mean	55	24	9	27	10	30	3.5

About 55 % of the dose of di(*p*-aminophenyl) sulphoxide was excreted in the urine as " total " diazotizable compounds. Considerable oxidation of di(*p*-aminophenyl) sulphoxide to dapsone occurs in the human body, about a quarter of the free amines excreted in the urine being due to the sulphone, but there was no evidence for the excretion of significant amounts of acid-labile sulphoxide conjugates.

DISCUSSION

The excellent absorption of dapsone previously reported by other workers is confirmed. In these studies about 75 % of the oral dose was excreted in the urine as " total " diazotizable compounds, which is similar to that reported by Smith (1949) in West Africa (83 %) and Dharmendra *et al.* (1950) in India (60 to 75 %). The absorption of dapsone is almost certainly greater than this, for both conjugation and oxidation of the amino groups of the sulphone probably result in the formation of compounds giving less colour than the drug itself. Chatterjee & Poddar (1957), using ^{35}S-labelled dapsone, concluded that about 78 % of an oral dose was excreted in the urine in 10 days.

The amount of unchanged dapsone excreted in the urine (about 15 % of the dose) is very similar to that reported by Lowe (1952) but greatly exceeds that found by Bushby & Woiwod (1955). About 26 % of the dose is excreted as a metabolite which could be hydrolysed to dapsone by dilute acid at room temperature. This metabolite is probably the acid-labile *N*-glucuronide of the drug (Bushby & Woiwod, 1955, 1956). Several *N*-glucuronides can be formed nonenzymatically and the rates of their synthesis and decomposition

vary considerably with *p*H (Bridges & Williams, 1962). The proportion of free dapsone excreted in the urine may therefore vary with the *p*H of the urine. In these studies the *p*H of most urine samples analysed was between 6 and 7. Thus about 41% of the dose of dapsone is excreted unmetabolized. A further 27% is excreted as a compound which could be hydrolysed by boiling with dilute hydrochloric acid to liberate a diazotizable compound extracting into organic solvents and dilute acid like dapsone. This metabolite might be an acetylated derivative of the drug or possibly the relatively acid-stable diazotizable glucuronide detected by Bushby & Woiwod (1955).

These findings suggest that dapsone is relatively slowly metabolized in man and that its antileprotic activity is due to the presence of the unchanged drug in the body.

The excretion of " total " diazotizable compounds in the urine after oral dosage with di(*p*-aminophenyl) sulphoxide (55%) is considerably less than that after dosage with dapsone (75%). This probably indicates that the absorption of the sulphoxide is less complete than that of dapsone. Considerable oxidation of di(*p*-aminophenyl) sulphoxide to the sulphone occurs in the human body and about 10% of the dose is excreted as free dapsone. Similar amounts of the free amines together with their acid-labile conjugates were excreted in the urine after dosage with either dapsone or the sulphoxide (37 and 41% respectively). These findings confirm the paper chromatographic studies of Jardin (1958) and suggest that some, but not necessarily all, of the antileprotic activity of di(*p*-aminophenyl) sulphoxide is due to its conversion in the human body to dapsone.

SUMMARY

1. The excretion of di(*p*-aminophenyl) sulphone (dapsone), di(*p*-aminophenyl) sulphoxide, their acid-labile metabolites and other diazotizable compounds was measured in the urine of leprosy patients being treated orally with dapsone and the sulphoxide.

2. About 41% of the dapsone given is excreted as the free compound plus acid-labile conjugates, and 27% as compounds which are hydrolysed to dapsone-like substances by boiling with dilute acid.

3. The absorption of the sulphoxide appears to be less complete than that of dapsone, only about 55% of the dose being excreted in the urine as diazotizable compounds compared with 75% for dapsone.

4. Considerable oxidation of di(*p*-aminophenyl) sulphoxide to dapsone occurs in the human body and about a quarter of the free amines excreted in the urine after dosage with the sulphoxide are due to dapsone.

5. These results are discussed in relation to the treatment of leprosy with these drugs.

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