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Analgesic activity of a thiomethyl metabolite of paracetamol

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Paracetamol (acetaminophen) is extensively metabolized, predominantly in the liver. Several studies have elucidated the metabolic pattern and the structures of its major and minor metabolites (cf. Forrest et al 1982).

A thiomethyl metabolite of both paracetamol and phenacetin (Focella et al 1972; Klutch et al 1978) has been isolated from urine of dogs and man (I) and shown to be derived in the hamster from the 3-glutathione adduct within the enterohepatic circulation (Gemborys & Mudge 1981). This compound (3MT-4HA) was described in a recent patent (Jollow 1980) as being equipotent with aspirin and more potent than paracetamol as analgesic in the mouse writhing test, and without hepatoxicity at doses up to 700 mg kg^{-1} (i.p.) in the hamster.

We have compared the analgesic effect of paracetamol and 3MT-4HA after oral administration to mice under strictly identical conditions.

Materials and methods

Male CD_1 mice (Charles River, France) 17–23 g were used at an ambient temperature of 22 ± 1 °C.

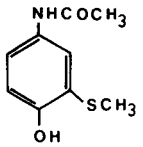
Compounds used were: paracetamol (acetaminophen Rhône-Poulenc, Paris, France) and 3MT-4HA (UPSA, Rueil Malmaison, France) were administered in a 2% aqueous suspension of gum arabic.

Control animals received vehicle. The algogenic substance phenylbenzoquinone (phenyl-*p*-benzoquinone) was from Sigma Chem. Co., U.S.A.

The analgesic effect was estimated using the writhing test described by Siegmund et al (1957). Responses were induced by intraperitoneal injection of 0·22 ml of a 0·02% aqueous-ethanolic solution of phenylbenzoquinone. The number of writhing and stretching movements were noted from the 5th to the 10th min after injection.

The activity kinetics were determined on groups of 6 mice treated orally (0·5 ml/20 g) with paracetamol (185 mg kg^{-1}) or 3MT-4HA (500 mg kg^{-1}), 5, 10, 15, 20, 30, 45 and 60 min before phenylbenzoquinone injection.

* Correspondence.



I. 3-Methylthio-4-hydroxyacetanilide (3MT-4HA).

The dose-effect relationship was evaluated on groups of 6–12 mice. Paracetamol and 3MT-4HA were administered respectively 1 h and 10 min before phenylbenzoquinone, orally (0·5 ml/20 g).

Results

The activity kinetics of paracetamol and of 3MT-4HA are represented in Fig. 1 as percentage inhibition of the writhing response. The doses used were 187·5 mg kg^{-1} for paracetamol and 500 mg kg^{-1} for the metabolite and were chosen after preliminary tests have shown that, at these doses, a reduction of the writhing response of 80–95% was attained at peak effect.

Two moles of the metabolite were equivalent to one mole of paracetamol. The peak effect was reached within 10 min of their administration.

The effect of 3MT-4HA waned in 30 min whereas paracetamol was still 50% active after 1 h.

In Table 1, the percent inhibition of the writhing response is given at different doses and the ED50 is calculated by the variance analysis of the regression line.

One hour after oral administration the ED50 of paracetamol was 266 mg kg^{-1} (174·8–403·6) but 3MT-4HA was inactive at 500 mg kg^{-1}. However, if given 10 min before the test, the 3MT-4HA metabolite shows a dose-effect relationship with an ED50 of 264 mg kg^{-1} (211·5–330·5).

Discussion

Our results confirm the analgesic effect of the 3-thiomethyl metabolite of paracetamol described by

Table 1: Effect of paracetamol and 3MT-4HA in the writhing test in mice.

Substance[a]	Dose mg kg^{-1} oral	Writhes/ mouse[b]	Inhibition (%)	ED50[c] mg kg^{-1} oral
Paracetamol	0	26·1 ± 2·22	—	266 (174·8–403·6)
	62·5	23·5 ± 2·31	10	
	125	18·2 ± 4·20	30	
	250	15·3 ± 2·42**	41	
	500	7·3 ± 2·22***	80	
3MT-4HA	0	22·5 ± 1·75	—	264 (211·5–330·5)
	125	18·4 ± 2·14	18	
	250	12·3 ± 2·89**	45	
	500	3·5 ± 2·12***	84	
	750	2·3 ± 0·59***	90	

[a] Paracetamol was administered 1 h and 3MT-4HA 10 min before phenylbenzoquinone.
[b] Mean value + s.e.m. for 6–12 mice.
[c] ED50 values were calculated as described in the 'results'; 95% confidence limits are indicated in parentheses.
** and *** indicates $P < 0.01$ and $P < 0.001$ when these values are compared with controls (Student's *t*-test).

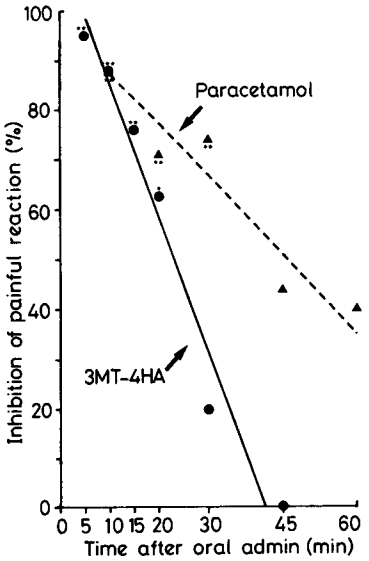


FIG. 1. Activity kinetics for paracetamol (187·5 mg kg^{-1} oral) and 3MT-4HA (500 mg kg^{-1}, oral). Each point represents the mean percentage value for 6 animals calculated from the mean writhing response per group for each time. *, ** and *** indicate $P < 0.05$, $P < 0.01$ and $P < 0.001$ for differences between these values and the values of corresponding control animals (Student's t-test).

Jollow (1980), but show that in mice its duration of action is much shorter than the parent compound even at twice the dose.

Since 3MT-4HA is a minor metabolite of paracetamol in man (Klutch et al 1978) and because of its apparent shorter duration of activity shown by the present study, its contribution to the analgesic effect of the parent compound appears to be negligible. The shorter duration of action of this *S*-methyl metabolite of paracetamol requires further investigation.

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Interaction of uridine 5′-diphosphoglucuronic acid (UDPGA) with cytochrome P 450

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It has been well established that many xenobiotics and endogenous compounds are hydroxylated and subsequently conjugated, for example with glucuronic acid, in the endoplasmic reticulum of the liver cell (Dutton 1980). For several substrates glucuronidation plays a decisive role in the extent in which these compounds display toxic properties.

Nemoto & Takayama (1977) reported that during metabolism of benzo[a]pyrene in rat liver microsomes, the addition of UDPGA selectively decreased the amounts of benzo[a]pyrene phenols. Bock (1978) established that the overall rate of benzo[a]pyrene monooxygenase is markedly increased by subsequent glucuronidation. In contrast, Von Bahr & Bertilsson (1971) did not find an effect of UDPGA on the hydroxylation rate of desmethylimipramine. Two tentative explanations have been put forward to explain these data. UDPGA has been thought to provide relief of product inhibition, which is sometimes caused by hydroxylated metabolites. As a result specific cytochrome P 450 catalysed oxygenation reactions can be enhanced considerably by subsequent glucuronidation (Bock 1978; Von Bahr & Bertilsson 1971; Levy & Ashley 1973). A selective inhibition of certain forms of cytochrome P 450 by UDPGA might also be involved (Nemoto & Takayama 1977). The latter has not been studied thus far and is subject of the present investigations.

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Methods

Adult Male Wistar rats (approx. 250 g TNO, Zeist) were used. Pretreated animals received either an i.p. injection of 80 mg kg^{-1} phenobarbitone 24, 48 and 72 h before killing. Liver microsomes were prepared as described previously (Bast & Noordhoek 1980), pooled and stored at −70 °C.

Spectral measurements in microsomal suspensions were carried out as described by Bast & Noordhoek (1981). Microsomal protein was assayed according to