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EFFECTS OF THE XANTHINE OXIDASE INHIBITOR ALLOPURINOL ON THE RENAL CLEARANCE OF NITROIMIDAZOLES

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Abstract—We have investigated the effects of the xanthine oxidase inhibitor allopurinol on the pharmacokinetics of nitroimidazoles in mice and dogs. Studies in mice showed that at a dose of 32 mg/kg given 30–60 min before, allopurinol had little or no effect on the clearance of misonidazole (MISO) or of the more lipophilic analogue Ro 07-0913, but did increase the blood concentrations of the hydrophilic dealkylation product desmethylmisonidazole (DEMIS). In addition, the clearances of administered DEMIS and the even more hydrophilic analogue SR-2508 were markedly reduced. This dose of allopurinol also caused a considerable fall in the clearances of ^{51}Cr–EDTA and ^{125}I–iodohippurate, normally used to measure glomerular filtration rate and effective renal plasma flow respectively. These data are consistent with a model in which allopurinol inhibits the renal clearance of hydrophilic nitroimidazoles. This leads to an increase in the acute toxicity of DEMIS and, to a lesser extent, of MISO. However, lower doses of allopurinol did not change the pharmacokinetics of MISO or DEMIS. A five-day pretreatment regimen (32 mg/kg/day) followed by a 66–76 hr recovery period was also without effect, thus demonstrating that the inhibition was reversible. Investigations in the dog showed that oral doses of 10–20 mg/kg allopurinol caused no change in the clearance of either ^{51}Cr–EDTA or DEMIS.

The clinical use of the hypoxic cell sensitizing drug misonidazole [1-(2-nitroimidazol-1-yl)-3-methoxypropan-2-ol;Ro 07-0582;MISO] is limited by its neurotoxicity [1, 2]. Considerable development is underway to identify less neurotoxic sensitizers [3–5]. An alternative is to use protective agents. Exposure to MISO can be decreased by pretreatment with the hepatic enzyme inducers phenobarbitone and phenytoin [6–8], and dexamethasone can reduce penetration of MISO into the brain [9]. These drugs are now under investigation as protective agents in man [7, 10].

The cytotoxicity shown by MISO *in vitro* appears to involve reduction of the nitro group, resulting in the production of toxic metabolites and a disturbance of cellular electron transfer processes [11–13]. It is conceivable that nitroreduction might also be responsible for MISO neurotoxicity.

The enzyme xanthine oxidase is known to catalyse the nitroreduction of MISO [14, 16]. With the aim that it might form a basis for decreasing MISO toxicity, Raleigh *et al.* [14] investigated the ability of allopurinol to inhibit the nitroreduction of MISO by xanthine oxidase. They were able to demonstrate inhibition using partially purified enzyme, and went on to study the effects of allopurinol on the pharmacokinetics of MISO and its demethylated metabolite DEMIS [1-(2-nitromidazol-1-yl)-2,3-propandiol; Ro 05-9963] in mice [14]. Some clear effects were observed, including increased serum concentrations of DEMIS, but these were rather difficult to interpret.

The present studies were initiated to clarify the influence of allopurinol on the pharmacokinetics of nitroimidazoles. Those investigated were MISO and another relatively lipophilic analogue Ro 07-0913, which are cleared predominantly by metabolism, and the hydrophilic analogues DEMIS and SR-2508 which are cleared by the kidney [17, 18]. The effects of allopurinol on renal function were also determined.

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MATERIALS AND METHODS

Animals. Adult male BALB/c and C3H/He mice were obtained from OLAC (Southern) Ltd (Bicester U.K.). They were housed in plastic cages on sawdust bedding from soft white woods, and allowed laboratory chow and water *ad lib.* Mice were used at 25–35 g body wt. The dogs used were terrier crossbred females weighing between 6 and 8 kg. Hepatic and renal functions were in the normal range. Food was withheld overnight prior to experiments.

Drugs and isotopes. The structures and sources of the nitroimidazoles used are given in Table 1, together with pertinent physicochemical and biological properties. Allopurinol (4-hydroxypyrazolo (3,4-*d*) pyrimidine) was obtained from Sigma (Poole, U.K.) and also in tablet form (Zyloric) from Wellcome (Beckenham, U.K.)

Isotopes were obtained from Amersham International (Amersham, U.K.). Chromium (^{51}Cr)-EDTA injection solution was supplied at a radioactive concentration of 100 μCi/ml and a specific activity of 1–2 mCi/mg chromium. Sodium iodohippurate (^{131}I) injection solution (BP) was supplied at a radioactive concentration of 500 μCi/ml and a specific activity of 10–50 μCi/mg sodium iodohippurate.

Table 1. Structures, physicochemical and biological properties of MISO analogues*

Compound	Source	Structure R =	Molecular Weight	$E_7^{\frac{1}{2}}$ (mV)	Partition Coefficient‖ (PC)	Acute LD_{50}§ (mmoles)kg)	Renal clearance (% Dose)
Misonidazole (Ro 07-0582)	Roche†	$-CH_2CH(OH)CH_2OCH_2CH_3$	215	−391	1.27	7.5	5
Ro 07-0913	Roche	$-CH_2CH(OH)CH_2OCH_3$	201	−389	0.43	8.9	7
Desmethylmisonidazole (Ro 05-9963)	Roche	$-CH_2CH(OH)CH_2OH$	187	−389	0.11	16.6	60
SR-2508	SRI‡	$-CH_2CONHCH_2CH_2OH$	214	−388	0.046	22.9	89

* See Ref. 17.
† Roche Laboratories, Welwyn Garden City, U.K.
‡ SRI International, Menlo Park, California, U.S.A.
§ BALB/c mice, i.p. route.
‖ Octanol–water.

Mouse pharmacokinetics. Drugs were dissolved in Hanks' buffered salt solution or saline (0.85%, w/v) and injected intraperitoneally (i.p.) or into the tail vein (i.v.) in volumes of 0.01–0.04 ml/g body wt. The nitroimidazoles were usually injected 60, 30 min or immediately after the allopurinol or vehicle control. In daily pretreatment experiments allopurinol was injected once daily for five days (days 1–5), and the nitroimidazoles were given on day 7, 66–76 hr after the last injection. The isotopes were diluted in Hanks' and injected i.v. in 0.005–0.01 ml/g to give doses of 100–500 μCi/kg. At appropriate time intervals blood samples were obtained from the tail, or by cardiac puncture using heparinized syringes [9]. The experiments were carried out in BALB/c mice unless stated otherwise.

Dog pharmacokinetics. Dogs were given bolus i.v. injections of 20 ml saline containing 200 μCi ^{51}Cr–EDTA and DEMIS to give a dose of 0.535 mmoles/kg. Allopurinol was given orally in tablet form to give doses of 10–20 mg/kg. Blood samples were collected into heparinized tubes.

Drug analysis. Nitroimidazole concentrations were determined by isocratic reverse-phase high-performance liquid chromatography (HPLC) as described previously [19] with minor modifications [9, 17]. Blood concentrations were analysed in mice, and control studies showed these to be equal to those in plasma. Plasma concentrations were analysed in dogs. ^{51}Cr and ^{125}I in plasma were measured using a Searle series 1185 automatic gamma counter. Procedures for the estimation of pharmacokinetic parameters have been described elsewhere [6, 17, 20].

Acute LD_{50}. Acute LD_{50} values were determined as described previously [6]. Mice were observed for one month after treatment, but deaths usually occurred within 3 days.

RESULTS

Effects of acute allopurinol pretreatment on nitroimidazole pharmacokinetics in mice

Figures 1 and 2 illustrate the effects of a single dose of allopurinol on the pharmacokinetics of MISO and DEMIS in the blood of BALB/c mice. The allopurinol dose was 32 mg/kg, and this was given 30 min before either high or low doses of nitroimidazoles. Data from two replicate experiments are shown to demonstrate the reproducibility of the effects. There was a tendency for the MISO concentration to be somewhat higher after allopurinol (Figs. 1A and 1C). However, the low dose $t_{\frac{1}{2}}$ was increased only slightly (16%) from 0.55 hr (95% confidence limits, 0.50–0.61 hr) to 0.64 hr (0.59–0.70 hr) ($0.05 > P > 0.02$) and there was no significant effect at the high dose ($P > 0.1$). There was no change in the volume of distribution (Vd) at either dose. In contrast the concentrations of the metabolite DEMIS were increased considerably, particularly with high dose MISO where they were doubled (Figs. 1B and 1D). Large effects of allopurinol were also seen with injected DEMIS (Fig. 2). The $t_{\frac{1}{2}}$ values were increased significantly ($P < 0.01$) at both low and high doses. For example, allopurinol increased the low dose $t_{\frac{1}{2}}$ from 0.69 (0.61–0.79) to 1.65 hr (1.24–2.44 hr). Peak DEMIS concentrations were enhanced by allopurinol (Fig. 2), but Vd was unchanged. Similar effects were obtained whether the allopurinol was given 60 min, 30 min or immediately before MISO or DEMIS (data not shown).

Experiments in which the allopurinol dose was varied (4, 16 and 32 mg/kg) showed that the kinetics of DEMIS and MISO were unaltered at doses below 32 mg/kg (data not shown).

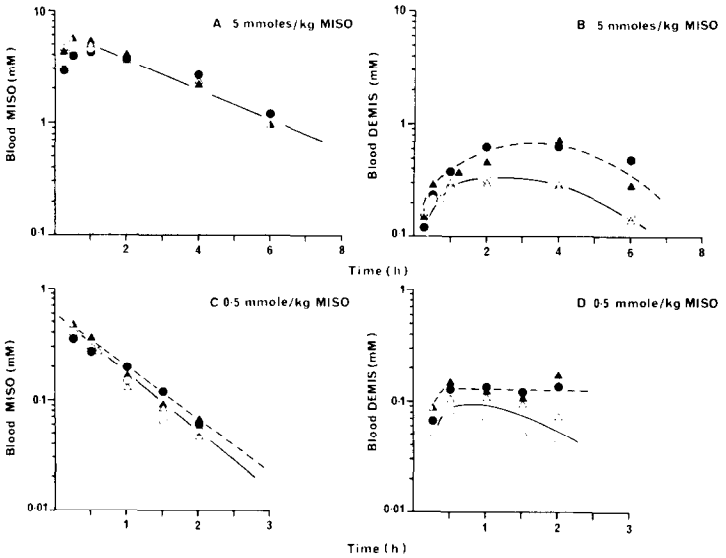

Fig. 1. Effects of allopurinol (32 mg/kg), given 60 min before, on the pharmacokinetics of MISO in BALB/c mice. (A) MISO and (B) DEMIS metabolite after 5 mmoles/kg MISO, (C) MISO and (D) DEMIS metabolite after 0.5 mmoles/kg MISO. Open symbols, vehicle control; closed symbols, allopurinol pretreated. Triangles and circles correspond to data from independent experiments. High dose, 5 mice per point; low dose, 3 mice per point.

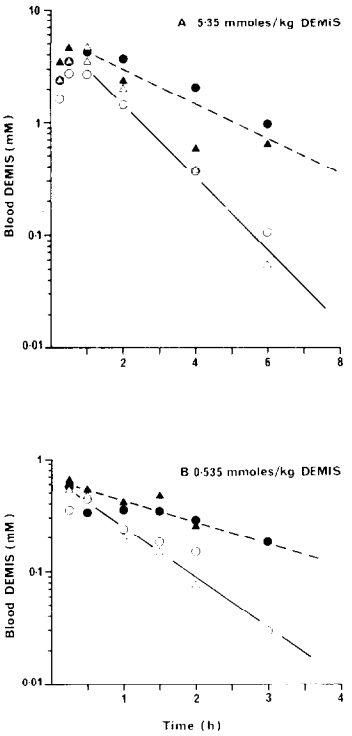

Fig. 2. Effects of allopurinol (32 mg/kg), given 60 min before, on the pharmacokinetics of DEMIS in BALB/c mice. (A) 5.35 mmoles/kg, (B) 0.535 mmoles/kg. Open symbols, vehicle control; closed symbols, allopurinol pretreated. Triangles and circles correspond to data from independent experiments. High doses, 5 mice per point; low dose 3 mice per point.

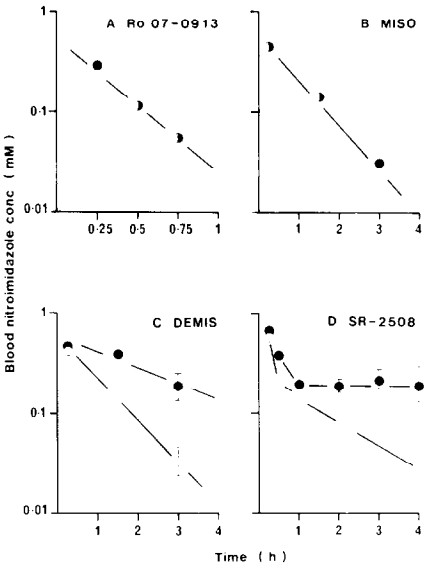

Fig. 3. Effects of allopurinol (32 mg/kg), given 30 min before, on the pharmacokinetics of (A) Ro 07-0913, (B) MISO, (C) DEMIS and (D) SR-2508 in BALB/c mice. SR-2508 was given i.v., the others i.p., all at 0.5 mmoles/kg. Data shown are pooled from 2 or 3 experiments, 3–10 mice per point. ○, vehicle control; ●, allopurinol. Error bars show 1 S.E.

To check for possible strain differences we also determined the effects of 32 mg/kg allopurinol, given 30 min before, on the pharmacokinetics of DEMIS (0.535 mmoles/kg) and MISO (0.5 mmoles/kg) in C3H/He mice. The results were very similar to those in BALB/c mice: the $t_{\frac{1}{2}}$ of MISO was unaffected, whereas that of DEMIS was increased by about 40%.

The preceding data show clearly that 32 mg/kg allopurinol markedly reduces the clearance of the relatively hydrophilic DEMIS but not the more lipophilic MISO. We therefore investigated the effects of allopurinol on the pharmacokinetics of two other analogues: Ro 07-0913 is more lipophilic than MISO, and SR-2508 more hydrophilic than DEMIS (Table 1). Figure 4 shows that, as well as reducing the clearance of DEMIS, allopurinol caused a major decrease in the elimination of SR-2508. Because of its poor absorption SR-2508 was given i.v., and it can be seen that whereas the distribution phase was unaffected the β-phase elimination was inhibited almost completely from 1–4 hr. In contrast, as for MISO, allopurinol had no effect on the pharmacokinetics of the more lipophilic Ro 07-0913 (Fig. 3).

Effect of daily allopurinol pretreatment on nitroimidazole pharmacokinetics in mice

BALB/c mice received daily injections of 32 mg/kg allopurinol for 5 days, and MISO (0.5 mmole/kg) or DEMIS (0.535 mmole/kg) given 66–76 hr after the last injection. In each of two experiments there was no change in the pharmacokinetics of the nitroimidazoles (data not shown).

Effects of acute allopurinol pretreatment on renal function in mice

In view of the selective inhibition of the clearance of the hydrophilic nitroimidazoles, we investigated the effects of acute allopurinol pretreatment on renal function in BALB/c mice. Renal function was assayed by the clearance of ^{51}Cr–EDTA, used to measure glomerular filtration rate, and ^{125}I-iodohippurate, used to measure effective renal plasma flow [21, 22]. Pooled data from several experiments are shown in Fig. 4. At a dose of 32 mg/kg 30 min before, allopurinol exhibits marked inhibition of the clearance of both isotopes. Clearance of ^{51}Cr–EDTA was reduced by a factor of 4.4, and ^{125}I-iodohippurate by a factor of 4.3.

Effects of acute allopurinol treatment in dogs

Dogs received bolus i.v. injections containing both ^{51}Cr–EDTA (200 μCi) and DEMIS (0.535 mmoles/kg). A week later the injection was repeated and allopurinol was also given. The allopurinol was administered orally, either at 10 mg/kg 30 min before the injection or at 20 mg/kg 2 hr after. In no case did allopurinol alter the pharmacokinetics of either DEMIS of ^{51}Cr–EDTA. In a typical experiment, values for the clearance of DEMIS were 3.3 ml/min/kg for the control and 4.18 after 10 mg/kg allopurinol, and those for ^{51}Cr–EDTA were 6.74 for the control and 6.50 with allopurinol. Likewise there was no change in $t_{\frac{1}{2}}$ or Vd.

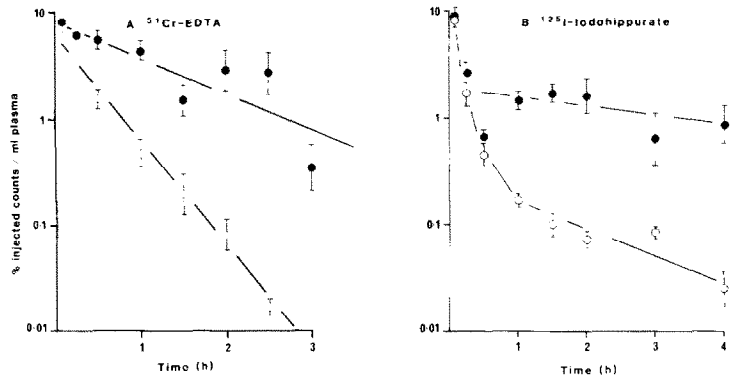

Fig. 4. Effects of allopurinol (32 mg/kg given 30 min before) on the clearance of (A) ^{51}Cr–EDTA and (B) ^{125}I-iodohippurate from the plasma of BALB/c mice. ○, vehicle control; ●, allopurinol. Data shown are pooled from 4 (A) or 5 experiments (B), 3–13 mice per point. Results are geometric means ± 1 S.E.

Table 2. Effects of allopurinol (32 mg/kg, 30 min before) on the acute LD_{50} of MISO and DEMIS*

Nitroimidazole	Pretreatment	Acute LD_{50} (95% confidence limits) (mmoles/kg) Experiment 1	Experiment 2	Experiments 1 + 2 combined
MISO	Hanks'	6.5 (5.5–7.7)	7.7 (7.1–8.2)	7.3 (6.8–7.7)
MISO	Allopurinol	5.4 (4.8–6.1)	4.3 (3.0–6.3)	4.9 (4.2–5.7)
DEMIS	Hanks'	22.1 (18.4–26.4)	22.5 (21.4–23.7)	21.9 (20.2–23.7)
DEMIS	Allopurinol	8.2 (6.2–10.7)	8.5 (6.7–10.8)	8.4 (7.0–10.0)

* In experiment 1, 6–8 dose levels of MISO were used for each pretreatment and 9–12 dose levels of DEMIS, with 3–6 mice per dose level. In experiment 2, 6 dose levels of MISO were used for each pretreatment and 6–7 dose levels of DEMIS, with 5 mice per dose level.

Effects of acute allopurinol pretreatment on acute LD_{50} of MISO and DEMIS in mice

Table 2 summarizes the effects of 32 mg/kg allopurinol, given 30 min before, on the acute LD_{50} of MISO and DEMIS. The LD_{50} for DEMIS was reduced by a factor of 2.6 (95% confidence limits, 2.1–3.3), and that for MISO by a factor of 1.5 (1.2–1.8). The acute LD_{50} of allopurinol itself was usually about 130 mg/kg.

DISCUSSION

The pharmacokinetic behaviour of the various nitroimidazoles in the absence of allopurinol was similar to that observed previously [17, 23, 24]. A dose of 32 mg/kg allopurinol to mice had little or no effect on the clearance of the lipophilic nitroimidazoles, MISO and Ro 07-0913. However, the blood levels of the more hydrophilic metabolite DEMIS were increased. Furthermore, this dose of allopurinol produced a marked reduction in the clearance of injected DEMIS and of the even more hydrophilic SR-2508.

Apart from their lipophilicity, the four analogues are similar in physicochemical properties (Table 1), and none shows significant binding to plasma proteins [17]. However, hydrophilic nitroimidazoles are eliminated predominantly by renal clearance, whereas lipophilic analogues are removed by metabolism [17, 18, and Table 1]. The data are thus consistent with a model in which allopurinol inhibits renal clearance of hydrophilic nitroimidazoles. Pretreatment with allopurinol can cause inhibition of hepatic drug metabolising enzymes other than xanthine oxidase [25, 26], but we found no inhibition of MISO metabolism by allopurinol when given as a single dose or daily for five days.

The allopurinol doses used here are similar to those of Raleigh *et al.* [14]. Using the same mouse strain they observed a persistence of DEMIS in the circulation but found no change in elimination $t_{\frac{1}{2}}$, and the effect was attributed to a reduced rate of absorption. A reduced MISO $t_{\frac{1}{2}}$ was also reported, and some changes in MISO pharmacokinetics were seen at lower doses. However, we were unable to confirm these latter effects.

The reduction in ^{51}Cr–EDTA clearance demonstrated a marked decrease in glomerular filtration rate by allopurinol, and the clearance of ^{125}I-iodohippurate was also considerably reduced. This may indicate a decrease in the effective renal plasma flow, or alternatively an inhibition of the active transport of iodohippurate at the renal tubules.

The mechanism for the renal clearance of hydrophilic nitroimidazoles is unknown, although glomerular filtration is most likely for these non-ionized

drugs. If so, the effects of allopurinol would be explained by the fall in gomerular filtration rate, perhaps through decreased renal plasma flow. However, inhibition of an active transport mechanism cannot be ruled out. The changes are clearly reversible, since the five-day pretreatment schedule was completely ineffective. We speculate that the acute effects of allopurinol in the kidney might result from precipitation of allopurinol or its less soluble metabolite oxypurinol [27], or alternatively of xanthine which can accumulate during inhibition of xanthine oxidase [28]. Crystallization of xanthine in the renal tubules is the dose-limiting effect with allopurinol in small animals [28].

The above effects of allopurinol on the mouse kidney were seen only at the highest dose tested, 32 mg/kg. The recommended dose for hyperuricaemia in dogs is 10 mg/kg orally twice daily, and we were unable to demonstrate any effect of 10–20 mg/kg allopurinol on the renal clearance of ^{51}Cr–EDTA or DEMIS in this species. Thus it seems unlikely that we would see effects of allopurinol on the renal clearance of nitroimidazoles in man with daily maintenance doses of 200–600 mg (3–9 mg/kg), and a preliminary study has shown no effect with MISO*. Isolated cases of nephrotoxicity associated with allopurinol therapy have been reported [29], but in general the drug has no effect on renal clearance in man [30]. The greater susceptibility of small animals to allopurinol nephrotoxicity is attributed to their higher level of purine metabolism [28].

In view of the increased toxicity of MISO and DEMIS in mice with reduced glomerular filtration care must be taken when using nitroimidazoles in patients with impaired renal function.

The present studies do not rule out the possibility that nitroreductase inhibitors might prevent reduction of MISO and so decrease its toxicity. They do, however, illustrate the problems that can arise in moving from an *in vitro* model to the whole animal. With high doses allopurinol in mice, any benefit from inhibition of nitroreduction could be masked by effects on the kidney. Changes in renal clearance might also complicate studies where allopurinol is used to inhibit purine catabolism; for example, doses up to 50 mg/kg have been employed to inhibit 6-mercaptopurine degradation in mice [31].

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