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Aldehyde Oxidase-catalysed Oxidation of Methotrexate in the Liver of Guinea-pig, Rabbit and Man

C. GERALDINE M. JORDAN, MOHAMMED R. RASHIDI, HUSSAIN LALJEE, STEPHEN E. CLARKE*, JOHN E. BROWN AND CHRISTINE BEEDHAM

*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Bradford, Bradford, West Yorkshire BD7 1DP and *Department of Drug Metabolism and Pharmacokinetics, SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, AL6 9AR, UK*

Abstract

Although 7-hydroxymethotrexate is a major metabolite of methotrexate during high-dose therapy, negligible methotrexate-oxidizing activity has been found in-vitro in the liver in man. The goals of this study were to determine the role of aldehyde oxidase in the metabolism of methotrexate to 7-hydroxymethotrexate in the liver and to study the effects of inhibitors and other substrates on the metabolism of methotrexate. Methotrexate, (±)-methotrexate and (−)-methotrexate were incubated with partially purified aldehyde oxidase from the liver of rabbit, guinea-pig and man and the products analysed by HPLC. Rabbit liver aldehyde oxidase was used for purposes of comparison.

In-vitro aldehyde oxidase from the liver of man catalyses the oxidation of methotrexate to 7-hydroxymethotrexate, but the turnover is low. However, formation of 7-hydroxymethotrexate from all forms of methotrexate by the liver in guinea-pig and man was significantly inhibited in the presence of 100 μM menadione and chlorpromazine, potent inhibitors of aldehyde oxidase. Allopurinol (100 μM) had a negligible inhibitory effect on liver aldehyde oxidase from guinea-pig and man. Allopurinol is a xanthine oxidase inhibitor. The production of 7-hydroxymethotrexate was enhanced in the presence of allopurinol. Although aldehyde oxidase is also responsible for some of this conversion, it is also possible that the closely related xanthine oxidase is responsible for the formation of 7-hydroxymethotrexate.

By employing potent selective inhibitors of aldehyde oxidase, menadione and chlorpromazine, we have demonstrated for the first time that liver aldehyde oxidase from man is minimally involved in methotrexate oxidation.

After high-dose therapy with the folate antagonist, methotrexate (10-methyl-4-pteroyl-L-glutamic acid), serum levels of its major metabolite, 7-hydroxymethotrexate, can be up to 100 times those of the parent drug, and up to 33% of the dose is excreted as 7-hydroxymethotrexate (Ertmann et al 1985; Winograd et al 1986). However, the metabolic origins of 7-hydroxymethotrexate, at least in man, are unclear. Rabbit liver aldehyde oxidase (aldehyde oxygen reductase oxidoreductase, EC 1.2.3.1) catalyses the rapid formation of 7-hydroxy-methotrexate from methotrexate but the same enzyme from the liver of man has negligible methotrexate-metabolizing activity (Johns 1967; Fabre et al 1986). This might be because of the instability of liver aldehyde oxidase in surgically excised and post-mortem samples from man (Johns 1967; Duley et al 1985). Alternatively, because rabbit liver aldehyde oxidase has substrate specificity very different from that of its counterpart in man (Beedham et al 1990, 1995), 7-hydroxymethotrexate formation might be attributed to another enzyme system, perhaps the closely related molybdenum hydroxylase, xanthine oxidase.

There are conflicting arguments on the contribution of 7-hydroxymethotrexate to the clinical toxicity of methotrexate. The metabolite is less water-soluble than the parent drug and might be implicated in the renal and hepatotoxicity associated with high-dose methotrexate therapy (Bremnes et al 1991). Ertmann et al (1985) have

Correspondence and present address: C. G. M. Jordan, School of Food Science and Environmental Health, Dublin Institute of Technology, Marlborough Street, Dublin 1, Ireland.

suggested that reduced production of 7-hydroxymethotrexate might lead to enhanced clinical toxicity which might not be predictable by monitoring methotrexate serum levels alone. It is thus important to determine whether other drugs which interact with aldehyde oxidase, either as substrates or as inhibitors, interfere with methotrexate metabolism. Inhibitors of aldehyde oxidase could potentially lead to increased methotrexate plasma levels and toxicity, or they might have a beneficial effect by reducing the variability in methotrexate clearance or reducing the toxicity caused by 7-hydroxymethotrexate, or both.

The goals of this study were twofold — to determine the role of aldehyde oxidase in the metabolism of methotrexate to 7-hydroxymethotrexate in the liver in man and to study the effects of inhibitors and other substrates on the metabolism of methotrexate. Experiments on the (±) and (−) forms of methotrexate (10-methyl-4-aminopteroyl-DL-glutamic acid and 10-methyl-4-aminopteroyl-D-glutamic acid, respectively) were performed for purposes of comparison and are completely novel. The drug described as methotrexate is the L or (+) configuration).

Materials and Methods

Chemicals

Methotrexate, (±)-methotrexate, (−)-methotrexate, allopurinol, menadione, chlorpromazine, dithioerythritol, phenylmethysulphonyl fluoride, leupeptin and EDTA were from Sigma (Poole, UK). All solvents were HPLC-grade from BDH (Poole, UK). Famciclovir, 6-deoxypenciclovir and penciclovir were supplied by SmithKline Beecham. 7-Hydroxymethotrexate was enzymatically prepared from methotrexate, in our laboratory, by use of partially (20-fold) purified aldehyde oxidase from New Zealand White rabbit liver.

Enzyme purification

Partially purified aldehyde oxidase was prepared, from freshly excised livers from New Zealand White rabbits or Dunkin-Hartley guinea-pigs, as described elsewhere (Johnson et al 1984). The enzyme from man was prepared in a similar manner from frozen liver (obtained from the International Institute for the Advancement of Medicine (IIAM), PA), except that samples were homogenized at 4°C in 2 mM Tris buffer, pH 7·0, containing 1 mM dithioerythritol, 50 mM phenylmethysulphonyl fluoride, 10 mM leupeptin and 1 mM EDTA. Aldehyde oxidase prepared as above has been purified 20- to 50-fold. Partially purified enzyme was stored as beads in liquid nitrogen until required.

Spectrophotometric determination of the enzyme activity of partially purified aldehyde oxidase

All spectrophotometric determinations were performed with a Pye-Unicam SP8-250 UV-Vis spectrophotometer fitted with a Pye-Unicam cell-temperature control-unit operated at 37°C. The enzyme activity of partially purified fractions was monitored by use of phenanthridine (a specific aldehyde oxidase substrate) and potassium ferricyanide as an electron acceptor. Test cuvettes contained 0·1 mL appropriately diluted enzyme and 50 μM phenanthridine in a total volume of 3·0 mL 67 mM Sørensen's phosphate buffer (pH 7·0) containing 0·1 mM EDTA. Substrate was omitted from control cuvettes and the reaction was monitored at 322 nm. The amount of enzyme used in the incubation depended on the activity of that particular sample.

Preparation of 7-hydroxymethotrexate from methotrexate using partially purified aldehyde oxidase from New Zealand White rabbit liver

Methotrexate (50 mg) was dissolved in a minimum amount of 0·1 M NaOH. Sørensen's buffer (pH 5·5, 70 mL) was added and the pH adjusted to 7·8 with 5 M NaOH initially and then 0·5 M NaOH. The enzyme used was partially purified aldehyde oxidase from New Zealand rabbit liver. This was added to the methotrexate solution in a water-bath at 37°C according to the regime: 0 min, 3 mL enzyme; 120 min, 2 mL; 240 min, 2 mL.

The enzyme was used directly and was not diluted with buffer. Samples were taken after 0, 120, 240 and 360 min. Sample (200 μL) was added to perchloric acid (3·6%, 100 μL) and the mixture was centrifuged at 9000 rev min^{-1} for 5 min. The reaction was monitored by HPLC. At this stage, most methotrexate was converted to 7-hydroxymethotrexate. The pH was now increased to 8·4 by use of 0·5 M NaOH, and the solution was left to stand overnight at 4°C. Next day the solution was centrifuged at 8000 rev min^{-1} for 10 min as a precipitate had formed. This precipitate was suspended in distilled water (6 mL) and heated in a boiling water-bath for 4 min. It was then centrifuged at 5000 rev min^{-1} for 10 min and the insoluble protein discarded. The supernatant from the first solution was chromatographed and found to contain a high

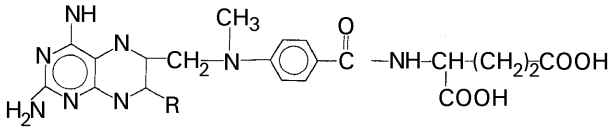

Figure 1. The structures of methotrexate (R = H) and 7-hydroxymethotrexate (R = OH).

concentration of 7-hydroxymethotrexate and some methotrexate. This solution was left to stand overnight at 4°C. It was then chromatographed and found to contain mostly 7-hydroxymethotrexate. Glacial acetic acid (4·8 mL) was added and the mixture was left to stand overnight at 4°C. Next day a yellow precipitate had formed in the solution. This precipitate was removed by vacuum-filtration, washed with acetone, and placed in a desiccator (P_2O_5) and dried overnight. This yellow precipitate was pure 7-hydroxymethotrexate; its structure was confirmed by spectroscopic analysis (nuclear magnetic resonance and mass spectrometry) and chromatographic analysis (HPLC). It was stored in a desiccator (P_2O_5) and used as required. The structures of methotrexate and 7-hydroxymethotrexate are shown in Figure 1.

Preparation of standard solutions of (±)- and (−)-methotrexate, 7-hydroxymethotrexate and inhibitors

Standards of (±)- and (−)-methotrexate and 7-hydroxymethotrexate are insoluble directly in aqueous buffer so must first be dissolved in HCl (0·1 M, 1·0 mL) which is then neutralized with NaOH (0·1 M, 1·0 mL). They are then diluted to volume with Sørensen's buffer, 63 mM EDTA (pH 7·0). Calibration curves for each solution were drawn and found to be linear within the range 0–300 μM ($r^2 = 0{\cdot}999$). A substrate concentration of 50 μM was chosen because this was relevant to therapeutic plasma concentrations and also furnished measurable concentrations of metabolite from partially purified rabbit and guinea-pig liver aldehyde oxidase. Substrate concentrations of 300 μM had to be used for aldehyde oxidase from the liver of man, because turnover was slow and this concentration was required to furnish measurable concentrations of metabolite. Concentrations of parent compound and metabolites are measured in micromoles (μM) instead of $mg\,L^{-1}$ or $\mu g\,mL^{-1}$; this is because the parent compound is prepared in buffer (pH 7·0) and μM is defined as weight of compound per volume of solution. By expressing concentration in μM it is easier to compare results in buffer only with those obtained in the presence of enzymes. With in-vitro studies it is always best to express concentration in μM.

Allopurinol was first dissolved in NaOH (0·1 M, 1·0 mL), then stirred, followed by addition of HCl (0·1 M, 1·0 mL), the final solution was diluted to volume with buffer (pH 7·0). Chlorpromazine was treated in the same manner as allopurinol. Chlorpromazine is light-sensitive and so was protected with aluminium foil. Because menadione is insoluble in aqueous buffer, it was prepared in absolute alcohol; the appropriate dilutions were then made with buffer (pH 7·0). This compound also is light-sensitive, and so was protected with aluminium foil; it was stable for only 2–3 h in buffer (pH 7·0). Famciclovir, penciclovir and 6-deoxypenciclovir were all soluble in aqueous buffer (pH 7·0). Each inhibitor (10, 50 and 100 μM of each) was used in incubations; because it was found that the 100 μM concentration caused the greatest inhibition, this concentration was used in all experiments.

Incubation conditions

(±)- or (−)-methotrexate (50 μM) was incubated with partially purified guinea-pig aldehyde oxidase (100 μL) in phosphate buffer (67 mM, pH 7·0; 3 mL) containing EDTA (0·1 mM) at 37°C. Reactions were terminated by addition of samples (200 μL) to perchloric acid (3·6%, 100 μL). Samples were centrifuged in a Beckman bench-top microcentrifuge for 5 min and the supernatant was subsequently analysed by HPLC. Experiments with liver aldehyde oxidase from rabbit and man were performed as for guinea-pig enzyme, with modifications: methotrexate only was incubated with rabbit liver aldehyde oxidase (20 μL) and methotrexate (300 μM) was incubated with liver enzyme from man (50 μL) in buffer (1·5 mL). These incubations were terminated with 6·0% perchloric acid. Incubations were also performed in the presence of allopurinol, menadione, chlorpromazine, famciclovir, penciclovir and 6-deoxypenciclovir (100 μM). Control incubations containing no substrate, no enzyme and no inhibitors were performed in parallel with normal incubations.

Chromatographic analysis

HPLC analysis was performed with a Waters Associates (Northwich, Cheshire, UK) system comprising a 717 Plus autosampler, UV 486 tunable absorbance detector and a 510 pump, all controlled by Millennium software. Samples from all incubations were analysed on a 25 cm × 4·6 mm (i.d.) 5 μM Spherisorb ODS2 column with a μBondapak C_{18} Guard-Pak insert; the mobile phase

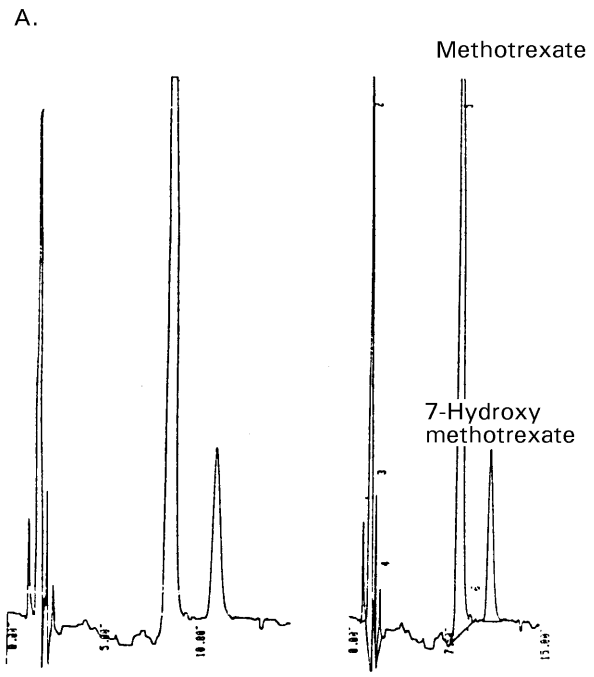

Figure 2. HPLC analysis showing the formation of 7-hydroxymethotrexate from methotrexate.

was 0·1 M ammonium acetate (pH 5·5)–methanol–acetonitrile, 87:5:8 (%v/v) at a flow rate of 1·5 mL min^{-1}. The reactions were monitored at 305 nm and room temperature. The concentration (μM) of 7-hydroxymethotrexate was calculated relative to the standard synthesized in our laboratory. Under these conditions methotrexate and 7-hydroxymethotrexate had retention times of approximately 8·6 and 11·1 min, respectively (Figure 2).

HPLC analysis of the oxidative conversion of 50 μM 6-deoxypenciclovir to penciclovir by partially purified liver aldehyde oxidase from man, was performed with a Waters Associates (Northwich, Cheshire, UK) system comprising a 510 pump, 710B WISP automatic injector, a Lambda-Max LC Spectrophotometer and a 740 data module. Samples from all incubations were analysed on a 25 cm × 4·6 mm (i.d.) 5 μM Spherisorb ODS2 column with a μBondapak C_{18} Guard-Pak insert; the mobile phase was 0·05 M ammonium acetate (pH 4·65)–1·4% acetonitrile at a flow-rate of 1·5 mL min^{-1}. The reactions were monitored at 280 nm and room temperature. Under these conditions the retention times of penciclovir and 6-deoxypenciclovir were 15 and 18 min, respectively.

Results and Discussion

In agreement with previous studies (Johns et al 1966; Fabre et al 1986) methotrexate was rapidly converted to 7-hydroxymethotrexate by rabbit liver aldehyde oxidase; disappearance of the substrate was complete within 30 min (Figure 3). However, when methotrexate was incubated with guinea-pig liver enzyme under similar conditions only 2% breakdown of methotrexate occurred within 240 min (Figure 4). Species differences in aldehyde oxidase activity towards methotrexate have been noted previously (Johns et al 1966). 7-Hydroxymethotrexate formation by guinea-pig enzyme was stereoselective with higher oxidation rates observed with the (−) isomer than with methotrexate or the racemate. Initial oxidation rates for methotrexate, (±)-methotrexate and (−)-methotrexate were in the ratio 2:3:4 (Table 1). The formation of 7-hydroxymethotrexate as a percentage of the starting concentration of the parent compound is shown in Table 2 for partially purified rabbit and guinea-pig

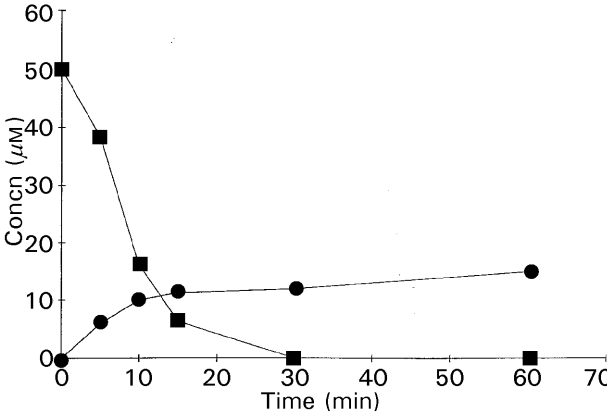

Figure 3. Formation of 7-hydroxymethotrexate from the incubation of 50 μM methotrexate with partially purified rabbit liver aldehyde oxidase for 1 h at 37°C. ■ (+)-Methotrexate, ◆ 7-hydroxymethotrexate.

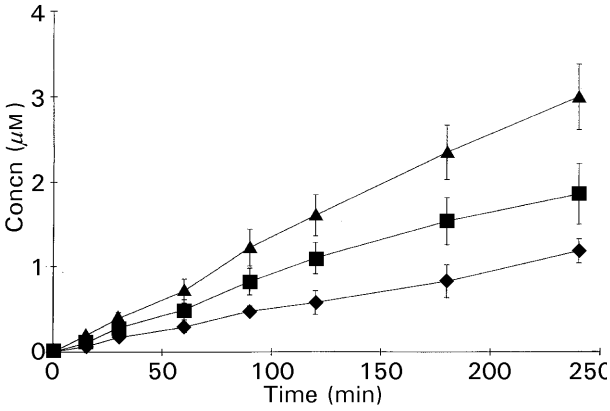

Figure 4. Formation of 7-hydroxymethotrexate from the incubation of 50μM methotrexate with partially purified guinea-pig liver aldehyde oxidase for 4 h at 37°C. Results are presented as mean ± s.e. (n = 4). ▲ (−)-methotrexate, ■ (±)-methotrexate, ◆ (+)-Methotrexate.

Table 1. The effect of inhibitors on the formation of 7-hydroxymethotrexate from 50 μM methotrexate catalysed by guinea-pig liver aldehyde oxidase.

Incubation	Concn (μM) of 7-hydroxymethotrexate formed in 180 min from:		
	Methotrexate	(±)-Methotrexate	(−)-Methotrexate
Control	1·90 ± 0·34	2·90 ± 0·22	3·70 ± 0·65
Menadione	0·30 ± 0·08**	0·40 ± 0·15**	0·60 ± 0·16*
Chlorpromazine	ND	ND	ND
Allopurinol	2·0 ± 0·27	2·90 ± 0·54	3·50 ± 0·57
Famciclovir	1·80 ± 0·26	2·70 ± 0·43	2·70 ± 0·36
6-Deoxypenciclovir	1·80 ± 0·27	2·60 ± 0·52	2·60 ± 0·21
Penciclovir	1·90 ± 0·24	2·80 ± 0·43	2·80 ± 0·34

Values are means ± s.e.m. (n = 4). *$P < 0·05$, **$P < 0·01$ compared with control. ND = not detectable.

Table 2. The formation of 7-hydroxymethotrexate as a percentage of the starting concentration of the parent compound (50 μM) in the presence of partially purified liver aldehyde oxidase.

Time (min)	Amount of 7-hydroxymethotrexate (%)		
	Methotrexate	(±)-Methotrexate	(−)-Methotrexate
Guinea-pig liver aldehyde oxidase			
0	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
15	0·10 ± 0·03	0·20 ± 0·05	0·40 ± 0·03
30	0·30 ± 0·02	0·60 ± 0·05	0·80 ± 0·07
60	0·60 ± 0·06	1·00 ± 0·12	1·40 ± 0·10
90	0·90 ± 0·06	1·60 ± 0·16	2·40 ± 0·22
120	1·10 ± 0·14	2·20 ± 0·18	3·20 ± 0·24
180	1·60 ± 0·19	3·10 ± 0·28	4·70 ± 0·32
240	2·40 ± 0·14	3·70 ± 0·36	6·00 ± 0·39
Rabbit liver aldehyde oxidase			
0	0·0		
5	11·90		
10	20·00		
15	22·60		
30	24·10		
60	29·90		

Mean ± s.e.m.

liver aldehyde oxidase. A comparison can be made between the isomers.

From the results of this study, the differences between aldehyde oxidase activity towards methotrexate and (−)-methotrexate are interesting because it seems that (−)-methotrexate is a better substrate than methotrexate for the liver enzymes from both guinea-pig and man. Although metabolite formation was faster from methotrexate with liver aldehyde oxidase from man, chlorpromazine and famciclovir had a greater inhibitory effect on metabolite formation from this isomer, indicating that it had lower affinity for the enzyme than did the (−) isomer. This might explain why (−)-methotrexate is less potent and considerably less toxic against leukaemia L1210 in the mouse than methotrexate (Skipper & Schmidt 1962), even though both isomers have a similar in-vitro potency toward dihydrofolate reductase from mouse L210 cells or leukaemia cells from man (Lee et al 1974).

Oxidation of methotrexate by liver aldehyde oxidase from man was even slower than that with guinea-pig enzyme. Consequently, higher concentrations of substrate and the enzyme from man were used to furnish measurable quantities of 7-hydroxymethotrexate and it was not possible to detect metabolite production in incubations up to 90 min. This is in agreement with the findings of other researchers (Johns 1967; Fabre et al 1986), who found that liver aldehyde oxidase enzyme from man has negligible methotrexate-metabolizing activity. This might arise from the instability of the enzyme in surgically excised and post-mortem samples from man (Johns 1967; Duley et al 1985). In contrast, almost complete conversion of 6-deoxypenciclovir to penciclovir occurred with

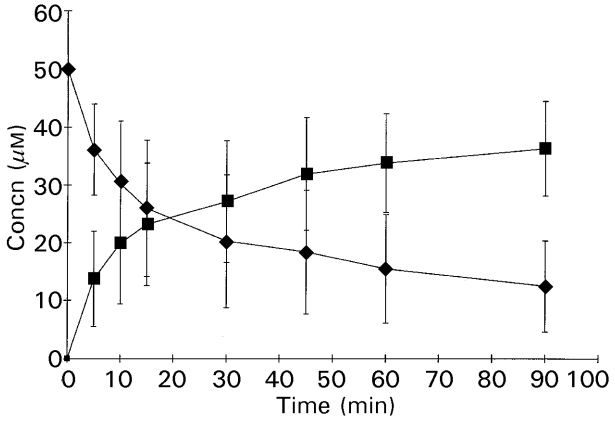

Figure 5. Oxidative conversion of 50 μM 6-deoxypenciclovir (◆) to penciclovir (■) by partially purified human liver aldehyde oxidase. Results are presented as mean ± s.e.m.

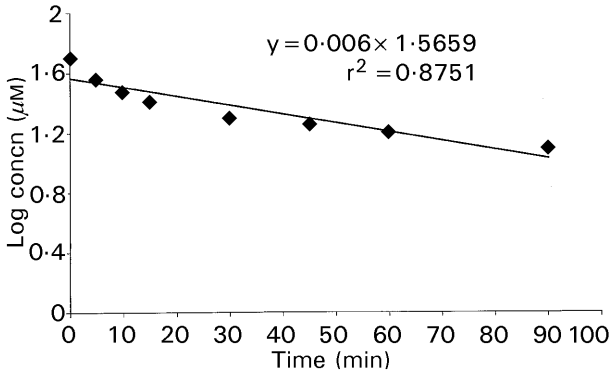

Figure 6. First-order plot for the degradation of 6-deoxypenciclovir to penciclovir by partially purified human liver aldehyde oxidase.

liver aldehyde oxidase from man (Figure 5), which shows that the enzyme preparation was still active. From Figures 6 and 7 it is clearly apparent that the degradation of 6-deoxypenciclovir follows second-order rate kinetics. The values of the rate constant (k) are shown in Table 3 for first- and second-order reactions, as is the half-life ($t\frac{1}{2}$) value for the first-

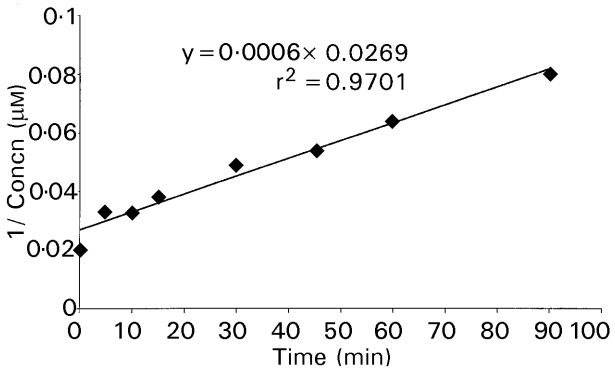

Figure 7. Second-order plot for the degradation of 6-deoxypenciclovir to penciclovir by partially purified human liver aldehyde oxidase.

Table 3. Rate constants and half-lives of the degradation of 6-deoxypenciclovir.

Physicochemical parameter	First-order reaction[a]	Second-order reaction[b]
Rate constant (min^{-1})	0·01	0·001
Half-life	50·20	–

[a,b]Rate constant, k, ±2·303 × slope. [a]Half-life, $t\frac{1}{2}$, = 0·693/k.

order reaction. The findings in Figure 4 served as a positive control. There was no significant difference in the results obtained from methotrexate, (±)-methotrexate, or (−)-methotrexate (Table 4). Absolute values of the concentrations (μM) were determined for substrate and metabolite in Figure 4. However, large errors are shown for the means of three experiments, because of large variations in aldehyde oxidase activity in liver samples from man.

Interaction of methotrexate, (±)-methotrexate and (−)-methotrexate with inhibitors

7-Hydroxymethotrexate formation from 50 μM methotrexate was monitored in the presence of 100 μM menadione and chlorpromazine, both of which are potent aldehyde oxidase inhibitors (Johns 1967). To account for the different catalytic activity of the liver enzyme from guinea-pig and man, incubations with that from the guinea-pig were performed for 180 min whereas those with the enzyme from man were performed for 240 min. Even with the increased incubation time 7-hydroxymethotrexate production by the enzyme from man was much lower than that by guinea-pig aldehyde oxidase. Menadione caused complete inhibition of 7-hydroxymethotrexate formation from all forms of methotrexate by liver fractions from man and reduced metabolite formation from each isomer by approximately 85% in guinea-pig incubations. Chlorpromazine, had a variable effect on 7-hydroxymethotrexate production, resulting in 100, 39 and 59% inhibition of oxidation of methotrexate, (±)-methotrexate and (−)-methotrexate, respectively, by the enzyme from man. This might indicate that in man different liver aldehyde oxidase isozymes are responsible for the oxidation of methotrexate and (−)-methotrexate or that (−)-methotrexate competes more effectively than methotrexate with chlorpromazine for the enzyme active site.

Allopurinol, which is a selective inhibitor of xanthine oxidase (Krenitsky et al 1986), had little effect on 7-hydroxymethotrexate formation from either isomer, confirming that xanthine oxidase is not involved in methotrexate metabolism. The

Table 4. The effect of inhibitors on the formation of 7-hydroxymethotrexate from 300 μM methotrexate catalysed by liver aldehyde oxidase from man.

Incubation	Concn (μM) of 7-hydroxymethotrexate formed in 240 min from:		
	Methotrexate	(±)-Methotrexate	(−)-Methotrexate
Control	0·20 ± 0·04	0·30 ± 0·05	0·20 ± 0·09
Menadione	ND	ND	ND
Chlorpromazine	ND	0·20 ± 0·01	0·10 ± 0·01
Allopurinol	0·20 ± 0·07	0·30 ± 0·06	0·30 ± 0·15
Famciclovir	0·10 ± 0·08	0·20 ± 0·01	0·10 ± 0·01
6-Deoxypenciclovir	0·20 ± 0·06	0·20 ± 0·06	0·10 ± 0·06

Mean ± s.e.m. (n = 4). ND = not detectable.

production of 7-hydroxymethotrexate was even enhanced in the presence of allopurinol (100 μM).

Neither famciclovir nor its metabolites (100 μM) significantly reduced 7-hydroxymethotrexate formation, by guinea-pig liver aldehyde oxidase, from methotrexate or (±)-methotrexate, although metabolite formation from (−)-methotrexate was reduced by 27%. 7-Hydroxymethotrexate production from (−)-methotrexate by liver aldehyde oxidase from man was also inhibited by famciclovir and 6-deoxypenciclovir and some inhibition was also found with methotrexate and (±)-methotrexate. However, it is unlikely that methotrexate metabolism would be affected in-vivo during combination therapy with famciclovir because in-vitro inhibition was only achieved with relatively high purine concentrations (100 μM). At 10 μM, famciclovir or its metabolites did not reduce methotrexate oxidation by guinea-pig aldehyde oxidase. Plasma concentrations of famciclovir and penciclovir would not be expected to exceed 10 μM (Filer et al 1994). Co-administration of potent aldehyde oxidase inhibitors, such as amsacrine or chlorpromazine, might enhance the therapeutic selectivity of methotrexate by reducing toxicity associated with 7-hydroxymethotrexate and by increasing the percentage of methotrexate available for polyglutamation, an important component of methotrexate cytotoxicity (Matherly et al 1987). In addition, inhibition of methotrexate 7-hydroxylation would lead to less variation in plasma levels of methotrexate and the success of leucovorin rescue would be easier to predict. Furthermore, although co-administration of amsacrine, an acridine antitumour agent, with methotrexate to rabbits reduces urinary excretion of 7-hydroxymethotrexate by 50% (approx.) (Lee & Chan 1988), amsacrine has a much higher affinity for aldehyde oxidase than famciclovir or 6-deoxypenciclovir. Activation of famciclovir, a recently introduced antiviral drug, involves hydrolysis of both acetyl groups to 6-deoxypenciclovir, then oxidation to the potent antiherpes agent, penciclovir (Vere Hodge et al 1989). The oxidation step is catalysed by hepatic aldehyde oxidase (Clarke et al 1995). Co-administration of famciclovir with methotrexate could potentially lead to increased methotrexate plasma levels and toxicity. Alternatively, this might have a beneficial effect by reducing the variability in methotrexate clearance or reducing toxicity caused by 7-hydroxymethotrexate, or both. Thus, the in-vitro interaction between famciclovir, or its metabolites, and methotrexate oxidation has been investigated. Amsacrine is a potent competitive aldehyde oxidase inhibitor with a calculated inhibition constant, K_i, of 2·5 μM (Lee & Chan 1988), whereas the K_m values for famciclovir and 6-deoxypenciclovir are 100–500 μM for liver aldehyde oxidase from guinea-pig or man (Rashidi et al 1994; Clarke et al 1995).

The different aldehyde oxidase activity toward methotrexate and (−)-methotrexate is of interest because it seems that the (−) isomer is a better substrate than methotrexate for the liver enzyme from both guinea-pig and man. Although metabolite formation was faster from methotrexate, with liver aldehyde oxidase from man, chlorpromazine and famciclovir had a greater inhibitory effect on metabolite formation from this compound indicating a lower affinity for the enzyme than that of the (−) isomer. This might explain why (−)-methotrexate is less potent and considerably less toxic against leukaemia L1210 in the mouse than methotrexate (Skipper & Schmidt 1962) even though both isomers have a similar in-vitro potency toward dihydrofolate reductase from mouse L210 cells or leukaemia cells from man (Lee et al 1974).

In conclusion, even though we have shown that, in-vitro, liver aldehyde oxidase from man catalyses the oxidation of methotrexate to 7-hydroxymethotrexate, turnover is low. During high-dose therapy 33% of the parent drug is

excreted as 7-hydroxymethotrexate (Ertmann et al 1985; Winograd et al 1986). Despite this, aldehyde oxidase is responsible for some of this conversion; perhaps the closely related xanthine oxidase is also responsible for the formation of 7-hydroxymethotrexate. By employing potent, selective inhibitors of aldehyde oxidase, menadione and chlorpromazine, we have demonstrated for the first time that liver aldehyde oxidase from man is minimally involved in methotrexate oxidation.

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