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The inhibitory effect of amiodarone and desethylamiodarone on dextromethorphan *O*-demethylation in human and rat liver microsomes

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Abstract—Amiodarone has been shown in-vitro to inhibit the activity of cytochrome P4502D6 (CYP2D6) in nonhuman primates. However, the influence of its major metabolite, desethylamiodarone, on this isozyme activity has not been studied. To determine the effect of these drugs on dextromethorphan *O*-demethylation, we carried out studies in 10 human and 10 rat liver microsomal preparations. In human microsomal studies, amiodarone and the metabolite competitively inhibited dextromethorphan metabolism with mean K_i values of $52{\cdot}70 \pm 5{\cdot}27$ and $34{\cdot}40 \pm 3{\cdot}30\,\mu M$, respectively. Similar studies in rat microsomes showed a competitive inhibitory effect of amiodarone and its metabolite on dextromethorphan metabolism. These data suggest that amiodarone and desethylamiodarone have an inhibitory effect on CYP2D6 in man and CYP2D1 in rats. However, it cannot be concluded that both of them are substrates of this isozyme activity.

Amiodarone has been reported to interact in-vivo with several other drugs that are often co-administered to achieve increased anti-arrhythmic efficacy (Leor et al 1988; Lesko 1989). One of the most important mechanisms of these pharmacokinetic drug interactions is the inhibition of hepatic metabolism. Previous studies showed that amiodarone is a potent inhibitor of hepatic microsomal mixed function oxidative drug metabolism in rats and markedly reduces cytochrome P450 (Duenas-Laita et al 1987). Moreover, recent studies on extensive metabolizers of dextromethorphan by nonhuman primate liver microsomes revealed that amiodarone inhibits the activity of cytochrome P4502D6 (CYP2D6) (Funck-Brentano et al 1991).

Desethylamiodarone (DEA), the major metabolite of amiodarone in man, has been found in high concentrations in plasma and tissues during long-term therapy of amiodarone (Holt et al 1983). However, it is not known whether DEA has an inhibitory effect on the activity of CYP2D6 in man or on cytochrome P4502D1 (CYP2D1) in rats.

The 4-hydroxylation of debrisoquine is catalysed by CYP2D6 in man and by CYP2D1 in rats and debrisoquine has been used as a model substrate to assess the activity of this enzyme (Gonzalez et al 1987, 1988 a,b). Dextromethorphan, an antitussive drug, is mainly metabolized by oxidative *O*-demethylation to form dextrorphan. Several studies have revealed that this reaction co-segregates with debrisoquine 4-hydroxylation, and it is claimed that the analysis of dextromethorphan and its *O*-demethylated metabolites in urine specimens is faster and simpler than that for debrisoquine (Küpfer et al 1984; Schmid et al 1985). Therefore, dextromethorphan has been proposed as an alternative to debrisoquine for both in-vitro and in-vivo studies (Küpfer et al 1984; Schmid et al 1985; Dayer et al 1989).

To determine the inhibitory effect of amiodarone and its metabolite on dextromethorphan *O*-demethylation we carried out additional studies in human and rat liver microsomes.

Materials and methods

Animals. Ten male Wistar rats, 200–500 g, were used in these experiments. All animals used in the study were inbred strains and were fed a commercial diet.

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Chemicals. Dextromethorphan hydrobromide and dextrorphan tartrate were generously donated by Hoffman La Roche (Nutley, NJ, USA) and DEA was generously donated by Wyeth-Ayerst (Princeton, NJ, USA). Amiodarone was purchased from Sigma Chemical Company (St Louis, MO, USA). All other chemicals were of analytical grade.

Liver microsomes and incubation conditions. The experimental protocol was approved by the institutional ethics committee. Histologically normal human liver samples were obtained from ten patients shortly after circulatory arrest, with permission from the relatives of the patients. Microsomes were prepared by a standard technique as described by Miners et al (1988), using differential ultracentrifugation. Protein concentrations of the liver preparations were measured by the method of Lowry et al (1951), using crystalline bovine serum albumin (fraction V) as standard. The microsomal incubation mixture was composed of dextromethorphan (10–54 μM) and an NADPH-generating system (25 mM $MgCl_2$, 5 mM NADP, 25 mM isocitrate and 5 units isocitrate dehydrogenase, type IV: Sigma Chemical Co., St Louis, MO, USA). All reagents were dissolved in 0·1 M sodium phosphate buffer (pH 7·4). After 5 min preincubation at 37°C, the reaction was initiated by the addition of 300 μg microsomal protein to make a total incubation volume of 1 mL. The reaction was terminated after 30 min by cooling on ice and the addition of 100 μL 60% $HClO_4$. Proteins were sedimented by centrifugation at 10 000 *g* for 5 min and the supernatant was stored at −80°C until analysis.

For inhibition studies, amiodarone or DEA was added to attain a final concentration of 15–150 μM.

Under the experimental conditions used, reaction rates were shown to be linear for incubation times to at least 1 h and for microsomal protein concentrations up to $1{\cdot}2\,mg\,mL^{-1}$.

Assay for dextrorphan. The concentration of dextrorphan was determined by reversed-phase HPLC. A sample (100 μL) of the microsomal supernatant from the incubations as described above was injected directly onto a Bondapak C_{18} column (Waters Associates). The mobile phase was 10 mM sodium phosphate buffer, acetonitrile (80/20, v/v), pH 3, at a flow rate of $1{\cdot}8\,mL\,min^{-1}$. The column effluent was monitored with a fluorescence detector (Jasco Model FP-210, Japan Spectroscopic Co. Ltd, Tokyo) at excitation and emission wavelengths of 270 and 330 nm, respectively, and data recorded on a Waters 745 Data Module integrator. The limit of detection of dextrorphan was 1 ng per injection.

Data analysis. The maximum velocity (V_{max}) and the apparent Michaelis–Menten constant (K_m) were obtained by graphical analysis of Lineweaver–Burk plots (Lineweaver & Burk 1934). Inhibitory constants (K_i values) were obtained graphically by the method of Dixon (1953).

Results

Analyses of dextromethorphan *O*-demethylation kinetics in human and rat liver microsomes are shown in Table 1. The

Table 1. The parameters of dextromethorphan *O*-demethylation kinetics and the K_i value for amiodarone and desethylamiodarone in human liver microsomes.

	V_{max} (nmol (mg protein)$^{-1}$ h^{-1})	K_m (μM)	K_i (amiodarone) (μM)	K_i (desethylamiodarone) (μM)
Human				
Liver 1	5·74	10·81	60	32
Liver 2	6·72	6·76	50	40
Liver 3	6·32	7·47	51	38
Liver 4	8·02	7·82	62	33
Liver 5	9·80	7·48	50	31
Liver 6	9·12	8·92	53	36
Liver 7	8·57	6·94	48	32
Liver 8	9·21	7·71	52	37
Liver 9	7·34	8·42	45	30
Liver 10	8·82	9·31	56	35
Mean ± s.d.	7·96 ± 1·37	8·16 ± 1·23	52·70 ± 5·27	34·40 ± 3·30
Rat				
Liver 1	9·05	15·90	50	32
Liver 2	6·77	11·21	60	37
Liver 3	7·07	8·24	88	44
Liver 4	7·89	8·05	85	39
Liver 5	8·42	11·42	70	38
Liver 6	7·24	9·31	66	42
Liver 7	9·01	10·74	76	35
Liver 8	5·92	9·24	59	41
Liver 9	7·56	8·75	80	45
Liver 10	8·06	10·31	67	40
Mean ± s.d.	7·69 ± 0·99	10·31 ± 2·29	70·10 ± 12·16	38·30 ± 3·65

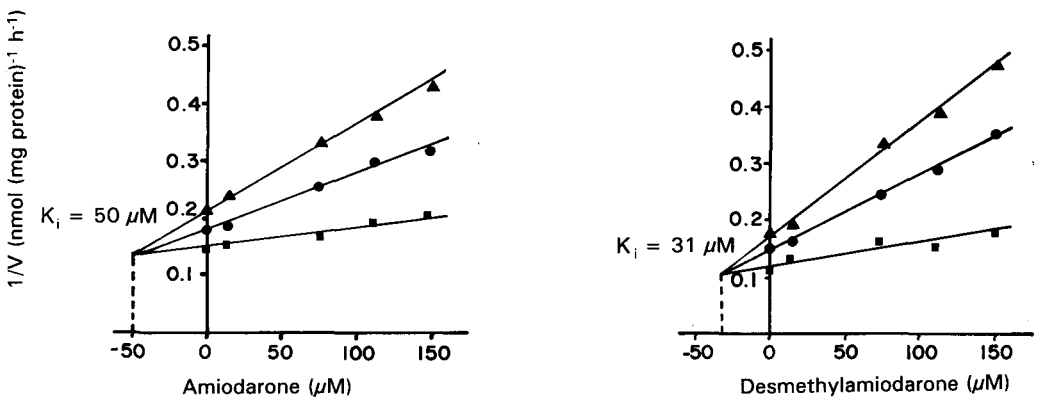

FIG. 1. Dixon plot of human liver dextromethorphan *O*-demethylation. The kinetics of competitive inhibition of dextromethorphan *O*-demethylation by amiodarone and desethylamiodarone (in human liver 5) in the presence of 27 (▲), 81 (●) and 135 (■) μM dextromethorphan are shown.

kinetics of inhibition of human and rat liver dextromethorphan *O*-demethylation activity by amiodarone or DEA was determined by incubating microsomes with various concentrations of dextromethorphan in the absence or presence of 100 μM amiodarone or DEA. Analysis of these data by double reciprocal (Lineweaver–Burk) plot, showing the same intercept on the Y(1/V) axis, indicated that inhibition was competitive. Further analysis was carried out by using varying concentrations of amiodarone or DEA in the presence of 27, 81 and 135 μM dextromethorphan. The K_i values for amiodarone and DEA inhibition of dextromethorphan *O*-demethylation are shown in Table 1 and Fig. 1.

Discussion

Previous studies showed that amiodarone inhibits dextromethorphan *O*-demethylase activity in monkey liver microsomes and may impair the elimination of dextromethorphan in extensive metabolizers of dextromethorphan (Funck-Brentano et al 1991). In the present studies in human liver microsomes, we have confirmed that amiodarone has a competitive inhibitory effect on dextromethorphan *O*-demethylation and have also shown that DEA has an inhibitory effect on the same isozyme. In addition, the results from our rat liver microsomal experiments are also in agreement with those of human microsomal studies. These findings suggest that amiodarone and DEA have an inhibitory effect on CYP2D6 in man and CYP2D1 in rats. During long-term administration of amiodarone, the concentrations of DEA in plasma and tissues are high (Holt et al 1983); therefore, we conclude that this metabolite may contribute to the inhibitory effect of the parent drug. Furthermore, both amiodarone and DEA have a very long plasma elimination half-life (Holt et al 1983), which suggests that the potential for drug interaction persists long after amiodarone treatment has stopped.

Many competitive inhibitors of CYP2D6 are substrates of

this enzyme activity, but some are not, such as quinidine and quinine (Brosen et al 1990; Wanwimolruk & Chalcroft 1991). Therefore, we cannot conclude that amiodarone and DEA are substrates of CYP2D6 in man or of CYP2D1 in rats. However, previous studies in female Dark-Agouti rats, an animal model of genetic debrisoquine hydroxylation deficiency, revealed that concentrations of amiodarone and DEA in plasma and liver were increased in this rat strain (Pirovino et al 1990). This suggested that the metabolism of amiodarone and DEA to other metabolites may in part co-segregate with debrisoquine 4-hydroxylation/dextromethorphan *O*-demethylation; further studies are required to investigate the clinical relevance of drug interaction between amiodarone and its metabolite and other drugs which co-segregate with debrisoquine/dextromethorphan, and to elucidate the metabolic pathways of amiodarone and its metabolite.

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Book Review

Indian Medicinal Plants. A Compendium of 500 Species. Vol. 1
Edited by P. K. Warrier, V. P. K. Nambiar and C. Ramankutty
Published 1993 Orient Longman Limited, Madras
394 pages
ISBN 0 86311 464 4 £25.95

When I was asked to review this book, my heart sank somewhat. What? Yet another volume on Indian medicinal plants? Not one volume exactly, but the first in a series of five volumes which will list alphabetically 500 plant species used in the Ayurveda system of alternative medicine. Volume 1 runs from *Abelmoschus esculentus* to *Carthmaus tinctorius*. Each entry includes Latin name, common names, distribution in India, morphological description, parts used and then medicinal properties and uses. For the Indian reader, there then follow some quotations from ancient verse texts. Notes follow if there is some uncertainty about the botanical identity of the plant in question. There are black and white line illustrations for each plant, as well as the occasional colour plate to brighten up the proceedings.

Plants used in the Ayurveda system include a number which are not normally classified as medicinal, such as the onion, pineapple, beetroot, peanut, cabbage and so on. Also, plants used chiefly as a source of colouring (e.g. *Bixa*) or insecticide (e.g. *Azadirachta*) are included. The possible hazards of imbibing some plant extracts are rarely mentioned. The entry under *Abrus precatorius* does not stress the very toxic nature of the seed protein or that the leaves are non-poisonous. What is also missing is any information on either the chemistry of the active principles or anything on the phytochemistry. Whether you buy this book or not will depend on how committed you are to the Ayurveda system of complementary medicine. Otherwise, it will provide the ethnobotanist with a list of plants and their reputed properties, as well as a means of checking the accurate identity of an uncertain plant specimen without going to the herbarium.

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