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SHORT COMMUNICATIONS

Characterization of the enzyme responsible for the metabolism of sumatriptan in human liver

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Abstract—Studies have been undertaken to investigate the enzymes responsible for the metabolism of [^{14}C]sumatriptan in man. Oxidative deamination of sumatriptan to form the indole acetic acid derivative is the only phase 1 pathway evident in man and both cytochrome P450 (P450) and monoamine oxidase (MAO) are capable of catalysing this type of reaction. The metabolism of [^{14}C]sumatriptan was therefore investigated *in vitro* in a preparation derived from human liver, which was shown, by the use of the probe substrates [^{14}C]testosterone (P450), [^{3}H]5HT (MAO-A) and [^{14}C]benzylamine (MAO-B) to be a rich source of both enzyme systems. Incubation with clorgyline and deprenyl, probe inhibitors of MAO-A and MAO-B, respectively, showed that [^{14}C]sumatriptan was metabolized by MAO-A; there was no evidence of P450 involvement in its metabolism. The data in this study therefore indicate that the enzyme MAO-A is the major enzyme responsible for the metabolism of sumatriptan in human liver.

Key words: cytochrome P450; monoamine oxidase; oxidative deamination

Sumatriptan succinate (IMIGRAN™; IMITREX™*) is a novel 5HT-1 like receptor agonist recently introduced for the episodic treatment of migraine and cluster headache [1]. Metabolism of the drug, the major clearance process, was recently described in animals and man [2], and is shown to involve oxidative N-deamination of the *N*-dimethyl side chain to form the indole acetic acid metabolite (Fig. 1). This was the only phase 1 route evident in man, with a portion of the metabolite being subsequently conjugated to form the glucuronide on the carboxylic acid moiety.

Oxidation of substituted amines can be mediated via several enzymes, including P450† [3] and MAO [4]. Both enzymes convert the amine moiety to the corresponding aldehyde, which may be oxidized to the respective acid by further enzymatic attack [5], prior to elimination or conjugation. Since sumatriptan shares some structural similarity to 5HT, which is metabolized predominantly by MAO, it was of interest to determine whether the drug was a substrate for this enzyme and/or for P450.

In order to investigate the potential involvement of the two enzyme systems in the metabolism of sumatriptan in man, we studied the metabolism of [^{14}C]sumatriptan *in vitro* using human liver. The *in vitro* preparation was characterized using [^{14}C]testosterone, a known substrate for several P450 isozymes including a major human form [6], to quantify P450 activity. Two probes, [^{3}H]5HT and [^{14}C]benzylamine, which are known substrates for MAO-A and MAO-B, respectively [7], were used to quantify the MAO activity present. Clorgyline and deprenyl, compounds known to preferentially inhibit MAO-A and MAO-B activity, respectively [7], were used as probe inhibitors, and the NADPH dependence of P450 activity was also used as a diagnostic tool.

* IMIGRAN™ and IMITREX™ are trade marks owned by the Glaxo Group of companies.

† Abbreviations: 5HT, 5-hydroxytryptamine; MAO, monoamine oxidase; P450, cytochrome P450.

Materials and Methods

Chemicals. [^{14}C]Sumatriptan succinate (sp. act. 2060 MBq/mmol, radiochemical purity >92%) and its metabolites (Fig. 1) were obtained from the Process or Chemical Research Departments, Glaxo Group Research Ltd (Ware, U.K.). [^{3}H]5HT creatinine sulphate (sp. act. 407,000 MBq/mmol, radiochemical purity >98%), [^{14}C]benzylamine hydrochloride (sp. act. 2000 MBq/mmol, radiochemical purity >98%) and [^{14}C]testosterone (sp. act. 2020 MBq/mmol, radiochemical purity >98%) were obtained from Amersham International plc (Little Chalfont, U.K.). 5HT creatinine sulphate, benzylamine hydrochloride, NADPH and clorgyline [*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)propylamine] were supplied by the Sigma Chemical Co. (Poole, U.K.). Deprenyl (selegiline hydrochloride) was a gift from Britannia Pharmaceuticals Ltd (Redhill, U.K.).

Preparation of the human liver in vitro *system.* Human liver samples (three male and one female) were acquired as surgical waste from essentially normal livers and 9000 *g* supernatants were prepared using standard homogenization and centrifugation methods. These supernatants were centrifuged at 100,000 *g* and suspensions prepared from the resulting pellets. The levels of protein (3–6 mg/mL) and total cytochrome P450 (0.4–0.7 nmol/mg protein) present in the suspensions were determined using modifications of the methods of Lowry *et al.* [8] and Omura and Sato [9], respectively. The presence of mitochondria in the suspensions was confirmed by quantifying the activity of the marker enzyme, succinate dehydrogenase [10], present in the suspensions (16–80 nmol/min/mg protein) using a modification of the method of Prospero [11].

The effect of NADPH on the metabolism of the substrates by the human liver in vitro *preparation.* The metabolism of all substrates was assessed in both the absence and presence of NADPH (500 μM), and compared to boiled suspensions. Each of the three probe substrates [^{3}H]5HT, [^{14}C]benzylamine and [^{14}C]testosterone, together with [^{14}C]sumatriptan, were incubated individually with the

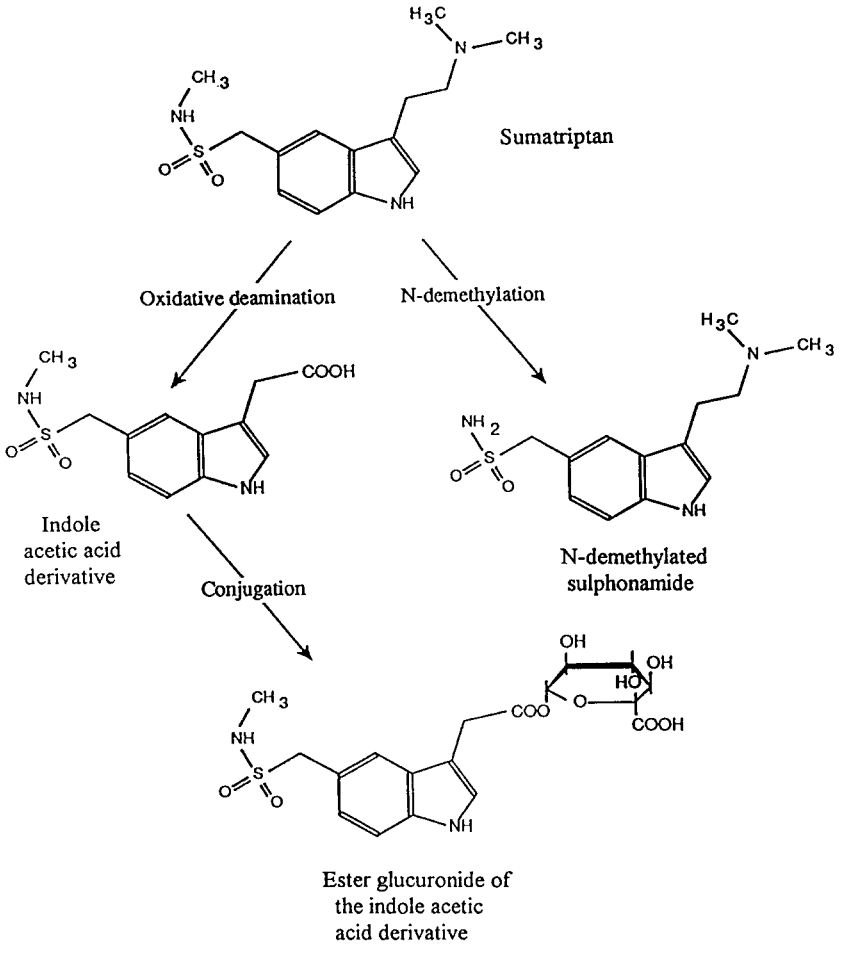


Fig. 1. The identified routes of metabolism of sumatriptan.

100,000 *g* suspensions at 37° for 30 min, 15 min, 30 min and 4 hr respectively, to achieve sufficient metabolism. Each incubate comprised a portion of suspension made up to 2.5 times its volume with 0.05 M phosphate buffer at pH 7.2. The final substrate concentration was 100 μM for all compounds (0.007, 0.004, 0.2 and 0.2 MBq/mL for the four substrates, respectively).

The metabolism of [^{3}H]5HT and [^{14}C]benzylamine was determined by acidification of the incubates with hydrochloric acid, to give a final concentration of 1 M, followed by selective extraction of the metabolites into water-saturated ethyl acetate. The metabolites were quantified by liquid scintillation counting.

The metabolism of [^{14}C]testosterone and [^{14}C]sumatriptan

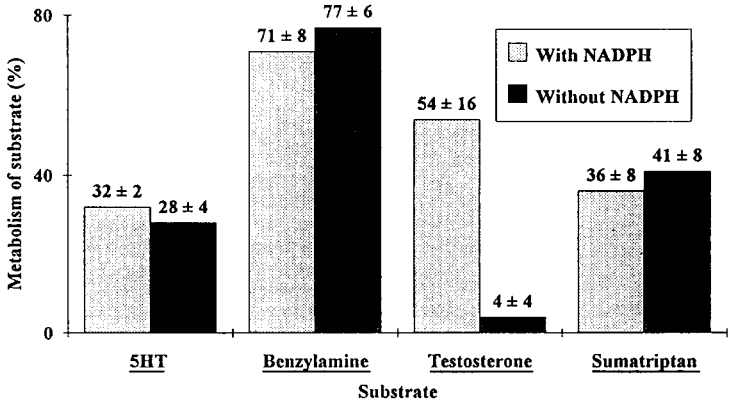


Fig. 2. The effect of NADPH on the metabolism of 5HT, benzylamine, testosterone and sumatriptan by the human liver *in vitro* preparation.

was determined by TLC [12, 13] of the incubates, following their dilution with an equal volume of acetonitrile. The major metabolite formed from [^{14}C]sumatriptan *in vitro* was isolated and identified by mass spectrometry.

The effect of clorgyline and deprenyl on the metabolism of the substrates by the human liver in vitro *preparation.* The metabolism of each of the four substrates in the presence of NADPH was also investigated in the presence of the two MAO inhibitors, clorgyline and deprenyl. The methods used were as specified above, except that the inhibitors were added to the incubates over a range of 2–200 μM. The percentage inhibition was calculated with reference to the metabolism observed in the absence of the inhibitors for each substrate.

Results

The effect of NADPH on the metabolism of the substrates by the human liver in vitro *preparation.* The mean (N = 4) results obtained for substrate metabolism in the presence and absence of NADPH (500 μM) are shown in Fig. 2. In the presence of NADPH all four substrates were extensively metabolized. In the absence of NADPH the percentage turnover of [^{3}H]5HT, [^{14}C]benzylamine and [^{14}C]sumatriptan was essentially unaltered. However, [^{14}C]testosterone metabolism was reduced more than 10-fold in the absence of NADPH.

The effect of clorgyline and deprenyl on the metabolism of the substrates by the human liver in vitro *preparation.* The mean (N = 4) results obtained for inhibition of

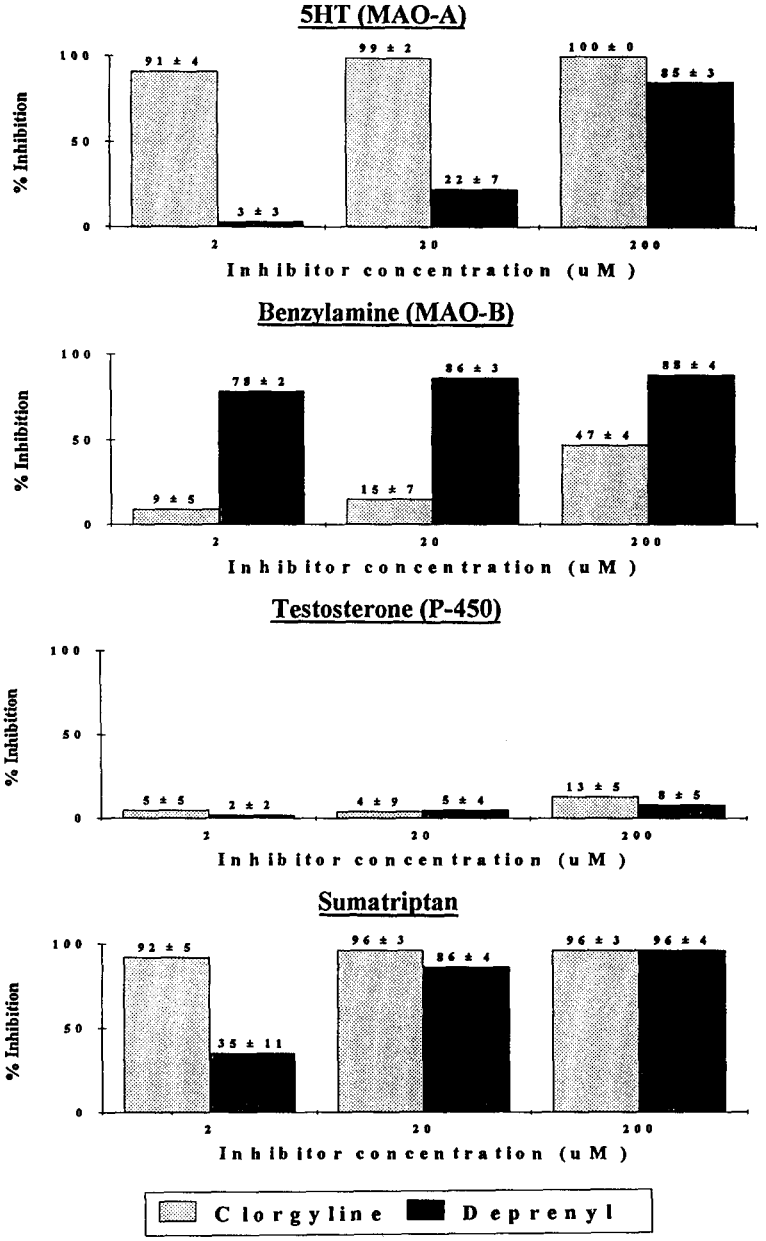
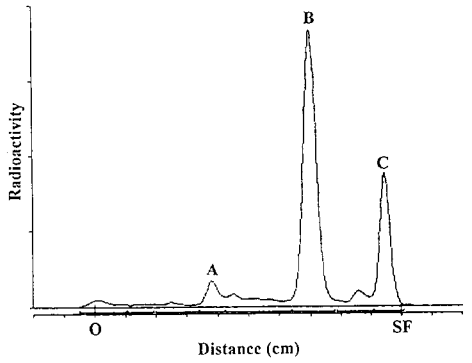


Fig. 3. The effect of clorgyline and deprenyl on the metabolism of 5HT, benzylamine, testosterone and sumatriptan by the human liver *in vitro* preparation.



O Origin A Indole acetic acid derivative
SF Solvent front B Sumatriptan
C Indole ethyl alcohol derivative

Fig. 4. The metabolic profile of [^{14}C]sumatriptan following incubation in the human liver *in vitro* preparation in the presence of NADPH.

substrate metabolism by clorgyline and deprenyl are shown in Fig. 3. The metabolism of [^{3}H]5HT was effectively blocked by the presence of clorgyline over the concentration range 2–200 μM, >90% inhibition being apparent at 2 μM. Extensive inhibition of [^{3}H]5HT metabolism was also observed in the presence of 200 μM deprenyl, although concentrations of $\leq$20 μM caused only minimal inhibition. [^{14}C]Benzylamine metabolism was only partially inhibited by the presence of clorgyline, with about 50% inhibition evident at 200 μM. However, deprenyl caused substantial inhibition of substrate metabolism over the concentration range 2–200 μM. Neither clorgyline nor deprenyl had a marked inhibitory effect on the metabolic turnover of [^{14}C]testosterone at inhibitor concentrations of $\leq$200 μM. The metabolism of [^{14}C]sumatriptan was effectively blocked by the presence of clorgyline at the lowest inhibitor concentration used. At the same concentration, deprenyl caused only limited inhibition, with total inhibition occurring when the deprenyl concentration was increased to 200 μM.

The metabolic profile of [^{14}C]*sumatriptan following incubation in the human liver* in vitro *preparation.* The metabolite profile of [^{14}C]sumatriptan in the presence of NADPH (500 μM) (Fig. 4) was qualitatively and quantitatively similar to that observed in the absence of NADPH. TLC demonstrated that unmetabolized [^{14}C]sumatriptan represented a mean of 60% of the drug-related material present in the extracts, with the major metabolite accounting for a further 26% of the material. The identity of this metabolite was determined by mass spectrometry to be the indole ethyl alcohol derivative of sumatriptan (Fig. 5). The chromatographic behaviour of the minor metabolite (6% of the total material) evident in the extracts was consistent with the indole acetic acid derivative of sumatriptan.

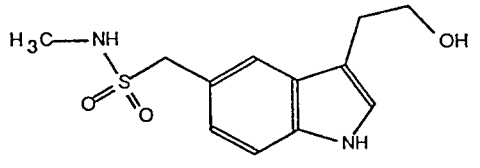


Fig. 5. The structure of the indole ethyl alcohol derivative of sumatriptan.

Discussion

The use of preparations from human liver and cell lines containing human drug metabolizing enzymes to characterize the enzymes responsible for metabolism of drugs is growing in importance. This information enables better prediction of drug interactions or potential impact of genetic polymorphisms in the clinical use of drugs. We have used an *in vitro* approach utilizing a human liver preparation to identify the major enzyme responsible for the metabolism of sumatriptan in man.

The major metabolite of sumatriptan in man is the indole acetic acid derivative formed by oxidative deamination [2]. This reaction may be catalysed by at least two enzyme systems, P450 or MAO. It was therefore appropriate to examine the metabolism of the drug in a matrix containing both enzymes. Our initial studies with probe substrates for P450 and MAO, i.e. testosterone (P450), [^{3}H]5HT (MAO-A) and [^{14}C]benzylamine (MAO-B), confirmed that the *in vitro* system used was rich in both enzymes. In addition, the co-factor requirements for turnover of the probe substrates confirmed their selectivity as probes, with [^{14}C]testosterone turnover alone being dependent on the presence of NADPH.

The inhibition of the MAO activities in the preparation by clorgyline and deprenyl was in agreement with the selectivity reported in the literature for these agents [7], with clorgyline showing marked specificity as an inhibitor of [^{3}H]5HT metabolism (MAO-A) and deprenyl of [^{14}C]benzylamine metabolism (MAO-B). These inhibitors did not influence the metabolism of [^{14}C]testosterone significantly, indicating a lack of inhibitory action on P450 at the concentrations used.

The results with sumatriptan indicate that the metabolism was independent of NADPH and was inhibited by the MAO inhibitors, with clorgyline being more effective than deprenyl. Taken together these results clearly indicate that the predominant enzyme responsible for the metabolism of sumatriptan in human liver is MAO-A.

The major product of metabolism of sumatriptan *in vitro* is consistent with MAO conversion to the aldehyde, prior to further conversion to the alcohol derivative. Although the primary product seen in excreta in man *in vivo* is the carboxylic acid, it is known that aldehydes may be converted to their alcohol derivatives [4]. The conditions present in this *in vitro* preparation would appear to favour this latter reaction.

In conclusion, these results, obtained using an *in vitro* preparation derived from human liver, suggest that MAO-A is the major enzyme responsible for the metabolism of sumatriptan in man.

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