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Metabolism of the monoamine oxidase-B inhibitor, MD 780236 and its enantiomers by the A and B forms of the enzyme in the rat

M. STROLIN BENEDETTI*, J. DOW, T. BOUCHER, P. DOSTERT, *Centre de Recherche Delalande, 10, rue des Carrières, 92500 Rueil-Malmaison, France*

MD 780236, 3-[4-((3-chlorophenyl)methoxy)-phenyl]-5-[(methylamino)methyl]-2-oxazolidinone methane sulphonate, is a selective inhibitor of MAO-B in the rat, which behaves as an irreversible inhibitor as well as a substrate in-vitro (Tipton et al 1983), and as a short-acting inhibitor ex-vivo although some residual inhibition is still present 48 h after administration, indicating the presence of an irreversible component (Strolin Benedetti et al 1982, 1983a). Its enantiomer with the *R* absolute configuration (MD 240928) is a totally short-acting inhibitor ex-vivo, as no inhibition of MAO-B is present at 24 h, whereas the *S*-enantiomer (MD 240931) retains the irreversible component of the inhibition seen with MD 780236 (Dostert et al 1983). Previous studies on MD 780236 in the rat (Strolin Benedetti & Dow 1983) showed that pretreatment with clorgyline 1 h before drug administration resulted in a long-lasting inhibition of MAO-B ex-vivo as inhibition at 24 h was as high as that at 1 h. In-vitro studies with the ^{14}C labelled compound in rat brain homogenates, demonstrated that selegiline ((−)-deprenyl) slightly inhibited the metabolism of MD 780236, whereas clorgyline almost completely inhibited its metabolism (Strolin Benedetti & Dow 1983). We concluded from these ex-vivo and in-vitro results that the A form of MAO played the dominant role in the metabolism of this selective MAO-B inhibitor.

This work has been continued using the *R*- and *S*-enantiomers of MD 780236. Studies on the ex-vivo inhibition of MAO-B in rat brain and liver after pretreatment with clorgyline followed 1 h later by administration of the *R*- or *S*-enantiomer have been carried out. In-vitro studies on the metabolism of the ^{14}C-labelled *R*-enantiomer, MD 240928, compared with that of the racemic compound, have also been carried out in rat brain and liver homogenates, with or without selegiline or clorgyline.

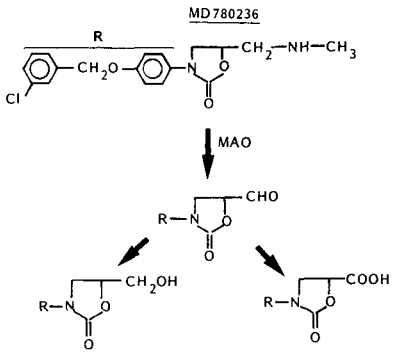

FIG. 1. Oxidative deamination of MD 780236 by monoamine oxidase (MAO).

* Correspondence.

Materials and methods

Chemicals

[^{14}C]-MD 780236 (spec. act. 23 mCi mmol^{-1}) and [^{14}C]-MD 240928 (spec. act. 18 mCi mmol^{-1}) both as the methane sulphonate salt, and with the ^{14}C label in the carbonyl group of the oxazolidinone ring were synthesized by C.E.A., Saclay, France. Both compounds were shown by t.l.c. to have a radiochemical purity >96%. All other chemicals were obtained as previously described (Dostert et al 1983; Strolin Benedetti & Dow 1983).

Animal treatment

Male Sprague Dawley rats (Charles River, CD, France) of approximately 150 g, were fasted overnight (16 h) before drug administration.

Metabolism in-vitro—linearity

Brains and livers of untreated rats were homogenized in 0·1M phosphate buffer (pH 7·4) to give a 5% w/v suspension. After preincubation of homogenate (1 ml) at 37 °C for 20 min, [^{14}C]MD 780236 or [^{14}C)]-MD 240928 was added to give a final concentration of 5×10^{-6} or $2{\cdot}5 \times 10^{-6}$M respectively. Homogenates with [^{14}C]MD 780236 or [^{14}C]MD 240928 were incubated in duplicate by agitation at 37 °C in presence of air for 0, 1, 2, 3, 5, 7, 10 and 15 min, and the reaction stopped with cold methanol (2 ml) containing non-radioactive reference compounds. Separation and quantification of reaction products was carried out by t.l.c. as previously described (Strolin Benedetti & Dow 1983), and the formation of the aldehyde metabolite was used to assess linearity.

Metabolism in-vitro—effect of selegiline, clorgyline or a mixture of both compounds

Incubations of homogenates (1 ml) of brain and liver with [^{14}C]MD 780236 (5×10^{-6}M), or [^{14}C]MD 240928 ($2{\cdot}5 \times 10^{-6}$M) were carried out in duplicate at 37 °C for 2 min (liver) and 10 min (brain) in the presence of selegiline (4×10^{-7}M) or clorgyline (4×10^{-7}M). The concentrations of selegiline and clorgyline have been

Table 1. Effect of selegiline (4×10^{-7}M) or clorgyline (4×10^{-7}M) on the in-vitro metabolism of [^{14}C]MD 780236 (5×10^{-6}M) or of [^{14}C]MD 240928 ($2{\cdot}5 \times 10^{-6}$M) by rat brain homogenates (5%, w/v) in 0·1M phosphate buffer (pH 7·4) incubated (1 ml) at 37 °C for 10 min. Selegiline or clorgyline were preincubated for 20 min. Results are expressed as the percentage of aldehyde metabolite formed in the incubation mixture (means of 2 separate incubations).

	[^{14}C]MD 780236 (5×10^{-6}M)		[^{14}C]MD 240928 ($2{\cdot}5 \times 10^{-6}$M)	
Incubation conditions	% aldehyde formed	% inhibition of aldehyde formation	% aldehyde formed	% inhibition of aldehyde formation
Controls	8·2		15·4	
+ selegiline (4×10^{-7}M)	7·0	15	12·4	20
+ clorgyline (4×10^{-7}M)	1·0	88	2·0	87

chosen taking into account results from previous experiments with rat brain homogenates (Fowler & Strolin Benedetti 1983) and rat liver homogenates (M. Strolin Benedetti & T. Boucher, unpublished results). Clorgyline and selegiline were preincubated in homogenates for 20 min before the addition of labelled compounds.

MAO-B inhibition ex-vivo—rat liver and brain homogenates

This was determined as previously described (Dostert et al 1983; Strolin Benedetti & Dow 1983) with [^{14}C] phenethylamine as substrate in rats pretreated with clorgyline (10 mg kg^{-1} oral) 1 h before MD 240928 (5 mg kg^{-1} oral) or MD 240931 (5 mg kg^{-1}, oral) and killed at different times after MD 240928 or MD 240931.

Results

Under the experimental conditions used, aldehyde formation in brain homogenates with [^{14}C]MD 780236 or [^{14}C]MD 240928 as substrate was linear for at least 15 min, whereas in liver homogenates product formation was linear for only 3 min. An incubation time of 10 min was therefore chosen for brain homogenates, and of 2 min for liver homogenates in all further experiments. Under these conditions, the acid, alcohol and other metabolites (Fig. 1) represented less than 2% of the radioactivity in the incubation mixtures.

The effect of selegiline and clorgyline on the in-vitro metabolism of [^{14}C]MD 780236 and [^{14}C]MD 240928 by brain homogenates is shown in Table 1. In the absence of clorgyline or selegiline, the percentage of aldehyde formed with [^{14}C]MD 780236 was roughly the half of that with [^{14}C]MD 240928, when the concentration of the *R*-isomer corresponded to that in the racemic mixture. Selegiline inhibited the formation of aldehyde between 15–20%, (and appeared to have a slightly greater effect on the inhibition of the metabolism of [^{14}C]MD 240928). Clorgyline produced a much greater inhibition of metabolism of both compounds, with about 88% inhibition of the formation of aldehyde.

In a similar experiment with rat liver homogenates (Table 2) selegiline again inhibited the formation of aldehyde from both compounds. In this tissue inhibition of the formation of aldehyde was about 30%. Clorgyline again produced a greater inhibition of metabolism, of between 66 and 78%. The difference in inhibition of aldehyde formation in brain and liver in the presence of selegiline or clorgyline may reflect the difference in the relative proportions of MAO-A and -B in these tissues (Fowler et al 1981; Houslay & Tipton 1976; Strolin Benedetti et al 1983b). Fig. 2a, b shows that MAO-B inhibition by the *R*-enantiomer MD 240928, measured at different times ex-vivo in both liver (a) and brain (b), was of relatively short duration, consistent with it acting as a fully reversible inhibitor, and that pretreatment with clorgyline had no significant effect on the time-course of MAO-B inhibition. In contrast, the time-course of ex-vivo inhibition of MAO-B by the *S*-enantiomer (MD 240931) showed a proportion of the initial inhibition to persist for a considerably longer time in both liver (Fig. 3a) and brain (Fig. 3b). This persistent proportion of the inhibitory effect of MD 240931 can be ascribed to the irreversible inhibition of MAO-B by this compound, as has been previously shown to be the case with the racemic mixture (MD 780236) (Dostert et al 1983). As we have previously observed with the racemic

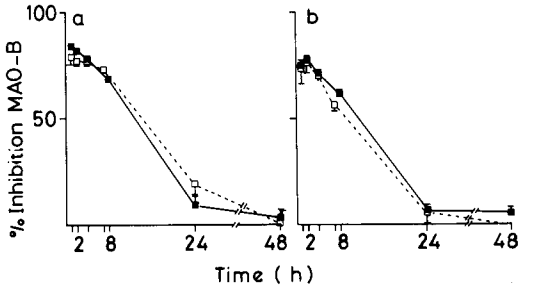

FIG. 2. Ex-vivo inhibition of (a) liver and (b) brain MAO-B in rats treated with MD 240928 (5 mg kg^{-1}, oral) (■) or pretreated with clorgyline (10 mg kg^{-1}, oral) (□) 1 h before MD 240928. The percentage inhibition of MAO-B by MD 240928 after pretreatment with clorgyline was calculated using clorgyline-pretreated rats as controls.

Table 2. Effect of selegiline (4×10^{-7}M) or clorgyline (4×10^{-7}M on the in-vitro metabolism of [^{14}C]MD 780236 (5×10^{-6}M) or of [^{14}C]MD 240928 ($2{\cdot}5 \times 10^{-6}$M) by rat liver homogenates (5%, w/v) in 0·1M phosphate buffer (pH 7·4) incubated (1 ml) at 37 °C for 2 min. Selegiline or clorgyline were preincubated for 20 min. Results are expressed as the percentage of aldehyde metabolite formed in the incubation mixture (means of 2 separate incubations).

	[^{14}C]MD 780236 (5×10^{-6}M)		[^{14}C]MD 240928 ($2{\cdot}5 \times 10^{-6}$M)	
Incubation	% aldehyde formed	% inhibition of aldehyde formation	% aldehyde formed	% inhibition of aldehyde formation
Controls	6·5		15·1	
+ selegiline (4×10^{-7}M)	4·4	32	10·7	29
+ clorgyline (4×10^{-7}M)	2·2	66	3·3	78

mixture (Strolin Benedetti & Dow 1983), pretreatment with clorgyline prolonged the initial MAO-B inhibition for at least 48 h.

Discussion

In-vitro inhibition studies with MD 780236 have confirmed our previous studies (Strolin Benedetti & Dow 1983), which were carried out with brain homogenates. In the present work, the oxidation of the inhibitor was studied under conditions where the formation of aldehyde product was linear with time. This metabolite (Fig. 1) represents the true product of the oxidative deamination of MD 780236 by monoamine oxidase (Tipton et al 1983; Strolin Benedetti & Dow 1983) and the production of further, or alternative, metabolites was small under these conditions. In absence of clorgyline or selegiline, the *R*-isomer (MD 240928) and the racemic mixture (MD 780236) appeared to be metabolized by brain and liver homogenates in a similar manner. Although the percentage of aldehyde formed was greater with the *R*-isomer than with the racemic mixture, the significance of this difference would require a more detailed knowledge of the kinetics of the oxidation of the two isomers by MAO and their effectiveness as inhibitors of the enzyme. The metabolism of the *R*-isomer, as that of the racemic mixture, was strongly inhibited by clorgyline which shows the A form of MAO to play the dominant role in the metabolism of these compounds under these conditions. Preincubation with selegiline produced a small inhibition of aldehyde formation, both in the case of the racemic mixture and in that of MD 240928, which is consistent with a smaller involvement of MAO-B in the metabolism of MD 240928.

Ex-vivo inhibition studies have shown a proportion of the inhibition given by the *S*-enantiomer (MD 240931) to be of relatively long duration, consistent with it being due to irreversible inhibition of MAO-B in liver and brain as has previously been observed with MD 780236 (Dostert et al 1983). In contrast, the MAO-B inhibition by the *R* enantiomer was of relatively short duration, as has been previously demonstrated (Dostert et al 1983). Pretreatment with clorgyline prolonged the initial high level of MAO-B inhibition observed with MD 240931 for at least 48 h. This is consistent with MAO-A playing the predominant role in the removal of MD 240931. Pretreatment with clorgyline produced essentially the same results with MD 780236 as with the *S*-isomer (Strolin Benedetti & Dow 1983), suggesting the observed long duration of MAO-B inhibition by the racemic mixture after clorgyline pretreatment to be due to its constituent *S*-isomer.

Pretreatment with clorgyline did not prolong the ex-vivo MAO-B inhibition by the *R*-enantiomer, showing MAO-A to play a less important role in terminating the activity of this compound.

Thus, both the ex-vivo and in-vitro results reported here are consistent with the two enantiomers being substrates for the A form of monoamine oxidase. The in-vitro experiments also showed the racemic mixture (MD 780236) and the *R*-isomer (MD 240928) to be poorer substrates for MAO-B. The behaviour of this system under initial-rate conditions may not, however, reflect the conditions in-vivo or ex-vivo, because much longer exposure of the enzyme to the inhibitor may be

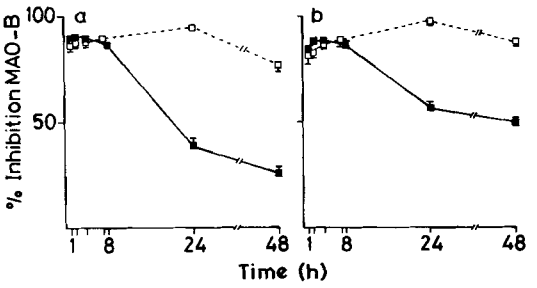

FIG. 3. Ex-vivo inhibition of (a) liver and (b) brain MAO-B in rats treated with MD 240931 (5 mg kg^{-1}, oral) (■) or pretreated with clorgyline (10 mg kg^{-1}, oral) (□) 1 h before MD 240931. The percentage inhibition of MAO-B by MD 240931 after pretreatment with clorgyline was calculated using clorgyline-pretreated rats as controls.

involved and MD 780236 has been shown to be a time-dependent irreversible inhibitor of MAO-B (Tipton et al 1983).

In conclusion, the *S*-isomer MD 240931 appears to be the agent responsible for the irreversible inhibition of MAO-B by MD 780236, although it can be metabolized by the A form. This isomer seems to be a selective inhibitor of the B form although it produces also a weak inhibition of the A form (Dostert et al 1983). The *R*-isomer, MD 240928, appears to be a selective inhibitor of the B form (IC50 = $3 \cdot 10^{-7}$M; IC50 value of $3 \cdot 10^{-8}$M appearing in Table 2 in Dostert et al 1983 was a transcription error), but substrate of both forms. It may be of interest to note that tricyclic antidepressants, like desipramine and nortriptyline, which are weak reversible inhibitors of MAO-B (Roth 1978), can also be metabolized via deamination of the aminopropyl side chain and oxidation of the resulting aldehyde (Beckett & Hutt 1982).

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