IN VITRO METABOLISM OF TRESPERIMUS BY HUMAN VASCULAR SEMICARBAZIDE-SENSITIVE AMINE OXIDASE

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ABSTRACT:

Tresperimus (Cellimis), a new immunosuppressive agent is mainly eliminated through an extensive nonhepatic metabolism, in which the oxidative deamination of the primary amine of the drug takes a preponderant part. We have previously demonstrated the ability of human plasma semicarbazide-sensitive amine oxidase (SSAO) to catalyze this reaction. Therefore, the suitability of human umbilical artery, a tissue combining a high SSAO activity with monooamine oxidase activity, to study tresperimus metabolism was tested, and the kinetic behavior of tissue-bound SSAO was compared with that of plasma soluble SSAO. All the oxidized metabolites resulting from the deamination of tresperimus and of two other metabolites, desaminopropyl derivatives of tresperimus and guanidinohexylamine, were formed in vascular homogenates. Chemical inhibition experiments demonstrated the major involvement of SSAO in the metabolism of these three compounds at physiologically relevant concentrations. The micromolar fraction was used to characterize tresperimus deamination. Tissue-bound and soluble SSAO exhibited similar $K_m$ values for the drug and $K_I$ values of tresperimus toward benzylamine metabolism, a classical SSAO substrate. The kinetic behavior of both enzymes seemed to argue in favor of a same catalytic entity. Human umbilical artery constituted a relevant in vitro model to demonstrate the predominant role of SSAO in tresperimus metabolism. Our results suggest that the possible role of SSAO as Phase I oxidative enzymes has to be considered in metabolism studies for drugs encompassing primary amine.

Tresperimus (Cellimis), a new immunosuppressive polyamine inducing a specific tolerance in rat cardiac allograft model after a short-term treatment. It is effective in the prevention of murine graft-versus-host disease (Dutartre et al., 1995; Andoins et al., 1996, 1997). The drug is mainly eliminated through an extensive nonhepatic metabolism that occurs mainly at the primary amino terminal group but also to a much lesser extent, at the middle (amide bond) of the molecule (Fig. 1) (Claud et al., 2001). The presence of oxidative deamination reactions in both biotransformation pathways suggests that the amine oxidases play a major role in tresperimus metabolism. Moreover, the significant increase of tresperimus plasma concentrations observed in one patient with graft-versus-host-disease, treated simultaneously with isoniazid and tresperimus, seemed to support this hypothesis. Indeed, the antitubercular drug isoniazid inhibits plasma and tissue-bound SSAO$^1$ with a relatively weak effect upon monooamine oxidase (MAO) (Blaschko, 1962; Lewinsohn et al., 1978). Besides, the ability of plasma SSAO to deaminate tresperimus has been shown in vitro (Claud et al., 2001).

$^1$ Abbreviations used are: SSAO, semicarbazide-sensitive amine oxidase; MAO, monoamine oxidase; LC/MS, liquid chromatography/mass spectrometry; HPLC, high-performance liquid chromatography; BzAO, benzylamine oxidase; ALDH, aldehyde dehydrogenase; M3, desaminopropyl derivative of tresperimus; M6, guanidinohexylamine; M8, acid derivative of M6; LF 07-0109, N-{4-(aminoproplylamino)-butyl}-N’-{6-guanidino-hexyl}-malonamide.

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The heterogeneous class of amine oxidases can be divided into two types according to the chemical structure of their cofactor (Strolin-Benedetti and Tipton, 1998). MAO-A and -B contain flavin adenine dinucleotide as a prosthetic group, whereas a second group is composed of diamine oxidase, lysyl oxidase, and SSAO with topaquinone as a cofactor (Janes et al., 1990; Buffoni and Isgnati, 2000).

Amine oxidases can be distinguished by their different sensitivities to inhibitors. Indeed, semicarbazide at concentrations around 1 mM has a relatively weak effect on MAO, whereas SSAO is not affected by acetylenic aromatic amines such as clorgyline or deprenyl, which cause selective inhibition of MAO-A and MAO-B at concentrations inferior to 1 $\mu$M (Johnston, 1968; Knoll and Magyar, 1972; Lyles, 1996).

As opposed to platelet-poor plasma previously used to study in vitro tresperimus metabolism, both amine oxidase classes are present within blood vessels. Indeed, smooth muscle cells within the vascular wall represent one of the main tissue location of SSAO, and MAO activity is also well characterized in this tissue (Precious and Lyles, 1988; Berry et al., 1994). The major proportion of tissue SSAO activity is membrane-bound with the greatest enrichment after subcellular fractionation being in microsomal fraction. On the other hand, MAO is primarily localized in the outer mitochondrial membrane although some MAO activity is also associated with microsomes (Gómez et al., 1988).

The umbilical artery could thus constitute a relevant in vitro model to assess the contribution of the different amine oxidase classes to tresperimus metabolism in man. The suitability of homogenates of human umbilical artery to study tresperimus metabolism was tested by...
determining the metabolic profiles of the unchanged drug and of two metabolites [desaminopropyl derivative of tresperimus (M3) and guanidinohexylamine (M6)] subject to oxidative deamination reactions (Fig. 1). Metabolites formed by vascular homogenates were identified by LC/MS analysis. The optimal pH for tresperimus and M3 metabolism was determined using an HPLC assay. Chemical inhibition experiments were then performed to identify the class of amine oxidase involved in tresperimus, M3, and M6 metabolism. The microsomal fraction of umbilical artery was used to characterize tresperimus and M3 deamination. The kinetic parameters of membrane-bound and plasma-soluble SSAO for the drug and the inhibitory constant of tresperimus toward benzylamine, the classical synthetic substrate of SSAO, metabolism were allowed to compare the kinetic behavior of both enzymes.

Experimental Procedures

Chemicals. Tresperimus HCl was synthesized at Synkem (Chenôve, France), M3, M6, and LF 07-0109 as internal standard were synthesized by Laboratoires Fournier (Dijon, France). Benzylamine, R-(−)-deprenyl, clorgyline, hydralazine, and semicarbazide were purchased from Sigma Chemicals (Saint Quentin Fallavier, France), and potassium phosphate was obtained from Merck (Darmstadt, Germany).

Methods. Preparation of Enzyme Sources. Umbilical cords were supplied by the Maternity Unit, Chenôve Hospital (Chenôve, France). Arteries were dissected, washed in saline to eliminate blood as a potential source of contaminating plasma amine oxidases, and stored frozen at −80°C for up to 1 month before use in subsequent experiments. They were then homogenized in 10 mM potassium phosphate buffer, pH 7.4 (1 g of tissue/30 ml), and centrifuged at 600 g for 10 min. The supernatants from this low-speed centrifugation were used as the enzyme source or centrifuged (12,000 g for 20 min at 4°C) to eliminate mitochondria and big cellular debris. Subsequent centrifugation of the resulting supernatant (105,000 g for 1 h at 4°C) produced the microsomal pellet, which was suspended in the same buffer (3 ml of buffer for 100 mg of membrane fractions). A pool of around eight arteries was constituted for each experiment. All the experiments were performed with an enzyme source freshly prepared.

Human blood was collected into heparinized tubes by Bruant Laboratories (Dijon, France). Platelet-poor plasma was then separated by centrifugation at 2,400 g for 5 min at 4°C. A pool of plasma, freshly collected, was prepared from at least 15 healthy human volunteers per experiment.

Tresperimus, M3, and M6 Incubation. The incubation mixtures consisted of 1 ml of enzyme source (homogenates or microsomes of umbilical artery prepared in 10 mM potassium phosphate buffer and plasma). Sodium hydroxide was added to the vascular preparations before use for adjusting to the appropriate pH. The addition of sodium hydroxide did not significantly modify

Fig. 1. Metabolic scheme of tresperimus in man (Claud et al., 2001).
the ionic strength of the incubation medium. The reaction was initiated by the addition of 10 μl of substrate dissolved in potassium phosphate buffer at 10 mM, pH 7.4. At each incubation time, the reaction was stopped by transferring 0.4 ml of reaction mixture into tubes previously placed in ice. An acidic reagent containing citric acid (0.5 M)NaH₂PO₄ (0.5 M) 70:30 (v/v) was added at a concentration of 5% (v/v), and the tubes were centrifuged at 2,400 g for 5 min at 4°C and stored at -20°C until analysis.

**Tresperimus, Desaminopropyl Tresperimus, and Guanidinoaldehydazylamine HPLC Assay.** After a liquid-solid extraction on a C₁₈ cartridge of the sample, a reversed-phase ion-pairing HPLC method, similar to that developed previously, was used to quantify the unchanged tresperimus, M3, and M6 (Claud et al., 2001).

The human plasma diluted at 1/50th with 10 mM potassium phosphate buffer, pH 7.4, has been used as a biological matrix to draw the calibration curves. The HPLC method has been fully validated, and the precision and accuracy of the method was lower than 8%.

**LC/MS Qualitative Analysis of Tresperimus Metabolism.** LC/MS was used to identify the metabolites of tresperimus, M3, and M6 produced during incubations in homogenates and microsomes of umbilical artery. The treatment of samples and analytical conditions were previously published (Claud et al., 2001).

The mass spectrometer operated in positive-ion with selective ion monitoring as previously described (Claud et al., 2001). The full scan detection mode was previously used to check that no other new metabolite was produced.

**Effects of Tresperimus upon Benzylamine Metabolism in Microsomes of Umbilical Arteries and in Human Plasma.** A typical incubation medium was composed of 300 μl of potassium phosphate (0.1 M, pH 7.8), 50 μl of 10 mM deprenyl in 0.01 M hydrochloric acid or hydrochloric acid alone, 50 μl of tresperimus in potassium phosphate buffer, pH 7.8, or buffer alone, and 50 μl of enzyme source. The reaction was started by the addition of 50 μl of benzylamine (0.01 M HCl). The reaction was terminated by the addition of 50 μl of 40% trichloroacetic acid, and the tubes were centrifuged for 5 min at 2,500g.

Seven concentrations of benzylamine ranging from 2 μM to 1 mM in the absence or presence of six tresperimus concentrations from 0 to 200 μM were used to study the kinetics of benzylamine metabolism. The incubation time was chosen so the rate of metabolite (benzaldehyde) formation was demonstrated linear.

The formation of benzaldehyde was used to determine the velocity of the substrate concentration (from 2 μM to 1 mM) relationship for benzylamine metabolism. Kₘ and Vₘₐₓ values were calculated from triplicate measurements by nonlinear regression using the software GraphPad PRISM version 2.0 computer program (GraphPad Software, San Diego, CA) according to the method of Michaelis-Menten. The Lineweaver-Burk plot was used to represent the inhibition of benzylamine metabolism by tresperimus. The Kᵢ for tresperimus was determined by linear regression from the slope replot.

The measurement of benzaldehyde formation was performed by HPLC with fluorimetric detection (Van Dijk et al., 1995). The HPLC method has been fully validated in human plasma and was precise (8.5%) and accurate (5.5%). Human plasma diluted at 1/50th, in 10 mM potassium phosphate buffer, pH 7.4, was used as biological matrix to quantify benzaldehyde in microsomes of umbilical artery since the fluorimetric response of benzaldehyde has been demonstrated to be equivalent in both biological matrices. Deprenyl, semicarbazide, and tresperimus did not interfere in the derivatization of benzaldehyde or in the chromatogram.

**Protein Assay.** Protein concentrations of homogenates and microsomes were measured by the method of Bradford with bovine serum albumin as the standard (Bradford, 1976).

**Results**

**Tresperimus, M3, and M6 Metabolic Profiles in Homogenates and Microsomes of Umbilical Artery.** We checked that the tresperimus metabolic profile was not modified according to the pH. From the LC/MS spectra, the tresperimus metabolic scheme could be drawn in homogenates and microsomes of umbilical artery. This scheme appeared similar in both biological matrices. Only the metabolites formed from the spermidine moiety of the drug were detected, namely the aldehyde and acid derivative of tresperimus, M3, and the corresponding aldehyde and acid derivative of this metabolite. However, in microsomes the acid derivative of tresperimus (M2) was only detected in very low amounts, near the limit of detection. M6 formation was not observed in these cellular fractions no matter what the incubation conditions were, including pH value.

In vivo studies on tresperimus metabolism in humans showed that M6 quantities excreted in urine were much lower than those of its acid derivative (M8) (data not shown). Thus an oxidative deamination reaction of M6 is likely to occur. Therefore, we determined the LC/MS metabolic profile of M6 in umbilical artery, concurrently with that of M3 (Fig. 2). During the incubation of these substrates, their aldehyde derivatives were detected, and the acid derivatives of both compounds were only formed in very low amounts.

**Effect of pH on Tresperimus and M3 Metabolism.** We first checked that chemical stability of tresperimus and M3 were not affected by the pH values we tested. The variations of initial velocity for tresperimus and M3 metabolism in homogenates as a function of pH values are presented in Fig. 3; they were very close for both substrates, with a similar optimum at pH 9.3 (Fig. 3B). On the other hand, the metabolism of both substrates was clearly reduced at physiological pH, especially for tresperimus metabolism. As shown by Fig. 3A, activity toward M3 metabolism appeared significantly higher than that for tresperimus no matter what the considered pH values were.

**Effects of Amine Oxidase Inhibitors on Tresperimus, M3, and M6 Metabolism.** To discriminate between MAO and SSAO activities involved in tresperimus, M3, and M6 metabolism, the effect of different specific and irreversible inhibitors was tested; clorgyline and deprenyl were used toward MAO activity, semicarbazide for SSAO, and hydralazine for both enzymatic activities in the conditions used. We previously ascertained that these amine oxidase inhibitors could not interfere with the measure of unchanged tresperimus, M3, and M6 as well as in the liquid-solid extraction as in the HPLC assay. Therefore, we showed that the quantities of unchanged tresperimus, M3, and M6, measured in diluted human plasma used as a biological matrix for the calibration curves, were not modified in the presence of the amine oxidase inhibitors.

Figures 4 and 5 show the effects of various amine oxidase inhibitors upon the metabolism of tresperimus, M3, and M6 in homogenates of umbilical arteries. Semicarbazide strongly inhibited tresperimus metabolism at 1 mM and 100 μM. A significant inhibition was still measured at 50 μM. Clorgyline and deprenyl also caused a decrease of tresperimus metabolism at 1 mM. However, this inhibition measured with MAO inhibitors decreased at a low level (around 25%) at 100 μM, and no trace of significant inhibition could be measured at 50 μM and below. Tresperimus metabolism was strongly suppressed by hydralazine up to 10 μM.

Similarly M3 and M6 metabolism was strongly inhibited by hydralazine up to10 μM. The metabolism of both compounds was also significantly inhibited by semicarbazide up to 50 μM. Clorgyline and deprenyl (1 mM) enhanced a decrease of M3 and M6 metabolism. However, 50 μM of MAO inhibitors did not affect M3 and M6 metabolism.

**Kᵢ and Vₘᵙₐₓ Determinations.** Kinetic constants for tresperimus and M3 metabolism were determined by nonlinear regression according to the Michaelis-Menten rate equation. Kinetic constants for tresperimus metabolism were Kᵢ (μM) 1.2 ± 0.3 at pH 9.3 in microsomes of umbilical artery and 1.9 ± 0.4 at pH 7.8 in plasma; the corresponding values for Vₘᵙₐₓ (pmol/min/mg of protein) were 355.1 ± 18.3 in microsomes of umbilical artery and 12.6 ± 0.8 pmol/liter of plasma (equivalent to 0.2 ± 0.01 pmol/min/ml of plasma).
FIG. 2. Selected ion monitoring.

Mass chromatograms at m/z 330, 331, and 346 for M3 metabolic profile (A) and 158, 159, and 174 for M6 metabolic profile (B). M3 (3.0 μM) was incubated at 37°C for 45 min and M6 (12.7 μM) for 2 h at 37°C in homogenates of umbilical artery at pH 7.4 and pH 9.3, respectively. The metabolic profile of each compound was determined by LC/MS as described under Experimental Procedures.
**TABLE 1**

**Effect of deprenyl on kinetic parameters for benzylamine metabolism in microsomes of umbilical artery and in human plasma**

The $K_m$ values are expressed in micromolar concentrations and the $V_{max}$ values are calculated in nanomoles of benzaldehyde formed per minute per milligram of proteins in microsomes and in nanomoles of benzaldehyde formed per minute per liter of plasma.

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (nmol/min/mg)</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (nmol/min/l)</th>
</tr>
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<tbody>
<tr>
<td>Without Deprenyl at 1 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsomes of umbilical artery</td>
<td>63.5 ± 9.5</td>
<td>52.1 ± 2.0</td>
<td>100 ± 6</td>
<td>51.5 ± 0.9</td>
</tr>
<tr>
<td>Human plasma</td>
<td>87.7 ± 12.1</td>
<td>300 ± 14</td>
<td>93.7 ± 6.4</td>
<td>280 ± 6</td>
</tr>
<tr>
<td>With Deprenyl at 1 mM</td>
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</table>
Kinetic constants for M3 metabolism were $K_m (\text{M})$ 3.3 ± 0.6 at pH 9.3 in microsomes of umbilical artery, and the corresponding value for $V_{\text{max}}$ (pmol/min/mg of protein) was 2770 ± 171.4.

As shown by the Hanes-Woolf plots of tresperimus and M3 metabolism by microsomes of umbilical artery and by human plasma, a straight line was obtained indicating that only one enzyme activity was responsible for the metabolism of these compounds in these biological matrices (Fig. 6).

**Kinetic Parameters for Benzylamine Metabolism.** Benzylamine is a substrate for MAO and SSAO. To evaluate the contribution of each enzymatic activity to benzylamine metabolism, kinetic data for benzylamine metabolism were determined in the presence or absence of MAO inhibitor (Table 1). In microsomes of umbilical artery, the $K_m$ value for benzylamine metabolism obtained in the presence of deprenyl was greater (by 60%) than the value determined without this inhibitor. A similar phenomenon was observed with another preparation in which the $K_m$ value without deprenyl (157 μM) was increased by 37% in deprenyl-treated samples ($K_m$, 215 μM). In contrast, $V_{\text{max}}$ values were relatively unchanged. These results showing increases in apparent $K_m$ values with deprenyl without any change in $V_{\text{max}}$ values could be indicative of an apparent competitive action of deprenyl upon the total benzylamine-metabolizing activity. This does not support the notion that deprenyl is selectively inactivating a small fraction of MAO.
MAO-B component contributing to the total benzylamine oxidase (BzAO) activity. Thus BzAO activity depends only on SSAO in our conditions.

The kinetic parameters for benzylamine metabolism by plasma SSAO were similar to those determined in microsomes. As opposed to membrane-bound enzyme, deprenyl did not affect these parameters for the plasma enzyme. Thus, SSAO seems also to be exclusively responsible for benzylamine deamination in this enzyme source.

**Effects of Tresperimus upon Benzylamine Metabolism in Microsomes of Umbilical Arteries and in Human Plasma.** The $K_i$ value of tresperimus on benzylamine oxidase activity in microsomes of umbilical artery was determined in the presence or absence of deprenyl since this MAO inhibitor seemed to interact on BzAO activity. On the other hand, the $K_i$ of tresperimus in plasma was determined only on total benzylamine oxidase activity since deprenyl did not seem to have an effect on plasma SSAO. Results were depicted as shown in the Lineweaver-Burk plot in Fig. 7. Tresperimus produced a mixed pattern of inhibition against benzylamine metabolism in both enzyme sources tested. The apparent $K_m$ values for benzylamine metabolism were increased by tresperimus whereas the $V_{max}$ values were decreased. The $K_i$ value of tresperimus for total benzylamine metabolism in microsomes of umbilical artery (31.9 ± 3.9 μM) was close to that in plasma (39.5 ± 4.8 μM). As for the determination of $K_m$ for benzylamine metabolism, this $K_i$ value (89.0 ± 2.4 μM) was greater for deprenyl-treated microsome samples.

**Discussion**

All the oxidative deamination reactions identified within the tresperimus metabolic scheme occurred in human umbilical artery. Indeed unchanged drug, M3, and M6 were deaminated in vascular homogenates as shown by the detection of the aldehyde and acid derivatives corresponding to these substrates. The deaminating activity toward tresperimus and M3 took place also in the microsomal fraction of this tissue. Besides, M6 metabolism by umbilical artery allowed the demonstration of the formation of the aldehyde derivative of M6, although this intermediary compound leading to the formation of M8 has never been detected in biological fluids in vivo. The oxidation of the aldehyde derivatives formed by these deamination reactions constituted clues to the presence of a functional NAD(P)-linked aldehyde dehydrogenase (ALDH; EC.1.2.1.3) despite the absence of supplementation in cofactors. On the other hand, the hydrolysis of the amide bond of tresperimus, leading to the release of M6, did not take place in these biological matrices no matter what the pH of the incubation medium was.

The use of umbilical artery confirmed the major involvement of SSAO in tresperimus deamination. Indeed, semicarbazide strongly inhibited tresperimus metabolism at 1 mM and 100 μM, whereas the oxidation of the drug was weakly sensitive to deprenyl or clorgyline at 100 μM and resistant to these inhibitors at 50 μM. The sensitivity of tresperimus metabolism to these inhibitors at 1 mM did not seem to reflect an MAO involvement in the drug-deaminating activity. Indeed,
the A-form of the enzyme is sensitive to inhibition by low (in the nanomolar range) concentrations of clorgyline but is not inhibited by l-deprenyl until micromolar concentrations of this inhibitor are used. The reverse is true for the B-form with deprenyl (Fowler et al., 1982). Both forms of MAO activities should be inhibited at 100 µM and still highly decreased at 50 µM. Therefore the decrease of tresperimus metabolism in the presence of MAO inhibitors at high concentration could rather reflect an interaction between these inhibitors and tissue-bound SSAO, as shown by the apparent competitive action of 1 mM deprenyl on benzylamine metabolism in the presence or in the absence of tresperimus. Similar inhibition has already been reported with clorgyline in umbilical artery (Precious and Lyles, 1988). The contribution of MAO to tresperimus metabolism thus appeared negligible at physiologically relevant concentrations and could be reflected by the low percentage of remaining activity (5%) in the presence of 1 mM semicarbazide. In any event, tresperimus metabolism was quite blocked when MAO and SSAO activities were inhibited as shown by hydralazine up to 10 µM. Indeed, the antihypertensive drug hydralazine acted as an inhibitor of both forms of amine oxidases in the experimental conditions used (Lyles et al., 1983).

Furthermore the mixed inhibition caused by tresperimus on benzylamine metabolism in human plasma and in microsomes of umbilical artery, a substrate only for SSAO in the assay conditions, supported that both substrates are metabolized by the same catalytic entity. Indeed a mixed inhibition is common in multisubstrate systems (Frieden, 1964).

SSAO seemed to be also predominantly responsible for M3 and M6 metabolism. Indeed, MAO inhibitors at 50 µM did not affect significantly M3 and M6 metabolism although the metabolism of these two compounds was strongly decreased by semicarbazide at 1 mM and 100 µM with around 85 to 90% and 50% inhibition, respectively. The incomplete inhibition of M3 and M6 metabolism by high semicarbazide concentrations could reflect a limited contribution (around 10 to 20%) of MAO to their metabolism. Anyway M3 and M6 metabolism was completely suppressed by hydralazine, an inhibitor of both MAO and SSAO in our experimental conditions.

The microsomal fraction of umbilical artery, an enzyme source with a high specific SSAO activity and the contaminating MAO activity being partly elminated, was used to determine the kinetic parameters of SSAO for tresperimus and M3 metabolism (Suzuki and Matsu-moto, 1984). As shown by the Hanes-Wolf plots, tresperimus and M3 metabolism was only dependent on SSAO activity in the microsomal fraction. Membrane-bound enzyme showed a high affinity of SSAO for both compounds as demonstrated by the $K_m$ values of 1.2 and 3.3 µM for tresperimus and M3 metabolism, respectively. These $K_m$ values were much lower than that for aminocetone metabolism, one of the lowest $K_m$ values (92 µM) for a human physiological SSAO substrate determined under comparable assay conditions (Lyles and Chalmers, 1992). On the other hand, the maximal velocity of microsomal SSAO for tresperimus (0.36 nmol/min/mg of protein) was very low compared with those for M3 and benzylamine metabolism (2.77 nmol/min/mg of protein and 52.1 ± 2.0 nmol/min/mg of protein, respectively).

The kinetic behavior of the soluble and the membrane-bound SSAO toward tresperimus metabolism was compared since the relationships between both enzymes remains unclear (Lizcano and Unzeta, 2000). The membrane-bound enzyme and the soluble form of SSAO exhibited similar $K_m$ values for the drug and $K_i$ values of tresperimus toward benzylamine metabolism. Thus, this kinetic behavior for tresperimus metabolism of membrane-bound and soluble SSAO seemed to argue in favor of a same catalytic entity.

Nevertheless, both forms of the enzyme seemed to behave differently according to the pH. Indeed tresperimus metabolism showed a strong pH dependence; it was almost abolished in umbilical artery at pH values inferior to pH 7.8, although the plasma enzyme activity could be studied at this pH value. The pH dependence of the reaction mechanism of SSAO has been often reported and was also observed for M3 metabolism (Farnum et al., 1986; Lindström et al., 1974; Medda et al., 1995). Besides, SSAO activity is reduced or even suppressed for substrates that carry ionized groups close to the terminal amino group (McEwen, 1965; Buffoni et al., 1972). The presence of the secondary amine of tresperimus as a protonated form at physiological pH could explain the low catalytic efficiency of membrane-bound SSAO for tresperimus at physiological pH. The behavior of the membrane-bound enzyme according to the pH could result from modifications of the micro-environment of the enzyme during microsomal preparation. Indeed the breaking up of the membrane architecture notably the electrostatic bonding with phospholipids of a membrane enzyme can cause a shift of pH optimum (Silmarn and Karlin, 1967; Coleman, 1973; Houssay and Tipton, 1973).

Umbilical artery provided qualitative and quantitative information on tresperimus metabolism in man. Crude homogenates of this tissue allowed to demonstrate the predominant role of SSAO in tresperimus, M3, and M6 metabolism and to exclude significant contributions of MAO to the metabolism of these compounds at physiologically relevant concentrations. Furthermore, ALDH represents an important group of detoxification enzymes, and characterization of the ALDH form(s) present in homogenates and microsomes of umbilical artery would constitute an important step to further assess the interest of such metabolic models (Sladek et al., 1989; Lindahl, 1992). Since membrane-bound SSAO and plasma-soluble SSAO exhibited similar kinetic properties, the use of microsomal fraction, an enzyme source with a high specific SSAO activity, could be relevant for the assessment of quantitative data on drug metabolism or drug interaction potential.

Tresperimus metabolism highlights the important role that SSAO could play as a Phase I oxidative enzyme to scavenge certain exogenous amines entering the bloodstream. Such an important contribution has been rarely demonstrated in xenobiotic metabolism. Indeed, the contribution of amine oxidases has been often neglected, especially due to the lack of appropriate in vitro models to clearly demonstrate their involvement (Strolin-Benedetti, 2001).

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