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COMMUNICATIONS

Elucidation of the structural requirements for the bioactivation of mianserin in-vitro

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Abstract—The aim of this study was to investigate structure-activity relationships among a series of compounds related to the antidepressant drug, mianserin, with respect to their ability to produce cytotoxic metabolites. Human peripheral lymphocytes were used as target cells and these were exposed to the individual compounds, in the presence or absence of a drug metabolizing system derived from human liver. The individual enantiomers of mianserin showed differences in their cytotoxicity profiles; the *R*-(−) isomer giving NADPH-dependent cytotoxicity while the *S*-(+) isomer showed direct cytotoxicity at high concentrations. Cytotoxicity was reduced by removal from mianserin of the nitrogen atom at the 5 position and by substitution of a methyl group for a hydrogen atom at position 14b. In contrast, insertion of an oxygen atom at position 10 of the drug molecule, precluding the formation of a carbonium ion, had little effect on cytotoxic metabolite formation. The data are consistent with the proposal that one or more iminium ions derived from mianserin are responsible for the cytotoxicity observed in this in-vitro system and that appropriate chemical modification may preclude bioactivation of mianserin by P450 enzymes.

The tetracyclic antidepressant mianserin has been shown to undergo in-vitro bioactivation by human liver microsomes to both protein-reactive and cytotoxic species (Lambert et al 1989; Roberts et al 1991). The reactive metabolites generated may be involved in the rare adverse reactions (agranulocytosis) observed with this drug in-vivo (Chaplin 1986).

The structural identity of reactive metabolites formed from mianserin would be useful in the diagnosis of patients at risk from the drug and could aid in drug development. It has previously been shown that cyanide will decrease the irreversible binding of mianserin to protein without decreasing overall metabolism of the drug, suggesting the generation of a reactive iminium species (Lambert et al 1989), but it was not possible to isolate a stable adduct. An alternative approach to the elucidation of the structure of an intermediate toxic drug metabolite is to study structure-activity relationships (SAR) with respect to toxicity. This method is used commonly in the development of a drug—e.g. cimetidine (Durant et al 1977)—and has also been used to elucidate the part of a molecule involved in its bioactivation to a reactive species—e.g. neurotoxicity observed with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Youngster et al 1989a, b).

The work described here uses an SAR approach to determine the part of the mianserin molecule involved in the generation of a cytotoxic metabolite.

Materials and methods

Mianserin and its separate enantiomers, Org GC94, Org 4564, Org 4596, Org 4597 and Org 4428 (Fig. 1) were all gifts from Organon International B.V. (Oss, The Netherlands). Reduced nicotinamide dinucleotide phosphate (NADPH, tetrasodium salt) was purchased from BDH Chemicals Ltd (Poole, Dorset, UK). Human serum albumin (HSA) was obtained from Sigma Chemical Co. (Poole, Dorset, UK). Solvents were of HPLC grade and were purchased from Fisons plc (Loughborough, UK). All other chemicals were purchased from Sigma Chemical Co. (Poole, Dorset, UK).

Preparation of human hepatic microsomes. Histologically normal human liver was obtained from a kidney transplant donor, cut into portions and frozen in liquid nitrogen until required for use. Ethical approval was granted and consent for removal of the livers was obtained from the donors' relatives. Microsomes were prepared by previously described ultracentrifugation techniques (Purba et al 1987) and stored at −80°C until required. All protein measurements were carried out using the method of Lowry et al (1951).

In-vitro cytotoxicity assay. Human peripheral mononuclear leucocytes (MNL) were isolated from heparinized blood freshly drawn from a single, healthy volunteer. The isolation procedure was essentially as described by Boyum (1984) and consisted of a one-step centrifugation through a sodium metrizoate/Ficoll solution (Lymphoprep) which was followed by several washing stages. MNL (10^6) were incubated with drug (3, 10, 30 and 100 μM) and 0·5 mg microsomal protein, in the presence or absence of NADPH, for 2 h at 37°C in a HEPES-buffered medium (pH 7·4). Direct cytotoxicity was determined by incubating 10^6 cells with drug (3, 10, 30 and 100 μM) alone. Cell death was assessed by trypan blue dye exclusion following a further incubation for 16 h at 37°C in a drug-free medium (HEPES buffer containing 5 mg mL^{-1} HSA) (Roberts et al 1991).

Determination of lipophilicity. The lipophilic nature of the compounds was determined by measuring the capacity ratio (K′). Log K′ is directly proportional to the octanol/water partition coefficient (Konemann et al 1979). Reversed-phase liquid chromatography was conducted isocratically on a μ-Bondapak C-18 column using an Altex model 110 pump and Altex model 160 absorbance detector (Altex Scientific Inc., Berkeley, CA, USA), equipped with a 254 nm filter, with an eluent flow rate of 1·0 mL min^{-1}. For the determination of retention times, authentic standards of mianserin and its structural analogues were dissolved in methanol (0·5 mg mL^{-1}) and 100 μL was injected onto the column. The eluent used was methanol: ammonium acetate (0·1 M, pH 4·6) at three different ratios (80:20, 85:15 or 90:10) with each measurement conducted in duplicate. The retention times measured (R_T) were corrected for eluent hold up in the column and dead volume by determining the retention time for methanol (R_0). K′ values were then obtained from the following equation:

$$K' = (R_T - R_0)/R_0$$

Statistics. Cytotoxicity data are presented as mean ± s.d. Comparison was made between values in the presence and absence of

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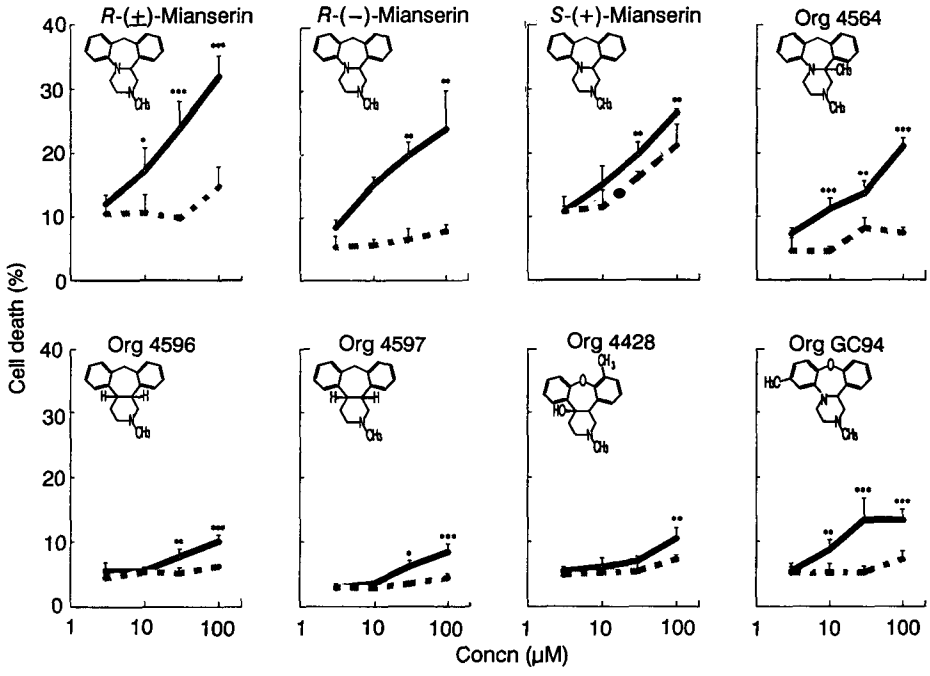

FIG. 1. Dose-activation curves showing the metabolism-dependent toxicity. Values are mean ± s.d. of quadruplicate determinations carried out in the absence (broken line) and presence (solid line) of NADPH. Significant differences between +NADPH and −NADPH curves are: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

NADPH using an unpaired Student's *t*-test. Least squares linear regression analysis was performed to determine correlations between cell death and lipophilicity.

Results and discussion

The generation of cytotoxic metabolites by bioactivation of mianserin, presumably by cytochrome P450 enzymes since it has been shown to be inducible by phenobarbitone and β-naphthoflavone (Riley et al 1990) and inhibitable by SKF 525-A (Lambert et al 1989), can be visualized in the present study by the divergence in the dose-toxicity curves obtained in the presence or absence of NADPH. A cytotoxic metabolite was generated from both (±)-mianserin and (−)-mianserin in an NADPH-dependent manner (Fig. 1). However, in agreement with previous studies (Riley et al 1989), a stereochemical difference was observed with NADPH-dependent cytotoxicity being greater with (−)-mianserin than with (+)-mianserin (Fig. 1); (+)-mianserin, in contrast, showed direct cytotoxicity at high concentrations. These data indicate that the bioactivation of mianserin by hepatic microsomes is sensitive to changes at the chiral centre (C14b) of the molecule. The involvement of the C14b atom is in agreement with the generation of an iminium ion between the N5 and C14b atoms (Fig. 2II) as proposed by Lambert et al (1989).

The possible generation of the N5-C14b iminium ion from mianserin was investigated using Org 4564 as a substrate in the cytotoxicity assay. Org 4564 differs structurally from mianserin by the addition of a methyl group at the C14b position which will block hydroxylation at this position and so prevent the formation of the N5-C14b iminium ion. However, a cytotoxic metabolite was formed from Org 4564 (Fig. 1) suggesting that the N5-C14b iminium ion is not solely responsible for the in-vitro cytotoxicity seen with mianserin. Although the N5-C14 iminium ion is precluded by the methyl group in Org 4564, an alternative iminium metabolite could be formed, however, from Org 4564, at the C4-N5 position. It is possible that this iminium ion, formed from mianserin (Fig. 2III), may be involved in the cytotoxicity seen with this drug. To investigate this second iminium ion theory, the metabolic activation of the two diastereomers Org 4596 and Org 4597 was investigated. These two molecules do not contain the N5 atom which is essential for the generation of either of the two iminium ions described above. Fig. 1 shows that the NADPH-dependent cytotoxicity was greatly reduced with these two molecules suggesting that the N5 atom in mianserin is essential for the generation of a cytotoxic metabolite. Furthermore, the lack of NADPH-dependent cytotoxicity observed with Org 4428, at concentrations up to 30 μM or below (Fig. 1), also suggests that the N5 atom is required for the formation of a cytotoxic metabolite as this compound does not possess a nitrogen atom at the five position.

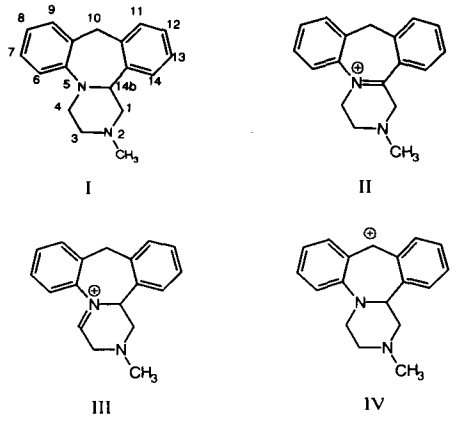

FIG. 2. The structures of mianserin (I) and putative reactive metabolites formed from mianserin in-vitro. II, iminium ion formed between N5 and C14b. III, iminium ion formed between C4 and N5. IV, carbonium ion formed at C10.

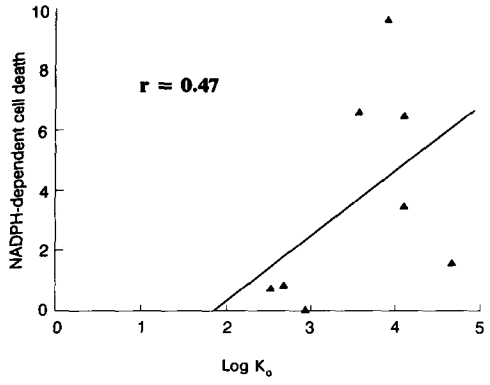

FIG. 3. Correlation of NADPH-dependent cell death with log K_0 for all compounds studied at a concentration of 10 μM.

Another possible cytotoxic metabolite which was postulated by Lambert et al (1989) was that of a carbonium ion at the C10 position (Fig. 2). To investigate this possibility, Org GC94 was included in the study. Org GC94 contains an oxygen atom at C10 thus preventing the formation of a carbonium ion. However, NADPH-dependent toxicity was observed with this compound (Fig. 1) indicating that the C10 atom is not essential for the generation of a cytotoxic metabolite from mianserin.

No correlation between toxicity and lipophilicity was apparent at a drug concentration of 10 μM (Fig. 3), and so it can be assumed that differences in the molecular structure of the compounds was responsible for differences in toxicity, rather than any simple difference in the rate of drug uptake by microsomes determined by lipophilicity (McMahon 1961).

In conclusion, we have shown that by making structural changes to the mianserin molecule it is possible to produce structural analogues with decreased metabolism-dependent cytotoxicity, compared with mianserin, in-vitro. Removal of the N5 atom in mianserin abolished the in-vitro NADPH-dependent toxicity observed with this compound at concentrations of 10 μM and less; concentrations that are far higher than would be achieved during mianserin therapy. It is postulated that the cytotoxic metabolite may be an iminium ion formed between C4 and N5 of mianserin, although we cannot discount the possibility of an iminium ion being formed between N5 and C14b.

Implicit in the study described is the assumption that all the compounds undergo metabolism to a similar extent, i.e. that the rate of biotransformation for all compounds was the same under the experimental conditions applied. Clearly, further work requires the use of radiolabelled material to determine the extent of metabolism, and the use of a structural analogue of mianserin containing a chemical group at C4 which would prevent the formation of an iminium ion at C4-N5, to determine whether there is a cytotoxic iminium ion formed at this position. A similar SAR approach could be adopted with other drugs which cause adverse drug reactions, and could aid in the synthesis of safer compounds with the same clinical efficacy.

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