naloxone at the end of 13 days morphine treatment enhanced the degree of ACh supersensitivity to 90 fold. These results and that observed in the present study clearly indicate that supersensitivity can be included as a reliable index to assess morphine tolerance and dependence. The inhibitory effect of morphine on ACh release in mouse ileum was suggested as a possible mechanism for morphine induced supersensitivity to ACh.

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J. Pharm. Pharmacol. 1984, 36: 276–277
Communicated September 21, 1983

Preliminary study of the disposition in man of acebutolol and its metabolite, diacetolol, using a new stereoselective hplc method

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A new stereospecific hplc method that is capable of simultaneously quantitating the *S*-(−)- and *R*-(+)-enantiomers of acebutolol and its major metabolite, diacetolol, in plasma and urine, is described. When applied to the assay of biological fluids collected during single and chronic oral dosing with acebutolol (Sectral), this procedure failed to reveal any important stereoselectivity in the disposition of either acebutolol or diacetolol in man. This may occur because acebutolol is metabolized by hydrolysis and *N*-acetylation, whereas the other β-blockers which exhibit some degree of stereoselective disposition (e.g. metoprolol and propranolol) are primarily metabolized by oxidation.

We have previously reported on the fate in man of the cardioselective β-adrenoceptor antagonist, acebutolol (Gabriel et al 1981). However, acebutolol and its major metabolite, diacetolol (the acetamido analogue), exist as *S*-(−)- and *R*-(+)-enantiomers, and, as with β-blocking drugs in general, most of the β-blocking activity resides in the *S*-(−)-enantiomer. For propranolol (Hermansson & Von Bahr 1980; Silber & Riegelman 1980; Tawara et al 1981; Silber et al 1982; Von Bahr et al 1982), alprenolol and metoprolol (Hermansson & Von Bahr 1982; Lennard et al 1983), bufuralol (Francis et al 1982) and moprolol (Harvengt & Desager 1982) there are higher circulating concentrations of the *S*-(−)-enantiomer compared with the corresponding *R*-(+)-enantiomer, and so we have re-examined the disposition of acebutolol in man by means of a new stereospecific hplc method.

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Because acebutolol has a major metabolite, diacetolol (Gulaid et al 1981), the stereospecific hplc method outlined below was designed to be capable of simultaneously quantitating the enantiomers of both acebutolol and diacetolol. The method involves the use of an optically pure derivatizing agent to form diastereoisomers, an approach previously described for other β-blocking drugs (see e.g. Hermansson & Von Bahr 1980 1982; Thompson et al 1982).

Method

The procedure is as follows: to each sample (1 ml) of plasma or urine is added 1 M NaOH (0·5 ml), the internal standard, M & B 17,764 which is the propionamido analogue of acebutolol (1 μg, as a concentrated methanolic solution), and a mixture (7 ml) of diethyl ether–chloroform (4 : 1 v/v), respectively. After having been shaken mechanically for 10 min the samples are centrifuged. The upper organic layer is removed and evaporated to dryness at about 60 °C under a stream of nitrogen (oxygen-free). To the dry residue is added chloroform (0·5 ml), triethylamine (10 μl) and the derivatizing agent (*S*-(−)-*N*-trifluoroacetylprolyl chloride (TPC), as a 0·1 M solution in chloroform; 100 μl). These are thoroughly mixed and then allowed to stand at room temperature (ca 20 °C) for about 25 min. To this solution is then added a mixture (7 ml) of diethylether–chloroform (4 : 1 v/v, respectively) followed by water (3 ml). After 10 min shaking and centrifugation, the upper organic layer is removed and evaporated as before. The dry residue is dissolved in

Table 1. Apparent elimination half-lives of the enantiomers of acebutolol and diacetolol following the administration of a single oral 400 mg dose of Sectral to a healthy volunteer.

	Apparent elimination half-life (h)	
	S-(−)-enantiomer	*R*-(+)-enantiomer
Plasma data		
Acebutolol	5·1	4·9
Diacetolol	9·0	9·7
Urinary data		
Acebutolol	9·2	8·7
Diacetolol	10·5	10·3

mobile phase (100 μl, see below) and suitable aliquots are injected into the chromatograph: column (15 cm × 4·6 mm i.d.) packed with Hypersil ODS (3 μm); mobile phase: 0·01 M aqueous potassium dihydrogen phosphate (adjusted to pH 2·0 with orthophosphoric acid) and acetonitrile, 7:3 v/v. Chromatography is at room temperature, and the flow rate of the mobile phase is 1·0 ml min^{-1} (producing a pressure of around 2500 p.s.i.). The diastereoisomers are detected by fluorescence (excitation 254 nm, emission filter 418 nm).

Results and discussion

This procedure achieves virtual base-line separation of the TPC derivatives of acebutolol, diacetolol and M & B 17,764 in about 20 min after injection. With optically pure *S*-(−)- and *R*-(+)-acebutolol, prepared previously (Basil, Jordan, Loveless, Pain & Wooldridge—unpublished work), the *S*-(−)-isomer eluted before the *R*-(+)-isomer, and it is reasonable to suppose that this would also occur with diacetolol and M & B 17,764. The concentration of each isomer of acebutolol and diacetolol in each sample of plasma and urine is determined by calculation of the peak height ratio of each derivatized enantiomer of acebutolol and of diacetolol to the corresponding derivatized enantiomer of M & B 17,764, and reference to linear calibration graphs obtained by adding known amounts of racemic acebutolol and diacetolol to blank plasma and urine spiked with racemic M & B 17,764. For plasma, the limit of detection is about 0·05 μg ml^{-1}, and coefficients of variation of less than 5% are obtained at a concentration of 0·5 μg ml^{-1} for each enantiomer of acebutolol and diacetolol. For urine the limit of detection is less than 1 μg ml^{-1}, and coefficients of variation of less than 10% are obtained at a concentration of 10 μg ml^{-1}.

With this stereospecific hplc procedure we have examined plasma and urine samples collected from a healthy volunteer given a single oral 400 mg dose of acebutolol (Sectral), and plasma samples obtained from eight patients receiving chronic oral Sectral therapy. From the volunteer's plasma and urinary data it was possible to estimate elimination half-lives of the isomers of acebutolol and diacetolol by using standard methods of regression analysis. The values obtained are given in Table 1. Apart from slightly higher plasma values of *S*-(−)-diacetolol compared with *R*-(+)-diacetolol being achieved, the plasma and urinary data showed the disposition of each isomer of acebutolol, and of diacetolol, to be virtually identical following a single oral dose of Sectral. There is support for this conclusion in the observation that in the patients' plasma samples collected during chronic oral dosing with Sectral the mean (±s.d.) ratios of S/R acebutolol and of diacetolol were 1·07 (± 0·07) and 1·04 (± 0·18), respectively. It is noteworthy that when these plasma samples were analysed by a non-stereospecific hplc method (Holt et al 1981) the values obtained were essentially the same as those obtained by the present procedure on summation of the isomer values.

In conclusion, therefore, in assessing both single and repeat dosage regimens with orally administered acebutolol this preliminary study has failed to reveal any important stereoselectivity in the disposition of either acebutolol or diacetolol in man. This may be because acebutolol is metabolized by hydrolysis and *N*-acetylation (Gulaid et al 1978), whereas the other β-blockers which exhibit stereoselective disposition (e.g. metoprolol and propranolol) are primarily metabolized by oxidation.

The authors thank Mr P. McKenna for technical assistance, and Professor Barber and Drs Walker and Poloniecki for obtaining the patients' plasma samples.

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