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Note

Monitoring of ethimizol and its metabolites in serum or saliva by means of high-performance liquid chromatography

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There is an extensive and rapidly growing utilization of high-performance liquid chromatography (HPLC) in the monitoring of many drugs to control dosage in order to achieve the optimum therapeutic effect and safety. The concentration of drugs is determined in various body fluids — whole blood, serum, plasma, and saliva as well. Samples of saliva may be taken painlessly and without the risk of iatrogenic infection and this substantiates the attempts to control the drug dosage on the basis of its salivary concentration.

Ethimizol (I), bis-methylamide of 1-ethylimidazole-4,5-dicarboxylic acid (see Fig. 1), is an original Soviet preparation [1]. In clinical practice ethimizol is mainly used in the treatment of respiratory disorders [2, 3].

The aim of this paper is to demonstrate that for monitoring purposes serum data of ethimizol and one of its metabolites (M_1) can be substituted by saliva data.

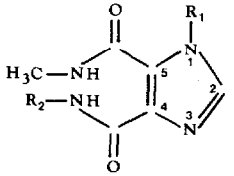

Fig. 1. Structural formula of ethimizol (I: $R_1 = C_2H_5$, $R_2 = CH_3$), antiffeine (II: $R_1 = CH_3$, $R_2 = CH_3$), and metabolite 1 (M_1:$R_1 = C_2H_5$, $R_2 = H$).

EXPERIMENTAL

Subjects

Four healthy male volunteers (for age and weight see Table I) participated in this study. After an overnight fast each received a single oral dose of 2 mg/kg ethimizol as a tablet. Blood was drawn 2, 3, 4, 5, and 6 h after ethimizol ad-

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ministration. Saliva was collected at the same time intervals. (The salivary flow was stimulated by chewing a piece of Parafilm.) Both serum and saliva were stored frozen until analysis.

Sample treatment

Serum or saliva sample (2.0 ml) diluted with a 2.0 ml aqueous solution (1.0 μg/ml) of the internal standard antiffeine (Fig. 1, II) was applied onto a disposable Sep-Pak C_{18} cartridge (Waters Assoc., Milford, MA, U.S.A.). (The cartridge was conditioned before use by washing with 5 ml of methanol followed by 5 ml of water.) After the diluted sample had passed through the cartridge, the packing was washed with 2 ml of water. The remaining water was blown off. The cartridge was connected to a disposable micro-column packed with silica gel (ca. 0.2 g) and the compounds under analysis were eluted with 4 ml of acetonitrile from the cartridge packing (C_{18}) through the silica sorbent into a conical glass vial. The acetonitrile eluate was evaporated to dryness under a stream of nitrogen at 50°C. The residue was redissolved in 20 μl of chloroform and 10 μl of centrifuged solution were chromatographed [4].

Chromatographic technique

A Spectra-Physics SP 8000 high-performance liquid chromatograph equipped with a variable-wavelength photometric detector (Model SP 770) was used. A stainless-steel column (25 cm × 4.6 mm I.D.) was packed with LiChrosorb SI-100, particle size 5 μm (Merck, Darmstadt, G.F.R.). The eluent, a mixture of *n*-heptane—dichloromethane—methanol—triethylamine (85:10:4.75:0.25, v/v) was pumped at a rate of 1.2 ml/min. Detection was effected at 262 nm. All separations were carried out at ambient temperature.

RESULTS AND DISCUSSION

A slightly modified sample treatment procedure developed previously [4] was used to preserve clogging of the HPLC column with any non-elutable co-preseparated endogenous compounds. As shown in Fig. 2, the preseparated blank human serum or saliva gave only a small peak of a compound (maybe caffeine which is eluted at 8.0 min), which did not significantly influence the evaluation of the peaks of I, (II), and M_1, which eluted at 6.3, (7.6), and 10.2 min, respectively. (The evaluation of the peaks of further ethimizol metabolites, eluted after 12 min until about 18 min, was initially omitted.)

Serum or saliva samples (2.0 ml) containing ethimizol (0.1 μg/ml) and metabolite 1 (1.0 μg/ml) were processed as described above in order to determine the within-day reproducibility of the method. The coefficients of variation calculated from quadruplicate determinations of I and M_1 in serum or saliva were 7.3% and 3.4%, respectively, or 6.9% and 3.6%, respectively.

The half-lives, calculated from the individual concentration—time curves of ethimizol (M_1) in serum or saliva, are listed in Table I. There is excellent agreement between the half-life values determined from serum and from saliva. Each corresponding pair of concentration dependence declines nearly in parallel and thus the saliva-to-serum concentration ratio ([Sa]/[Se]) of ethimizol (M_1) is almost constant (see Table I). The mean [Sa]/[Se] ratio for M_1 is significantly higher than that for I.

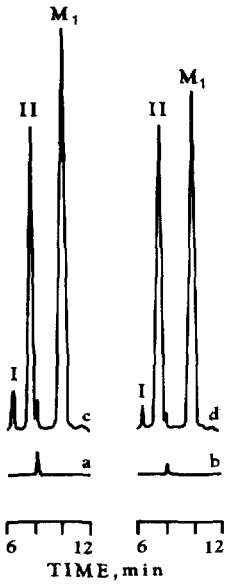

Fig. 2. Normal-phase HPLC elution profile of preseparated blank human serum (a) or saliva (b), and a serum (c) or a saliva (d) sample obtained 2 h after ethimizol administration. The chromatograms are recorded between the start of the ethimizol elution curve and the end of an HPLC analysis run time (12 min).

TABLE I

SERUM (Se) AND SALIVA (Sa) HALF-LIVES OF ETHIMIZOL AND METABOLITE 1, AND THEIR [Sa]/[Se] CONCENTRATION RATIOS

Subject			Half-life of I in:		[Sa]/[Se] ratio of I	Half-life of M_1 in		[Sa]/[Se] ratio of M_1
Code	Age (years)	Weight (kg)	Se (h)	Sa (h)		Se (h)	Sa (h)	
J.R.	28	74	0.78	0.82	0.42—0.47	3.41	3.46	0.88—0.89
Z.K.	29	80	0.67	0.72	0.51—0.67	1.82	1.85	0.86—0.89
L.L.	33	77	0.97	0.97	0.40—0.40	3.22	3.47	0.84—0.89
V.S.	36	65	1.33	1.35	0.60—0.63	2.50	2.51	0.84—0.84

The results presented demonstrate that the chromatogram of the saliva sample fully substitutes the serum HPLC analysis not only qualitatively but also quantitatively. The saliva data may be used reliably for the determination of the terminal half-life of I (M_1) after a single oral administration of ethimizol tablets in a commonly used therapeutic dose. Since the concentration of many drugs (metabolites) in saliva equals their free (non-protein-bound) concentration in plasma, the saliva levels might be more relevant for their pharmacologic action than the corresponding serum (plasma) concentrations [5]. In patients under long-term treatment, the validity of controlling ethimizol dosage by saliva rather than serum analysis has still to be verified.

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