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Note

Capillary gas chromatographic method for determination of flumecinol in plasma and saliva

I. KLEBOVICH*, M. KAPÁS and L. VERECZKEY

Pharmacological Research Centre, Department of Pharmacokinetics and Drug Metabolism, Chemical Works of Gedeon Richter Ltd., 10. Pf.27, H-1475 Budapest (Hungary)

Flumecinol (Zixoryn®, 3-trifluoromethyl-α-ethyl-benzhydrol [1] is a selective hepatic enzyme inducer both in animal experiments and in human clinical studies. For example, flumecinol induces the endoplasmic reticulum-bound mixed-function oxidase system of the liver [2—6]. The main indicating area of flumecinol therapy is recommended for the treatment of icterus neonatorum.

Our preliminary experiments showed that flumecinol seems to bind very intensively to plasma protein(s) [7]; therefore, the study of in vivo protein binding and the determination of the plasma flumecinol concentration in rats during chronical toxicity studies required a more sensitive method than our earlier one [8].

This new simple method is appropriate for routine analysis; the human pharmacokinetics of the drug and its in vivo protein binding could be calculated from the saliva/plasma level, and the plasma level of flumecinol can be determined in chronic toxicity experiments from 250 μl of rat plasma.

EXPERIMENTAL

Materials

Zixoryn® was manufactured by Chemical Works of Gedeon Richter, Budapest, Hungary. The specific activity of [1-^{14}C]flumecinol was 1467.4 MBq/g (39.6 mCi/g).

Potassium hydroxide and diethyl ether puriss. were from Merck (Darmstadt, G.F.R.). Diethyl ether, chloroform and methanol were carefully purified by bidistillation before use. Parafilm® was from American Can. Co., Greenwich, CT, U.S.A. All other chemicals were the products of Reanal (Budapest, Hungary) and were of analytical grade.

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Preparation of calibration curves

To 1 ml of human plasma or 2 ml of saliva, 200 ng (for 250 μl of rat plasma 100 ng) of 3-trifluoromethyl-benzhydrol (internal standard) were added. The flumecinol concentration in the calibration curves was between 10 and 200 ng for human plasma, 10 and 50 ng for saliva, and 10 and 100 ng for rat plasma samples, the flumecinol being added in methanol. To these samples 0.6 ml of 2 *N* potassium hydroxide was added and they were extracted with 5 ml of diethyl ether, shaken for 20 min and centrifuged at 3000 *g* for 15 min.

The diethyl ether was transferred to conical test-tubes and evaporated at room temperature. The dry residue was dissolved in 20 μl of chloroform under shaking and 1—2 μl were injected into the gas chromatograph.

For quantitation the ratio of peak height of drug to that of internal standard was used. The recovery of flumecinol was detected by liquid scintillation counting of [^{14}C] flumecinol.

Determination of radioactivity

Radioactivity of liquid samples was counted in a Packard Tri-Carb liquid scintillation spectrometer Type 2660. The [^{14}C] flumecinol in aqueous samples (0.25—2 ml) was counted in 5 ml of Insta-Gel® (Packard, Downers Grove, IL, U.S.A.) and in the diethyl ether samples (5 ml) in 5 ml of toluene-based liquid scintillation solution (5 g of PPO, 0.1 g of dimethyl POPOP, 1000 ml of toluene). For the determination of the absolute activity the external standard—channel ratio method was used.

Gas—liquid chromatography

A Hewlett-Packard 5736A gas chromatograph equipped with a flame ionization detector (FID) was used. The electronic parameters were set at maximum sensitivity: range 1, attenuation 1 for the plasma and saliva samples.

Nitrogen of high purity was used as carrier and auxiliary gas. The methyl silicone Sp 2100 — Carbowax 20M deactivated — flexible fused-silica capillary column was 25 m × 0.20—0.21 mm I.D. (Hewlett-Packard). The capillary column was operated at 160°C (human and rat plasma) and 150°C (human saliva) with a nitrogen flow-rate of 1.6 ml/min. The capillary column pressure was 196.6 kPa (28.5 p.s.i.) (plasma) and 207.0 kPa (30 p.s.i.) (saliva). The flow-rate was 18.2 ml/min at the split vent and 1.5 ml/min at the septum vent. The flow-rates of the detector gases were 40 ml/min for hydrogen and 150 ml/min for air. The temperature settings were as follows: capillary injector port in split mode 250°C (split insert was unpacked), FID 300°C.

In vivo experiments

For the pharmacokinetic examination healthy volunteers received Zixoryn® (flumecinol) in a single 100-mg oral dose; for the saliva kinetics 400 mg of Zixoryn were administered orally. Venous blood and saliva samples were obtained up to 72 h (on 14 occasions). Salivary flow was stimulated by chewing Parafilm® until at least 3 ml had been collected. The rats received Zixoryn mixed in the daily food in a dose of 125 mg per kg per day during the chronic toxicity studies.

Sodium citrate (3.8% w/v) was added to the human blood samples in a ratio of 1:9, to prevent coagulation. The plasma and saliva samples were kept deep-frozen until analysis.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of human plasma extract. The retention time for internal standard (3-trifluoromethyl-benzhydrol) was 3.1 min, and for flumecinol 4.1 min.

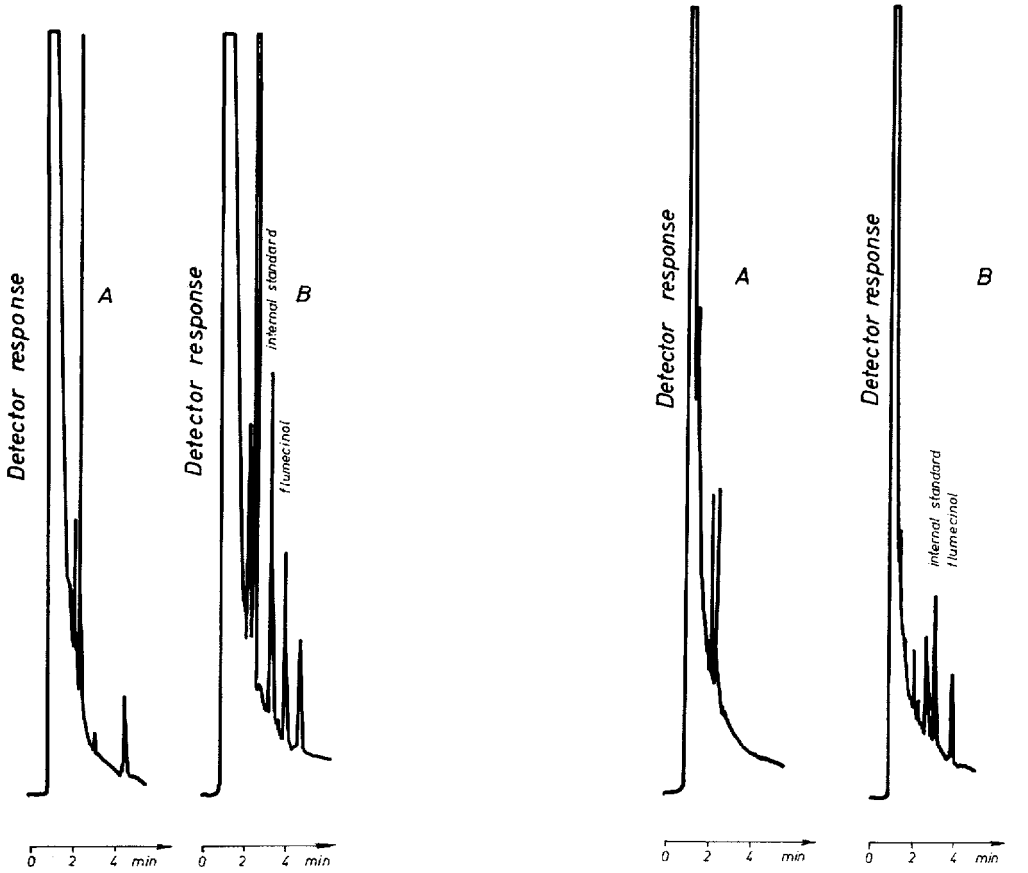

Fig. 1. Chromatograms of human plasma extract: (A) control plasma; (B) plasma obtained 2.5 h after oral administration of flumecinol.

Fig. 2. Chromatograms of rat plasma extract: (A) control plasma; (B) plasma obtained after 125 mg per kg per day oral administration of flumecinol.

Fig. 2 shows a chromatogram of rat plasma extract. The retention times are the same as for human plasma chromatograms. The method can also be applied to human saliva; Fig. 3 shows a typical chromatogram of human saliva extract. The endogenous compounds can be separated at lower temperature

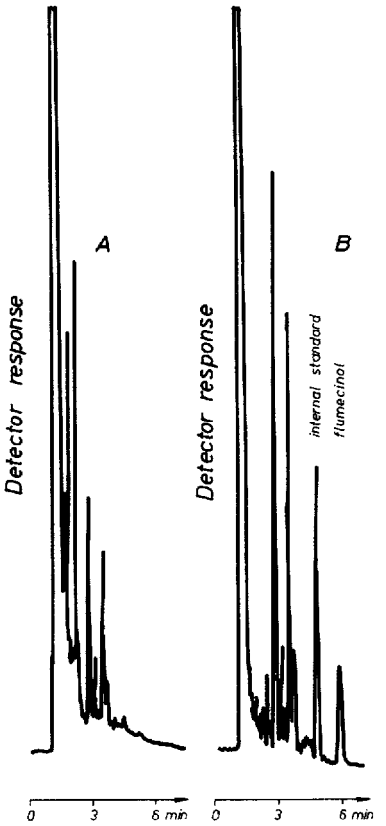

Fig. 3. Chromatograms of human saliva extract: (A) control saliva; (B) saliva obtained 3 h after oral administration of flumecinol.

and therefore the retention times are longer (4.8 and 6.0 min) than in the case of plasma samples.

The calibration curves obtained with human and rat plasma and saliva show a good linearity within the range 10—200 ng/ml flumecinol (Fig. 4). The correlation coefficient of the calibration curve for human plasma was 0.990317, for saliva 0.990028, and for rat plasma 0.994828.

Recovery of flumecinol from plasma and saliva samples was checked using radiolabelled drug and was found to be 78.11 ± 3.452% (S.D.), 75.03 ± 1.056% and 88.10 ± 2.442% for human plasma, saliva and rat plasma, respectively.

In Fig. 5 the concentration—time curves are shown for human plasma and saliva, demonstrating that the method allows monitoring of plasma and saliva concentrations up to the 72nd and 30th hour, respectively.

The advantages of the extraction and flexible fused capillary gas—liquid chromatographic method are that it has a sensitivity of 0.5 ng/ml and permits the determination of flumecinol in a volume as small as 250 μl of rat plasma too. From the same volunteers the saliva/plasma concentration ratio could be determined and the in vivo protein binding of flumecinol could be calculated. The retention time for flumecinol determination is short (4.5 min).

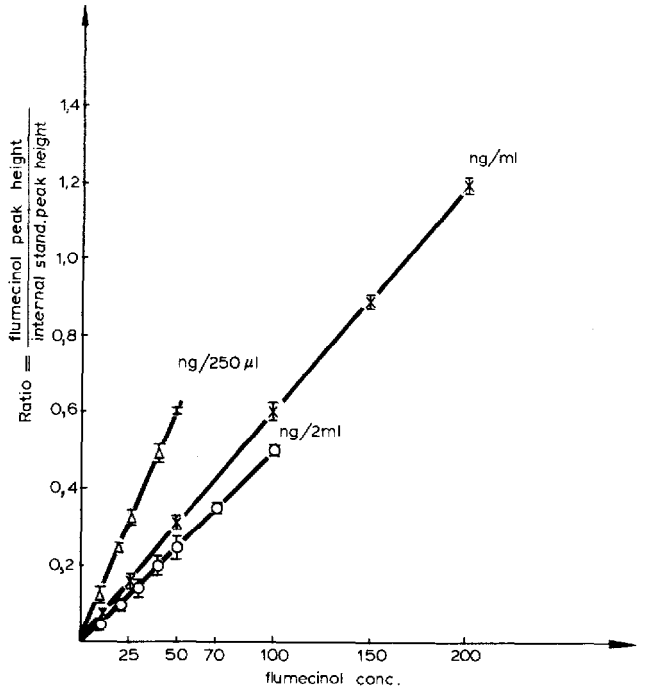

Fig. 4. Calibration curves for human plasma (x——x), human saliva (○——○) and rat plasma (△——△) (mean ± S.D.).

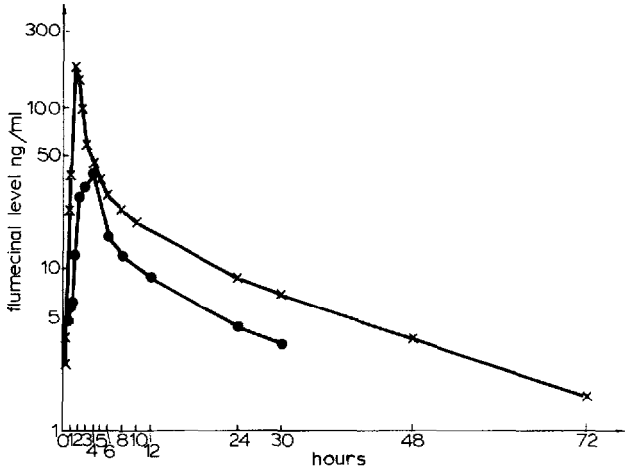

Fig. 5. plasma (x——x) and saliva (○——○) flumecinol concentration—time curves after oral administration of 100 mg (plasma) and 400 mg (saliva) of flumecinol to healthy volunteers.

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