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Note

Detection of some antihypertensive drugs and their metabolites in urine by thin-layer chromatography

II. A further five beta blockers and dihydralazine

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In Part I[1] we described a simple method of monitoring antihypertensive drug compliance by tests on urine from patients prescribed metoprolol, acebutolol, oxprenolol, propranolol, nadolol and hydralazine. The method has now been applied successfully to urine specimens from patients or volunteers taking atenolol, labetalol, pindolol, alprenolol, sotalol and dihydralazine. The structures of these compounds are given in Fig. 1.

MATERIALS AND METHODS

Thin-layer chromatography (TLC)

This was as described in Part I except that, for the detection of atenolol, plates with an added fluorescent indicator were used (Polygram Sil N-HR UV 254; Macherey, Nagel & Co., Düren, G.F.R.).

Reagents and materials

To test for atenolol, pindolol, alprenolol and dihydralazine, the method described in Part I was followed exactly. For labetalol, an ammonia–HCl buffer (1 *M*, pH 8.5) was substituted for 7 *M* NaOH. Because of the low lipid solubility of sotalol a different extraction procedure had to be introduced. This used a carbonate–bicarbonate buffer (0.1 *M*, pH 10.0) and a charcoal slurry made by suspending 0.5 g of activated charcoal (acid washed, BDH, Poole, Great Britain) in 100 ml of carbonate–bicarbonate buffer. Atenolol was supplied by Stuart (Cheadle, Great Britain), labetalol by Allen and Hanburys (London, Great Britain), pindolol by Sandoz (Leeds, Great Britain), alprenolol by Hässle (Mölndal, Sweden), sotalol by Bristol (Slough, Great Britain) and dihydralazine by Leiras (Turku, Finland). Visualizing reagents used were 38% formaldehyde in concentrated sulphuric acid (1:10) and a saturated solution of ammonium metavanadate in concentrated sulphuric acid.

Extraction procedure

For atenolol, pindolol, alprenolol and dihydralazine this was exactly as

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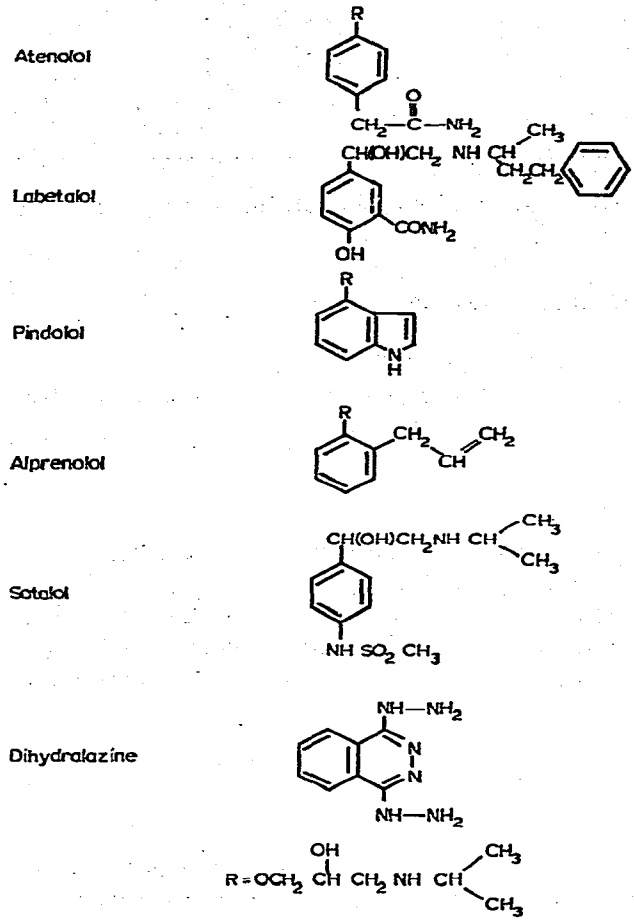

Fig. 1. Structures of the antihypertensive drugs, studied.

described in Part I. For the extraction of labetalol, 2 ml of ammonia HCl buffer were substituted for 0.05 ml of 7 *M* NaOH. Because of its very low solubility in non-polar organic solvents, sotalol could not be adequately extracted from urine by the method described in Part I and the procedure described below was used.

To a glass screw-cap tube, 4 ml of urine, an equal volume of carbonate–bicarbonate buffer and 0.1 ml of charcoal slurry are added. The tube is mixed by rotation at 30 rpm for 5 min and centrifuged at 300 *g* for 5 min. The supernatant is decanted and discarded. 10 ml of distilled water are added to the tube and mixing is carried out for 2 min followed by centrifugation for 5 min. The supernatant is again discarded and 2 ml of methanol are added to the tube and the contents mixed in a whirlimixer for 10 sec. The tube is allowed to stand for 5 min, then centrifuged at 300 *g* for 5 min. The methanol is decanted and evaporated to dryness under a stream of nitrogen at 50°C. The extract is redissolved in two drops of methanol, spotted on to a TLC plate and developed in the solvent system used for all the beta blockers. Sotalol is visualized by pouring down the plate a saturated solution of ammonium metavanadate in concentrated sulphuric acid.

RESULTS AND DISCUSSION

The R_F values of the drugs studied, their colours under UV light or after treating with visualizing reagent, and minimum detectabilities are given in Table I. To measure the minimum detectabilities, visualization was carried out by using UV light or the formaldehyde–sulphuric acid reagent. The only exception to this was sotalol, which did not fluoresce or give a colour with formaldehyde–sulphuric acid. In order to visualize sotalol, the ammonium vanadate reagent had to be used and this proved much less sensitive giving a minimum detectability in urine of 50 mg/l. The minimum detectabilities are sufficiently low to allow detection in urine of all the drugs when taken orally, by patients or volunteers, at the recommended therepeutic doses. This is true even for sotalol, which is normally given orally in doses of 80 mg or more twice daily and is excreted in urine largely unchanged[2]. When 80 mg of sotalol were taken twice daily for two days by a volunteer, the unchanged drug could easily be demonstrated in an early morning urine specimen collected on the third day. In the case of the other beta blockers, it is also the unchanged drug that is detected. The method has been successfully applied to urine specimens from patients taking atenolol and labetalol. We did not have access to patients taking sotalol and the other antihypertensives, pindolol, alprenolol and dihydralazine are used less frequently or not at all in Great Britain. Urine specimens were collected from volunteers taking oral doses in these cases, and the usefulness of the method can be judged from the observation that pindolol 5 mg or alprenolol 100 mg taken twice daily for two days can be demonstrated without difficulty in an early morning urine specimen collected on the third day.

When a single 25-mg oral dose of dihydralazine (Pressalin®, Leiras) was taken by a healthy volunteer and the urine collected 8 h later, a fluorescent spot, R_F 0.55, was found which was not present in a urine specimen collected immediately before dosing. A search of the literature revealed nothing on the metabolism of dihydralazine in man.

TABLE I

R_F VALUES, CHARACTERISTIC COLOURS AND MINIMUM DETECTABILITY IN URINE

Solvent systems: 1, ethyl acetate–methanol (40:5); 2, ethyl acetate–methanol–concentrated ammonia (40:5:5).

Compound	R_F		*UV 254*	*Colour with formaldehyde–conc. sulphuric acid*	*Colour with ammonium metavanadate–conc. sulphuric acid*	*Minimum detectability in urine (mg/l)*
	Solvent system 1	*Solvent system 2*				
Atenolol	0.0	0.54	dark spot*	—	brown	0.5
Labetalol	0.30	0.76	pale blue	—	green	0.5
Pindolol	0.20	0.84	—	yellow	brown	1.0
Alprenolol	0.10	0.90	—	red brown	red brown	0.1
Sotalol	0.0	0.70	—	—	brown	50
Dihydralazine metabolite	0.55	—	pale blue	—	—	see text

* No native fluorescence, dark spot when run on a fluorescent plate.

Assuming that its transformation was similar to that of hydralazine[3], a symmetrical triazolo or a methyl triazolo metabolite would be expected. Unfortunately the synthesis of these derivatives proved difficult. Dihydralazine reacts with formic acid to give, not the expected *s*-triazolophthalazine, but 6-(2-formylhydrazino)-*s*-triazolo-[3,4-*a*]phthalazine[4]. Dihydralazine and acetic anhydride are reported to produce a diacetyl derivative if the reaction is carried out at 95°C, and a triacetyl derivative if the reaction temperature is 130°C[5]. However, we were unable to prepare a pure compound on treating dihydralazine with acetic anhydride and are unable, consequently, to report a minimum detectability in urine. The R_F value and fluorescence of the dihydralazine metabolite in the urine of a volunter were similar to those of one spot obtained on chromatography of the mixture obtained on the reaction of dihydralazine with acetic anhydride.

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