J. Pharm. Pharmacol. 1982, 34: 264–266
Communicated December 17, 1981

0022–3573/82/040264–03 $02.50/0

Metabolism of [^{14}C]papaverine in man

GUNILLA WILÉN*, PAULI YLITALO†, *Research Laboratories of Star Ltd., P.O. Box 33, 33721 Tampere 72, Finland, †Department of Biomedical Sciences, University of Tampere, and Tampere University Central Hospital, Tampere, Finland.

There are few data available concerning the fate of papaverine. Axelrod et al (1958) showed it to be almost completely metabolized in vivo, while different animal species (Belpaire & Bogaert 1973, Belpaire & Bogaert 1975) excreted metabolites predominantly in the bile as conjugated demethylated metabolites. Only in rabbit, guinea-pig and man the excretion in urine was important.

Rossel & Belpaire (1977) identified five papaverine metabolites in human urine by mass spectrometry and Belpaire et al (1978) recognized and gas-chromatographically quantified 50% of orally given papaverine in urine.

Since labelling the drug has advantages for pharmacokinetic studies, we have investigated the appearance of [^{14}C]papaverine in the circulation and its fate in patients.

Material and methods

Subjects and collection of samples. Six female and three male volunteer patients, all over 60 years of age and with normal gastrointestinal, liver and kidney functions, were given two 100 mg tablets of papaverine HCl with 300 ml of mineral water. Each tablet consisted of unlabelled (Star Ltd., Tampere, Finland) and ^{14}C-labelled papaverine purity >99%, and spec. act. 1·41 μCi mg^{-1}, Res. Lab. Medica Ltd., Helsinki, Finland). The final radioactivity was 2·2–3·5 μCi per tablet.

The patients fasted 2 h before and 2 h after drug administration. Venous blood samples were drawn before and 1, 2, 4, 6, 8, 12, 24, and 48 h after the drug was given. Urine was collected at 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, and 24–48 h. Heparinized blood specimens were centrifuged and the plasma was separated. All the plasma and urine samples were stored at −20 °C until analysed.

Measurement of papaverine and its metabolites. Papaverine concentrations in blood were measured by high-pressure liquid chromatography. To 1 ml of blood 1·5 ml of 1 M NaOH was added and the mixture was extracted twice with ether. Aliquots were evaporated by nitrogen and the residue was redissolved in 0·1 M HCl. Samples were injected in duplicate into a high pressure liquid chromatograph (Waters Associates Inc.), fitted with Mikro-Bondapak C-18 column. The mobile phase was 30% acetonitrile in 0·1 M NaH_2PO_4 (pH = 5·0).

The extraction of papaverine and its metabolites from urine was performed as follows. Radioactivity was measured from free papaverine and free basic compounds after 5 ml of urine had been extracted twice with 10 ml n-heptane containing 1·5% isoamylol at pH 12·5 (or 10 ml $CHCl_3$ at pH 9). Free acidic compounds were counted after extraction of the urine with 10 ml $CHCl_3$ at pH 4. Conjugated metabolites of papaverine were assessed after incubation with β-glucuronidase/arylsulphatase (18 h pH 7·2, 37 °C) followed by extraction with 20 ml $CHCl_3$ at pH 9 to give the basic papaverine metabolites. These were assayed by g.l.c. or t.l.c. or the radioactivity counted. Acid metabolites were extracted from the incubation mixture with 20 ml $CHCl_3$ at pH 4 and counted. The aqueous remainder was also counted. The amount of papaverine and the degree of its conjugation in urine were estimated by measuring the radioactivity in organic extracts before and after the enzymatic cleavage with 10 000 Fishman units of β-glucuronidase/17 500 units of arylsulphatase per sample (Koch-Light Lab., Ltd., Colnbrook, England) or with pure β-glucuronidase (10 000 Fishman units per sample, Sigma, Saint Louis, USA). Radioactivity was measured using Lumagel (Lumac Systems A.G., Schaesberg, Holland) scintillation solution and a Wallac 1210 Ultrobeta scintillation counter (Turku, Finland).

To quantify 4′-desmethylpapaverine in basic urine extracts, a Hewlett Packard gas chromatograph 5750G equipped with a nitrogen detector and a 3% OV-17 glass column was used. The injection port was 320 °C, the column 270 °C, and detector block 370 °C.

For thin-layer chromatography (t.l.c.) samples were spotted on silica gel 60 F 254 plates. Papaverine, 4′-desmethyl- and 3′-desmethylpapaverine (Star Ltd.) were used as reference compounds. The carrier solvent was chloroform–dioxan–ethylacetate–ammonia (25:60:10:5). The spots were visualized under u.v. irradiation. The

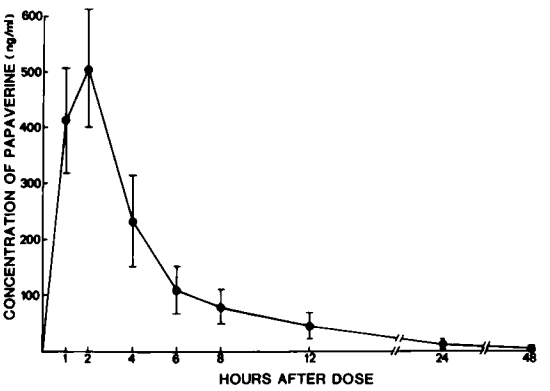

FIG. 1. The concentration of unchanged papaverine in circulation (mean ± s.e.) after the administration of a single oral dose (200 mg) of [^{14}C]papaverine HCl in 9 patients.

* Correspondence.

Table 1. Papaverine and released metabolites extractable into chloroform from pooled urine (0–48 h) at pH 9 after β-glucuronidase/arylsulphatase treatment in 7 patients (mean ± s.e.) expressed as % of total extractable radioactivity. Corresponding R_F values on the t.l.c. chromatogram are also listed.

Papaverine	Metab. I 4′-desmethyl-papaverine	Metab. II 3′-desmethyl-papaverine	Metab. III	Metab. IV	Metab. V	Metab. VI
0·6 ± 0·1	16·3 ± 0·6	1·8 ± 1·1	0·9 ± 0·1	23·3 ± 0·5	9·2 ± 1·2	0·19 ± 0·1
R_F 0·67	0·58	0·51	0·40	0·31	0·26	0·12

corresponding areas were scraped off and extracted into chloroform and the radioactivity measured as described.

Results and discussion

The concentration of unchanged papaverine in circulation after the single oral dose (200 mg) of [^{14}C]papaverine HCl is shown in Fig. 1. The peak concentration of papaverine (503 ± 108 ng ml^{-1}, mean ± s.e.) appeared in 2 h, and it corresponded to 8·3 ± 2·4% of the total plasma radioactivity at this time. Thereafter the fraction of unchanged drug declined, and at 12 h it was only 3·1 ± 1·3% of the total activity. During the 48 h after administration 80 ± 4% of the radioactivity appeared in urine indicating that papaverine is almost completely absorbed from the gastrointestinal tract and thereafter rapidly metabolized.

After the absorption phase the initial half-life of papaverine in circulation was 1·7 ± 0·2 h, and the second one 6·6 ± 0·9 h ($T_{1/2}$ β). The half-lives of total radioactivity were 2·7 ± 0·3 and 11·8 ± 0·6 h, respectively. Thus the disappearance of papaverine and its metabolites from circulation seemed to occur in two phases: a rapid disappearance during the first 6–8 h, followed by a slower elimination during the next 24 h. This is in accordance with the studies by De Graeve et al (1977), who found the plasma half-life of papaverine during the first 6 h was about 1 h, followed by a slower elimination. Our results also suggest that the elimination half-lives of papaverine in the circulation are shorter than those of its metabolites ($P < 0·01$, paired t-test).

The metabolic pattern of papaverine in urine was studied by estimating the radioactivity in organic solvent extracts before and after β-glucuronidase/arylsulphatase treatment. Only small amounts, 0·82 ± 0·1% of the recovered radioactivity, were extractable into organic solvent before the enzyme treatment. In agreement with Axelrod et al (1958) and Belpaire et al (1978), this suggests that only traces of papaverine and its free lipid-soluble metabolites are excreted in urine.

After the incubation with β-glucuronidase/arylsulphatase, 60 ± 2% of the recovered radioactivity (= 48% of the given dose) was extractable into chloroform at pH 9. This fraction consisted of basic glucuronide and/or sulphate conjugates. At least six metabolites could be separated in this fraction by t.l.c. and quantified by counting the radioactivity (Table 1). Two of the metabolites, 4′-desmethyl- and 3′-desmethyl-papaverine, were characterized by comparing them with synthesized reference substances. 4′-Desmethylpapaverine, which was considered by Axelrod et al (1958) as the major urinary metabolite of papaverine, was quantified additionally in three subjects by g.l.c. They were found to excrete, in 48 h, 7·8 ± 0·5% of the given dose as 4′-desmethylpapaverine. With t.l.c. the 4′-desmethylpapaverine excretion in seven patients represented 13·2 ± 0·5% of the dose. These findings are in some agreement with the results on 4′-desmethylpapaverine excretion obtained by Belpaire et al (1978), but Axelrod et al (1958) reported this metabolite to represent about 48% of the dose administrated (10 mg kg^{-1}). We found, one of the unidentified basic metabolites represented a larger fraction than 4′-desmethylpapaverine, accounting for 18·6% of the dose (23·3% of the excreted radioactivity, see Table 1).

The ratio of glucuronide to sulphate conjugation was investigated in the 48 h urine of three patients. The urine was treated with pure β-glucuronidase, and extracted as described earlier. Thereafter the urine was further treated with β-glucuronidase/arylsulphatase, and the released compounds were again extracted. In two of the patients only about 10% of the metabolites were excreted as sulphate conjugates, whereas one patient excreted more than half of the metabolites sulphate-conjugated.

Only small amounts of papaverine seem to be excreted as acidic glucuronide and/or sulphate conjugates, since after enzyme treatments no more than 3 ± 0·3% of the recovered radioactivity (2·4% of the dose) appeared in organic solvent at pH 4. About one third (35%) of the total urinary radioactivity remained in the aqueous residue and could not be identified.

Therefore, after oral administration, papaverine is well absorbed and most is excreted as glucuronide and/or sulphate conjugates. Metabolism is rapid and only a negligible amount of drug is excreted as free papaverine or unconjugated lipid-soluble metabolites.

The authors thank Dr Aneri Penttilä, Medica Pharmaceutical Company Ltd, Helsinki, for preparing the ^{14}C labelled papaverine, and Heikki Nupponen, M.Sc., Star Ltd, Tampere, for synthetizing the reference substances. We also wish to thank Lasse Siitonen, M.D. and Lauri Pöyhönen, M.D., Tampere University Central Hospital, for smooth collaboration during the study. We are grateful to Mrs Arja Ollila and Mr Pauli Mäkinen for their technical assistance.

REFERENCES

Axelrod, J., Shofer, R., Inscoe, J. K., King, W. M., Sjoerdsma, A. (1958) J. Pharmacol. Exp. Ther. 124: 9–15

Belpaire, F. M., Bogaert, M. G. (1973) Biochem. Pharmacol. 22: 59–66

Belpaire, F. M., Bogaert, M. G. (1975) Xenobiotica 5: 421–429

Belpaire, F. M., Rossel, M. T., Bogaert, M. G. (1978) Ibid 8: 297–300

DeGraeve, J., van Countfort, J., Gielen, J. (1977) J. Chromatogr. 133: 153–160

Rossel, M. T., Belpaire, F. M. (1977) in: De Leenheer, A. P., Roncucci, R. R. (eds). Quantitative Mass Spectrometry in Life Sciences. p. 133. Amsterdam: Elsevier Scientific Publishing Company.

J. Pharm. Pharmacol. 1982, 34: 266–268
Communicated May 7, 1981

0022–3573/82/040266–03 $02.50/0

Pre- and postsynaptic effects of sulpiride in the rat isolated vas deferens

A. BADIA*, P. BERMEJO, F. JANÉ, *Departament de Farmacologia i Terapèutica, Facultat de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain*

It is known that many sympathetic nerve terminals have not only α-adrenoceptors but also dopamine receptors, that regulate the stimulation-evoked release of noradrenaline by means of a negative feed-back mechanism (Enero & Langer 1975; Hope et al 1978; Dubocovich & Langer 1980). In the rat vas deferens, in addition to the classical postsynaptic α-adrenoceptors, the existence of presynaptic α-adrenoceptors (Drew 1977) and postsynaptic and presynaptic dopamine receptors has been described (Simon & Van Maanen 1976; Tayo 1977, 1979). On the other hand, it has been reported that some differences do exist between pre- and postsynaptic dopamine receptors (Goldberg et al 1978) as occurs with α-ardrenoceptors (Langer 1979). In order to obtain more information about the nature of the two types of dopamine receptors in the rat vas deferens, we have investigated the activity of several benzamides, known as potent dopamine antagonists (Jenner & Marsden 1979). This report describes the effects of sulpiride, a substituted benzamide, on pre- and postsynaptic α-adrenoceptors and dopamine receptors in the rat isolated vas deferens.

Male Wistar rats (300–325 g) were killed by cervical dislocation and exsanguination. Both vasa deferentia were removed and carefully cleaned. The whole vasa were set up in isolated organ baths containing 20 ml of Krebs solution, as modified by Huković (1961). The solution was maintained at 32 ± 0·5 °C and gassed with 95% O_2–5% CO_2. 1 h was allowed to elapse before starting the experiment. The organ responses against 0·5 g tension were recorded by means of an isotonic Ealing transducer on an Omniscribe pen-recorder.

Presynaptic studies. Platinum ring electrodes were placed above and below of the vas deferens and continuous field stimulation was carried out with an Ealing Stimulator (0·1 Hz, 3 ms and 20–30 V). When the twitch responses to field stimulation became stable, the agonists were added to the bath in a cumulative concentration schedule every 3–5 min. When the concentration-response curve with the agonist was obtained, a full recovery after washout was difficult to obtain and it was only possible to evaluate one concentration-response curve in each tissue. In order to resolve this problem, the following procedure was used. When a maximal inhibitory effect was obtained with the agonist, the initial twitch response was restored by the cumulative addition of sulpiride in a concentration-dependent manner. Using the method described by Davis et al (1980) for isolated bronchi, it was possible to determine at least three dose-ratios of agonist from the isoresponse points of the two curves obtained in each preparation, and the pA_2 and slopes of Arunlakshana & Schild (1959) were calculated.

Postsynaptic studies. Cumulative concentration-response curves of isotonic contractions were obtained in each vas deferens with the agonist. The concentration-response curves as control and in the presence of sulpiride ($3 \cdot 10^{-5}$, $1 \cdot 10^{-4}$ and $3 \cdot 10^{-4}$ mol litre^{-1} added 5 min before obtaining each curve) were constructed for each preparation. In this situation, it was possible to calculate the pA_2 and the slopes of the Schild plots (Arunlakshana & Schild 1959) in each experiment.

All the results are given as the mean ± s.e.m. The means were statistically compared using Student's *t*-test and the differences were significant when $P < 0{\cdot}05$.

Clonidine and apomorphine were used as presynaptic α-adrenergic and dopaminergic agonists respectively because they do not induce contractile effects at the concentrations used. Both agonists inhibited in a concentration dependent fashion the twitch contraction of the rat isolated vas deferens obtained with continuous field stimulation, and sulpiride restored this to the initial value after graded additions (Fig. 1). Calculating, by means of interpolation, the concentration of the agonist used as control equivalent to the restored response with sulpiride, at least three dose-ratios were determined in each experiment. With the calculated values, the Schild plots were constructed and the pA_2 and the slopes of the regression lines were obtained for the interaction of sulpiride with both agonists (Table 1). As can be seen, the two slopes were near the theoretical value of 1 and the differences between the two pA_2 values were not statistically significant.

Noradrenaline and dopamine were used as postsynaptic α-adrenergic and dopaminergic agonists respectively

* Correspondence.