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Note

Rapid gas chromatographic determination of pyrimethamine in human plasma and urine

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Pyrimethamine (5-*p*-chlorophenyl-6-ethylpyrimidine-2,4-diamine) possesses cytotoxic activities like many other 2,4-diaminopyrimidines. It passes the blood—brain barrier and inhibits dihydrofolate reductase (EC 1.5.1.4) [1]. It has gained widest use as a malarial suppressant in combination with a sulphonamide (synergistic effect) [2, 3]. It is also used in meningeal leukaemia [4, 5] and as a veterinary additive to rabbit and poultry feeds for the prevention of coccidiosis [6, 7]. The good permeability of pyrimethamine in rat and dog brain tissue [5, 8] has attracted interest in its possible antitumor activity [5, 9—11].

Previous analytical methods were biological [11—13], photometric [9, 14, 15] and fluorimetric [16, 17]. The gas chromatographic method with electron-capture detection described by Cala et al. [18] greatly improved the specificity and sensitivity. The method described was used on chicken tissue and the sensitivity limit was about 100 ng/g. Analysis of pyrimethamine in plasma was first described by DeAngelis et al. [10], who used thin-layer chromatography and an ultraviolet absorption scanning instrument. The methodology was refined by the use of high-performance liquid chromatography [7, 19], and the minimum concentrations measurable [19] were approximately 10 ng/ml. Jones et al. [20] reverted to gas chromatography with the aim of enhancing the sensitivity. They reported the minimum detectable quantity of the pure compound to be 50 pg injected onto the column. However, the analytical procedure involved two extractions from the plasma with the poorly evaporable toluene, programming of the column temperature and a chromatography time of 26 min. Bonini et al. [21] published at the same time gas chromatographic methods to determine some antimalarials including pyrimethamine in biological fluids. The analytes were added and extracted from the cellular whole blood or

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urine and subjected to gas chromatography with a nitrogen—phosphorus detector. No genuine samples were analyzed. Recently, description of a liquid chromatographic method with fluorescence detection to determine pyrimethamine in plasma appeared [22]. The limits of detection were in both cases [21, 22] reported to be about 5 ng calculated per ml of specimen.

This paper describes analytical methods for the rapid and selective determination of pyrimethamine in plasma and urine. The techniques were applied in a pilot study on a female volunteer given a single oral dose of 25 mg.

EXPERIMENTAL

Chemicals and solutions

Pyrimethamine and 2-chlorotriphenylmethanol (internal standard) (Fig. 1) were supplied by the Synthesis Laboratory of Dumex Ltd. All other chemicals were of analytical grade. Methanol was used as solvent for stock and standard solutions of pyrimethamine and 2-chlorotriphenylmethanol.

For the pilot in vivo study a tablet containing 25 mg of pyrimethamine and 500 mg of sulfadoxine was supplied by the Pharmaceutical Research Laboratory of Dumex Ltd.

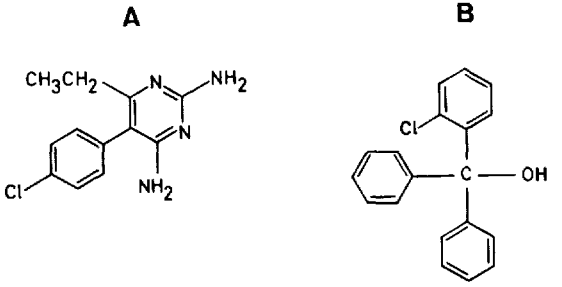

Fig. 1. Chemical structure of (A) pyrimethamine and (B) 2-chlorotriphenylmethanol (internal standard).

Gas chromatographic equipment

A Pye 104 gas chromatograph with an electron-capture detector (^{63}Ni) was used. The glass column was 150 cm long and had an internal diameter of 6.2 mm. The column material was Chromosorb 750 (80—100 mesh) with 3% OV-17.

Analytical procedures

Assay I: plasma concentrations. A 1-ml volume of sample was added to the evaporated residue of 0.6 ng or less of 2-chlorotriphenylmethanol dissolved in methanol. After alkalinization with 150 μl of 0.15 mol/l NaOH the glass tube was rotated at 60 rpm for 5 min. Extraction of the two compounds was performed by adding 6 ml of dichloromethane and rotating the tubes. After centrifugation at about 2000 *g* at ambient temperature and discarding the plasma phase, 5 ml of the remaining organic phase were transferred to a conical glass tube and evaporated under a stream of nitrogen. The residue was redissolved in 100 μl of acetone and 1—2 μl were injected onto the column. The temperature

of the column oven was 235°C, the detector 350°C; the temperature setting of the injection port was position 1. The flow-rate of the carrier gas (argon—methane, 9 : 1) was 60 ml/min.

Assay II: urine concentrations. Urine samples were treated in the same way as plasma, except for the following differences. First, only 100 μl of 0.15 mol/l NaOH were added. Secondly, the organic phase resulting from the extraction was washed with 1 ml of distilled water with a pH of 7.0, after which the internal standard was added. The temperature settings were as given above except that the temperature of the column was 220°C. The carrier gas flow-rate was 50 ml/min.

Quantitation of pyrimethamine. Plasma standard curve samples were prepared by adding drug-free sample to known amounts of pyrimethamine and the internal standard. When urine was analysed only pyrimethamine was added before the extraction (see above). The standard concentrations (ng/ml) ranged from 0 to 400 ng of pyrimethamine whereas the amount of internal standard added was held constant at, for example, 0.6 ng (plasma and 0.3 ng (urine). The peak area ratios of the two analytes in each of the standards were related to the pyrimethamine concentrations and a straight line (linear regression by method of least squares) was obtained. The concentrations of the samples were then computed from this line.

Analytical selectivity. The developed methods were without interferences from commonly prescribed drugs such as acetylsalicylic acid, acetaminophen, acetophenetidin and diazepam.

RESULTS AND DISCUSSION

Analytical procedures

By employing these methods for pyrimethamine quantitation, plasma concentrations were measured in less than 6 min after a simple extraction process. Figs. 2 and 3 demonstrate the ready ability of the methods to separate and identify the two compounds in plasma and urine. The extraction recovery of pyrimethamine from plasma and urine was fully quantitative. The internal standard was extracted from plasma with a yield of about 70% (n = 10, S.E.M. < 5%) and from urine with a yield below 50%. The latter was considered to be unfavorable for an internal standard and therefore this compound was not included in the extraction process of urine.

The concentration limit of quantitation could be lowered to 5 ng/ml when extracting 1 ml of the sample and reducing the volume of redissolution to about 30 μl. The degree of linearity of the plasma standard curve and the results of repetitive analyses of control samples (pools) served as quality characteristics (i.e. accuracy and precision) of the method. From normal plasma standard curves it appeared that the mean coefficient of determination was 0.996, with an interassay coefficient of variation (C.V.) below 1% (n = 10). The mean pool concentration was 95 ng/ml with an interassay C.V. of 7% (n = 10). When an enhancement of the sensitivity was accomplished by reduction of the redissolution volume to 30 μl, an increase in C.V. to about 12% was observed. The quality characteristics of the urine assay differed solely concerning the precision in that the interassay C.V. increased to about 10% (n = 10) at the same concentration.

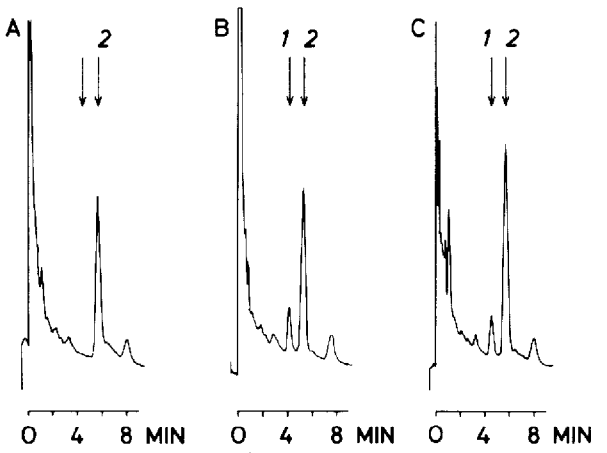

Fig. 2. Chromatograms of (A) blank plasma, (B) plasma pyrimethamine standard of 150 ng/ml, and (C) plasma sample from two days after oral administration of 25 mg of pyrimethamine to an adult volunteer. The peak area ratio was estimated to represent approximately 110 ng/ml. Sample volumes were 0.5 ml and injection volumes 1 μl in all cases. 1 = pyrimethamine, 2 = internal standard.

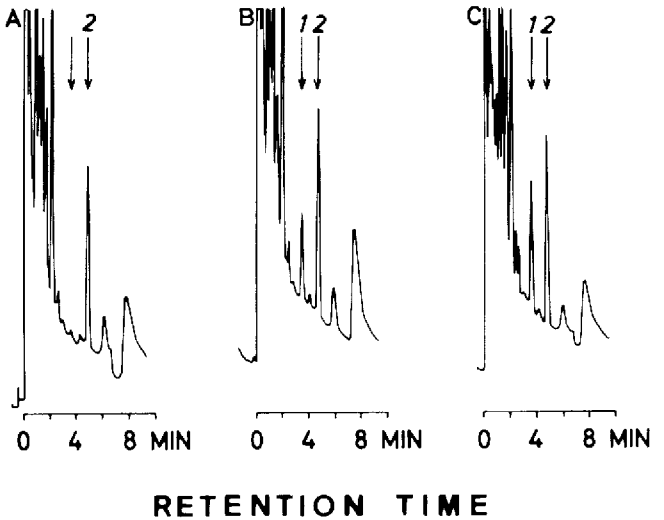

Fig. 3. Chromatograms of (A) blank urine, (B) urine pyrimethamine standard of 150 ng/ml, and (C) urine sample collected 0—3 h after oral administration of 25 mg of pyrimethamine to an adult volunteer. The peak area ratio was estimated to represent approximately 250 ng/ml. Sample volumes were 1 ml and injection volumes 1 μl in all cases. 1 = pyrimethamine, 2 = internal standard.

When developing the methods, different approaches were explored with respect to the extraction and chromatographic procedures. The final extraction procedure resulted from a series of experiments with extraction mixtures of dichloromethane and *n*-hexane at varying pH. Concerning the chromatography there were some difficulties in that extraneous peaks appeared in the chromato-

gram after about twenty injections of plasma samples. However, the retention times of these peaks were 20—25 min, thus far beyond that of pyrimethamine and the internal standard. Raising the temperature of the column oven to about 280°C for 1 h was found to diminish this disturbance.

Application

The methodology was applied in a pilot study in which a female volunteer ingested a tablet of 25 mg of pyrimethamine and 500 mg of sulfadoxine. The analysis of sulfadoxine will be reported later. The plasma concentration of pyrimethamine versus time curve and the corresponding urinary excretion rate curve are shown in Fig. 4.

Assuming that distribution is complete after the third day, the elimination half-life of the drug appears to be about 96 h. This value agrees with other assessments [5, 19], which range from 35 to 175 h. The urinary excretion rate during the first 24 h is estimated as three mean values of about 50 μg/h covering the periods 0—3, 3—8 and 8—24 h. This implies an excretion of about 14% of the dose within 0—24 h and is in agreement with data published on doses of 100 mg of pyrimethamine [23]. To the author's knowledge no data have been published on pyrimethamine urinary excretion after intake of 25 mg of the drug.

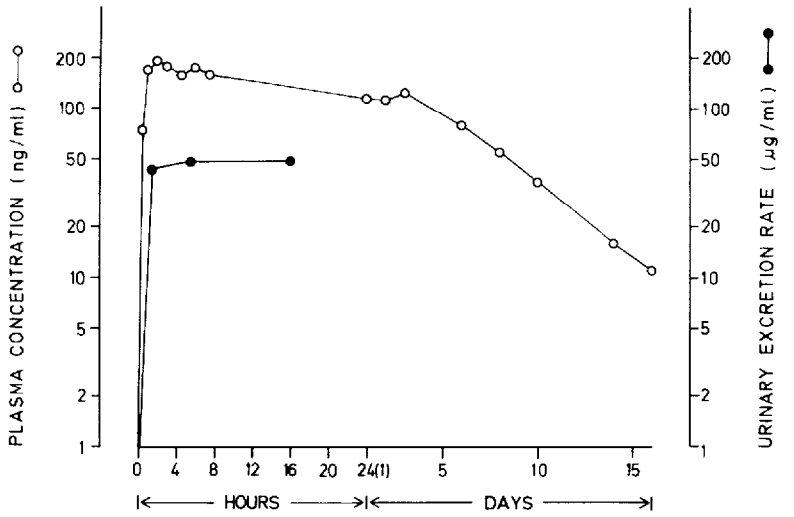

Fig. 4. Plasma concentration and urinary excretion rate versus time curve for a female volunteer who had ingested a 25-mg oral dose of pyrimethamine. The excretion rates are plotted in the midpoints of the collection periods.

CONCLUSION

This paper describes selective and rapid gas chromatographic procedures which permit measurement of pyrimethamine in human plasma and urine for up to at least 16 days after a 25-mg single oral dose of the drug.

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