THE INFLUENCE OF DIPHENHYDRAMINE ON THE ABSORPTION OF METHAQUALONE IN MAN

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- 1 The effects of diphenhydramine on the buccal absorption, *in vivo* absorption and the *in vitro* dissolution of methaqualone have been studied.
- 2 Diphenhydramine significantly reduced the buccal absorption of methaqualone and the effect was dose and pH dependent. *In vivo*, diphenhydramine did not alter the rate of absorption or the distribution of methaqualone in blood. *In vitro*, the presence of diphenhydramine increased the rate of dissolution of methaqualone and the effect was more marked when the particle size was small.
- 3 The reasons for and the implications of these apparently contradictory results are discussed and it is concluded that any increased efficacy resulting from combining diphenhydramine with methaqualone cannot be due to increased plasma drug levels.

Introduction

Studies carried out in these laboratories have shown that Mandrax preparations yielded higher plasma methaqualone concentrations than four other products (Williams, Kendall, Davis, Poxon & Mitchard, 1974). Formulation factors were important and a two-fold difference in peak plasma levels was obtained from the two different preparations of Mandrax tablets which were tested. Mandrax contains diphenhydramine hydrochloride 25 mg in addition to methaqualone 250 mg. On clinical grounds, it has been claimed that diphenhydramine improves the hypnotic potency of methaqualone (Beaubien, Kristof, Lehmann & Ban, 1968; Hoffmeister & Koller, 1970). We have therefore investigated the influence of diphenhydramine on the dissolution, absorption and distribution of methaqualone. This was of particular interest since diphenhydramine has recently been shown to inhibit the gastrointestinal absorption of p-aminosalicylate (Lavigne & Marchand, 1973).

The investigation has been carried out using *in vivo* and *in vitro* techniques. We have studied the effects of diphenhydramine on the distribution in blood, and on buccal and gastrointestinal absorption of methaqualone. We have also investigated its effect on the *in vitro* dissolution of methaqualone and the influence of particle size on this process.

Methods

Methaqualone as the free base was obtained from Roussel Laboratories and diphenhydramine hydrochloride was supplied by Parke, Davis & Company.

In vivo studies

Buccal absorption. This was investigated by a modification of the method of Beckett & Triggs (1967). The following aqueous solutions were prepared:

- (1) Methaqualone 1 mg/ml; (2) methaqualone 1 mg/ml plus diphenhydramine hydrochloride 0.1 mg/ml; (3) methaqualone 1 mg/ml plus diphenhydramine hydrochloride 0.2 mg/ml.

These solutions (1 ml) were added to 20 ml of each of two buffers (Sorensen's phosphate buffers pH 5.0 and pH 8.0; Documenta Geigy, sixth edition), to provide a total of six different test solutions 1a, 1b, 2a, 2b, 3a, 3b, in which a and b correspond to pH 5.0 and 8.0 respectively. These six solutions were given to 10 volunteers (male and female aged 20-40 years) in the listed sequence. The tests on each volunteer were all performed on the same day, allowing at least 30 min to elapse between each experiment. These solutions were circulated round the mouth approximately 100 times/min for 5 min, after which the solution was

expelled. The mouth was quickly rinsed out twice with buffer solution (10 ml) at two 1 min intervals. The combined expelled solution and mouth washings were made up to 250 ml with distilled water and an aliquot (5 ml) of this was assayed by the method of Mitchard & Williams (1972). All samples were analysed on the same day. The results obtained were related to the concentration of the original methaqualone test solution (1 mg/ml) by taking 1 ml of the test solution through the same dilution procedure and 6 x 5 ml aliquots of the diluted standard being taken for analysis; the mean result was taken as the 100% unabsorbed or 0% absorbed value.

Gastrointestinal absorption, distribution and protein binding studies. Three male volunteers (aged 23-25 years) who had fasted overnight, were given methaqualone powder (4 mg/kg) orally washed down with 100 ml water. Blood samples (10 ml) were collected into lithium heparin tubes from an indwelling cannula in a forearm vein through which 5% dextrose was delivered at a constant rate of 500 ml/hour. The volunteers did not eat during the first 6 h of the study. A basal blood sample was taken, and then one at 30 min after swallowing the methaqualone. Subsequent samples were collected every 15 min for 90 min, twice at 30 min intervals and then three samples at hourly intervals. A further sample was collected at 24 hours. The plasma was separated immediately by centrifuging for 5 min at 3,000 g, stored where necessary at 0-4°C and the total plasma methaqualone levels determined within 12 hours. Protein binding and distribution of methaqualone were determined by taking an extra blood sample (10 ml) at 60, 90, 120 and 150 min after ingestion of the powder. Protein binding was estimated in plasma (5 ml) by equilibrium dialysis in Kostenbauder cells fitted with visking cellophane (36/32") as described by Patel & Foss (1964). Each plasma sample (5 ml) was placed in one compartment of the four dialysis cells with 0.065 M Sorensen's phosphate buffer pH 7.4 (5 ml) in the other compartment. The four cells were shaken (100 times/min) at 37°C for 20 hours. Erythrocytes (2.5 ml) were haemolysed by the addition of cold water (2.5 ml) and stored at 0-4°C until analysed. All samples were analysed by the method of Mitchard & Williams (1972). One week later the experiment was repeated under the same conditions, except that each volunteer was given diphenhydramine hydrochloride powder (0.4 mg/kg) together with the methaqualone.

In vitro studies

Dissolution. The effect of diphenhydramine on

the dissolution of methaqualone was followed using an *in vitro* dissolution test based on the flask method of Poole (1969) and the beaker method of Levy & Hayes (1960). One litre of dissolution medium of pH 2.0 buffer solution (0.1 M potassium chloride made up to pH 2.0 with 0.1 N hydrochloric acid) was placed in a one litre reaction vessel and maintained at $37^{\circ} \pm 0.5^{\circ}\text{C}$ in a constant temperature water bath. The vessel cover had five necks, to allow for sampling, maintenance of volume, temperature control, and agitation. Constant, smooth agitation of the medium was obtained by the use of a small controllable stirrer magnet placed on the bottom of the reaction vessel. Methaqualone powder (250 mg) was introduced through one of the necks in the reaction vessel. Samples (5 ml) were removed at 5 min intervals for 20 min then at 10 min intervals for 1 h from the dissolution media via one of the side arms by a long sampling pipe attachment set at a constant height and angle. The volume of the reaction vessel was maintained constant by the addition of 5 ml of dissolution medium after the removal of each sample.

Methaqualone (in the presence of diphenhydramine) was assayed spectrophotometrically at 281 nm, path length 1 cm, against a blank of pH 2.0 buffer, and the extinction related to a previously constructed calibration graph.

Diphenhydramine hydrochloride (25 mg) was dissolved in one litre of dissolution medium prior to the addition of the methaqualone powder and the experiment repeated to compare the dissolution data obtained from the methaqualone powder alone to that obtained in the presence of diphenhydramine hydrochloride.

Particle size. The influence of particle size of methaqualone on the dissolution process with and without diphenhydramine was studied. Methaqualone powder was graded by sieving to give a range of particle sizes from 63 μ to 724 μ . The particle size of diphenhydramine powder was unknown but was constant throughout the experiments.

Results

In vivo studies

Buccal absorption. Table 1 shows the percent methaqualone absorbed from the buccal mucosa from the three different solutions at pH 5.0 and 8.0. A three-way analysis of variance on the seven subjects, who completed the series of tests, is given in Table 2.

This shows that:

(a) The percentage methaqualone absorbed depended upon the pH of the buffer solution; significantly more methaqualone was absorbed from an alkaline solution.

(b) The presence of diphenhydramine decreased the amount of methaqualone absorbed; and this reduction depended upon the concentration of diphenhydramine.

(c) There was a significant intersubject variation.

(d) The effect of diphenhydramine was significantly greater in some individuals.

Gastrointestinal absorption, distribution and protein binding studies. Figure 1 shows the mean plasma methaqualone concentrations against time in the three volunteers who took the methaqualone powder with and without diphenhydramine. Without diphenhydramine the time taken to reach the peak plasma methaqualone level of 2.49 $\mu\text{g/ml}$ (s.e. mean ± 0.33) was 120 min and with diphenhydramine the peak of 2.53 $\mu\text{g/ml}$ (s.e. mean ± 0.22) was reached in 105 minutes. Thus the presence of diphenhydramine produced no significant difference in the peak plasma methaqualone levels and a striking similarity existed

Table 1 Influence of pH and diphenhydramine on the buccal absorption of methaqualone

Subject number	Methaqualone (1 mg/ml)		% Methaqualone absorbed			
			Methaqualone (1 mg/ml) +		Methaqualone (1 mg/ml) +	
			Diphenhydramine (0.1 mg/ml)		Diphenhydramine (0.2 mg/ml)	
	pH 5.0	pH 8.0	pH 5.0	pH 8.0	pH 5.0	pH 8.0
1	23.58	22.60	15.75	20.38	15.52	18.60
2	11.38	26.37	10.84	14.35	8.86	10.35
3	—	—	23.06	22.06	9.31	15.89
4	11.31	16.88	20.52	22.20	17.33	18.84
5	33.08	34.31	22.31	19.22	13.11	13.58
6	19.80	31.49	18.55	27.56	11.42	17.81
7	30.03	33.43	26.04	21.78	10.10	14.76
8	21.73	43.52	28.40	29.31	—	—
9	27.23	20.53	19.96	19.74	—	—
10	33.52	39.84	22.15	21.41	5.04	9.04
Mean	23.52	29.89	20.76	21.80	10.07	14.85

Table 2 Three-way analysis of variance of buccal absorption results

Source	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Main effects				
pH	1	132.43	132.43	20.18*
Dose	2	1201.56	600.78	91.54*
Subject	6	379.57	63.26	9.64*
Interaction				
Dose x subject	12	794.89	66.24	10.09*
Subject x pH	6	94.91	15.82	2.41
pH x dose	2	36.58	18.29	2.79
Residual				
pH x dose x subject	12	78.77	6.56	1.00
Total	41	2718.71		

* Significant at the 0.1% probability level.

Fig. 1 Mean plasma levels of methaqualone following the oral administration of methaqualone powder (4 mg/kg) (○) or methaqualone powder (4 mg/kg) with diphenhydramine hydrochloride (0.4 mg/kg) (●) to three subjects.

between the two profiles during both the absorption and elimination phases. At the peak blood levels of 2.5 µg/ml both in the presence and absence of diphenhydramine approximately 20-25% of the blood methaqualone was present in the erythrocytes, 58-63% was protein bound and

about 17% was free. Total plasma methaqualone values indicate that 30% of the drug was adsorbed on to the dialysis membrane, a phenomenon which has been discussed by McArthur & Smith (1969).

In vitro studies

Figure 2 shows the typical dissolution rate profiles of methaqualone powder of various particle sizes from 63 µ to 724 µ with and without added diphenhydramine. The dissolution characteristics

Fig. 2 Effect of methaqualone particle size, 63 µ (square symbols); 262 µ (circles) and 724 µ (triangles), on the dissolution profile of methaqualone (open symbols) and methaqualone with diphenhydramine hydrochloride (solid symbols).

Table 3 Influence of particle size and added diphenhydramine on the *in vitro* dissolution $t_{50\%}$ of methaqualone

Powder	$t_{50\%}$ (min)
Methaqualone 724 µ	35.0
Methaqualone 724 µ + diphenhydramine	33.5
Methaqualone 280 µ	29.0
Methaqualone 280 µ + diphenhydramine	25.5
Methaqualone 262 µ	27.0
Methaqualone 262 µ + diphenhydramine	24.0
Methaqualone 194 µ	26.0
Methaqualone 194 µ + diphenhydramine	20.0
Methaqualone 76 µ	16.0
Methaqualone 76 µ + diphenhydramine	9.0
Methaqualone 68 µ	12.0
Methaqualone 68 µ + diphenhydramine	9.0
Methaqualone 63 µ	11.0
Methaqualone 63 µ + diphenhydramine	5.5

Fig. 3 *In vitro* correlation between $t_{50\%}$ of methaqualone (○) and methaqualone with diphenhydramine (●) and log particle size of methaqualone.

of the two powders have been characterized by the time taken for half of the methaqualone to dissolve as determined from the whole of the dissolution time profile ($t_{50\%}$). The addition of diphenhydramine to the medium produced a decrease in the $t_{50\%}$ of methaqualone.

The influence of particle size and added diphenhydramine on the dissolution $t_{50\%}$ of methaqualone are shown in Table 3. A reduction in the methaqualone particle size produced more rapid dissolution. This effect was more pronounced at the finer methaqualone particle sizes and in the presence of added diphenhydramine.

Linear relationships are demonstrated between the $t_{50\%}$ of methaqualone and the logarithm of particle size in Fig. 3, both with and without diphenhydramine. The calculated correlation coefficients of 0.90 and 0.86 respectively against the theoretical value of 0.75, at a probability level of $P = 0.05$, show a significant correlation.

Discussion

We have demonstrated that diphenhydramine influences the dissolution and buccal absorption of methaqualone. There was, however, no effect on protein binding and distribution, and diphenhydramine did not alter the *in vivo* absorption of methaqualone.

The buccal absorption model of Beckett & Triggs (1967) has been used to study drug transfer across physiological membranes to obtain informa-

tion on the absorption characteristics of drugs. The buccal absorption data in Table 1 indicates that diphenhydramine reduces the absorption of methaqualone. The three-way analysis of variance (Table 2) demonstrates that not only does diphenhydramine decrease the amount of methaqualone absorbed, but that this decrease is significantly influenced by both pH and the concentration of diphenhydramine. At the lower pH there will be less methaqualone (pK_a 2.46) in the unionized form and the rate of absorption will be correspondingly reduced. Statistical analysis did not show that the effect of diphenhydramine was significantly different at the different pH values which suggests that the ionization equilibrium of methaqualone was unaltered and that the effect of diphenhydramine was due to a direct action on the process of absorption. Analysis also demonstrated a significant intersubject variation in the amount of methaqualone absorbed and the influence of diphenhydramine on this process was more marked in certain individuals.

The rate of absorption of a sparingly soluble drug is largely determined by its rate of dissolution; and it has been shown that the rate of dissolution of griseofulvin is largely determined by particle size (Atkinson, Bedford, Child & Tomich, 1962a, b). Methaqualone is also sparingly soluble and like griseofulvin a reduction in particle size promotes dissolution (Table 3). Of particular interest was the effect of diphenhydramine on this process. Not only did it increase the rate of dissolution but this effect became more marked as the particle size decreased.

Another mechanism by which diphenhydramine could exert an effect on plasma methaqualone levels is by competing for protein-binding sites thereby increasing the concentration of the pharmacologically active free drug. Our results show that the distribution and protein binding characteristics of methaqualone were not affected by the presence of diphenhydramine.

Diphenhydramine has weak anticholinergic properties which may reduce gut motility and might therefore be expected to delay the absorption of methaqualone from the small intestine. However, Fig. 1 shows that diphenhydramine had no effect on the absorption of methaqualone, in fact, the similarity of the data obtained on the two occasions was remarkable. This may have been because factors tending to promote absorption, such as more rapid dissolution, may have been opposed by other factors, such as the impaired uptake demonstrated by the buccal studies or reduced gut motility. Alternatively, when considering drug interactions, information obtained from buccal studies or from predictions on the knowledge of their pharmacological properties

cannot be used as a guide to the likely consequences *in vivo*. Thus, though diphenhydramine delays buccal absorption it may have no effect in the intestine where it will be greatly diluted and where the surface area for absorption is so large. Similarly, whereas diphenhydramine is surface active (Attwood, 1972) and can act as a wetting agent, this surfactant effect would be small compared with that of bile.

Methaqualone is claimed on clinical grounds to be a more effective hypnotic in the presence of diphenhydramine (Beaubhen *et al.*, 1968; Hoffmeister & Koller, 1970). Our results show that this effect is not due to higher plasma levels. The only

alternative mechanism producing an enhanced hypnotic effect would appear to be one mediated through a central synergistic pharmacological action of methaqualone with diphenhydramine as suggested by Harman (1970).

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References

- ATKINSON, R.M., BEDFORD, C., CHILD, K.J. & TOMICH, E.G. (1962a). Effect of particle size on blood griseofulvin levels in man. *Nature, Lond.*, **193**, 588-589.
- ATKINSON, R.M., BEDFORD, C., CHILD, K.J. & TOMICH, E.G. (1962b). The effect of griseofulvin particle size on blood levels in man. *Antibiotics and Chemotherapy*, **12**, 232-238.
- ATTWOOD, D. (1972). Micelle formation by some antihistamines in aqueous solution. *J. Pharm. Pharmac.*, **24**, 751-752.
- BEAUBHEN, J., KRISTOF, F.E., LEHMANN, H.E. & BAN, T.A. (1968). A comparison of the hypnotic properties of Mandrax and its two constituents. *Curr. Ther. Res.*, **10**, 231-232.
- BECKETT, A.H. & TRIGGS, E.J. (1967). Buccal absorption of basic drugs and its application as an *in vivo* model of passive drug transfer through lipid membranes. *J. Pharm. Pharmac.*, **19**, Suppl., 31s-41s.
- HARMAN, J.B. (1970). Preparations containing methaqualone. *Prescriber's Journal*, **10**, 129-131.
- HOFFMEISTER, von W. & KOLLER, S. (1970). Clinical trial of a combination of methaqualone and diphenhydramine and methaqualone in a double blind test. *Arzneimittel-Forsch.*, **20**, 261-264.
- LAVIGNE, J.G. & MARCHAND, M. (1973). Inhibition of gastrointestinal absorption of *p*-aminosalicylate (P.A.S.) in rats and humans by diphenhydramine. *Clin. Pharmac. Ther.*, **14**, 404-413.
- LEVY, G. & HAYES, B.A. (1960). Physicochemical basis of the buffered acetylsalicylic acid controversy. *New Eng. J. Med.*, **262**, 1053-1058.
- McARTHUR, J.N. & SMITH, M.J.H. (1969). The determination of the binding of salicylate to serum proteins. *J. Pharm. Pharmac.*, **21**, 589-594.
- MITCHARD, M. & WILLIAMS, M.E. (1972). An improved quantitative gas liquid chromatographic assay for the estimation of methaqualone in biological fluids. *J. Chromatogr.*, **72**, 29-34.
- PATEL, N.K. & FOSS, N.E. (1964). Interaction of some pharmaceuticals with macromolecules. I. Effect of temperature on the binding of parabens and phenols by polysorbate 80 and polyethylene glycol 4000. *J. Pharm. Sci.*, **53**, 94-97.
- POOLE, J.W. (1969). Some experiences in the evaluation of formulation variables in drug availability. *Drug Inform. Bull.*, **3**, 8-16.
- WILLIAMS, M.E., KENDALL, M.J., DAVIS, S.S., POXON, R. & MITCHARD, M. (1974). Availability of methaqualone from commercial preparations: *in vitro* and *in vivo* studies in man. *Br. J. clin. Pharm.*, **1**, 99-105.

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