PLASMA LEVEL OF DIETHYLCARBAMAZINE IN JIRDS AND HAMSTERS

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INTRODUCTION

Diethylcarbamazine (DEC) is widely used for the treatment of human filariasis. Although many experimental studies have been carried out, the mode of action of DEC remains to be clarified (Hawking, 1979). For many years, several different species of rodents and filarial worms have been used for the studies of antifilarial activity of DEC. The metabolism of DEC in rats was investigated by Bangham (1955), Faulkner and Smith (1972), and Edwards *et al.*, (1981). However, there is little information about the blood level of DEC in rodents, which is one of the indispensable factors to evaluate the antifilarial activity of the drug. This seems to be simply due to the lack of sensitive and reliable method to determine DEC levels in the biological fluids.

Recently, Allen *et al.*, (1979) described a new method using the gas-liquid chromatography. By using their method, Hillman *et al.*, (1983) reported the blood levels of DEC in cats by several routes and protocols of DEC administration. By this method the concentration of DEC as low as 10 ng/ml of blood could be measured. Studies were carried out to determine the relation between dosage and plasma level of DEC in jirds and hamsters, which are commonly used as experimental hosts for lymphatic-dwelling filarial worms, *Brugia malayi* and *B. pahangi*. The excretion of DEC in the urine and feces was also studied.

MATERIALS AND METHODS

Blood, urine and feces from animals after administration of DEC citrate were collected. The animals used in this study were Mongolian jirds and hamsters which had been maintained in our laboratory for many years. Supatonin^R solution (Tanabe Ltd.) containing DEC citrate at 200 mg/ml was diluted with saline or distilled water to the required concentrations and given to animals intraperitoneally or orally (by stomach intubation). The dosages varied from 6 to 300 mg/kg of body weight (B.W.). Blood samples were taken from the retro-orbital sinus with heparinized haematocrit tubes at scheduled intervals. As 0.5 ml of plasma was required for the determination of DEC level, each animal could be bled only twice. Thus, in each series of experiment, 5-10 animals were used. Some jirds were individually placed in a metabolic cage to facilitate the separate collection of urine and feces at intervals of 12 hours for 48 hours.

The extraction procedure for plasma and urine was carried out by the method described by Allen *et al.*, (1979) with minor modifications. To each sample (0.5 ml) in a glass-stoppered tube were added 0.5 ml of 2 M sodium hydroxide solution and 5 ml of ethyl acetate. The solution was shaken well by a vibrator for 10 min. and then centrifuged at 3,000 rpm for 10 min. The upper organic layer was transfered to another test tube as completely as possible. To lower aqueous

layer, another 5 ml ethyl acetate was added and the extraction was repeated. After centrifugation, the upper layer was added to the first extract. The ethyl acetate was dried under nitrogen gas at room temperature and the residue was redissolved in 200 µl of hexan just before injection to the gas chromatograph. For feces, the sample was homogenized in distilled water and made up to a known volume, and the sample was processed as for plasma and urine. Powdered DEC citrate, a generous gift by Tanabe Ltd., was used to prepare the standard plasma, urine and feces solutions (0.2 µg/ml, 1.0 µg/ml. 10.0 µg/ml, and 100 µg/ml).

Shimazu gas-liquid chromatograph (GC-R1A) equipped with a flame ionization detector was used in this study. The column used was the same as described by Allen *et al.*, (1979), consisting of 2% Carbowax 20M, 5% KOH, on Chromosorb G AW DMCS (100-120 mesh). The column temperature was 160°C and the injection temperature 180°C. The carrier gas was nitrogen at a flow rate of 60 ml/min. Five µl of sample was injected. Under these conditions, the retention time of DEC was 8.3 min. The amount of the drug in samples was calculated from the calibration graph set up with the standard solutions containing known amounts of DEC. In this paper, the dosage given to animals refers to citrate salt and plasma concentration and the amount of drug excreted into urine and feces were expressed as DEC base.

RESULTS

The plasma level of DEC following single intraperitoneal dose of DEC citrate is shown in Fig. 1. When DEC citrate was given to jirds at 100 mg/kg B.W., the blood level (DEC base) reached the maximum of 20-25 µg/ml at 10 minutes after dosing, and thereafter, the concentration of drug fell rapidly. At 4 hours, no DEC could be detected by gas chromatography. Reduced dosage of 30 mg/kg produced a lower peak level and resulted in more quick diminution from the blood. When larger dosage (300 mg/kg) was given, the plasma level reached the maximum of 43-61 µg/ml at 30 minutes and disappeared from the blood by 8 hours.

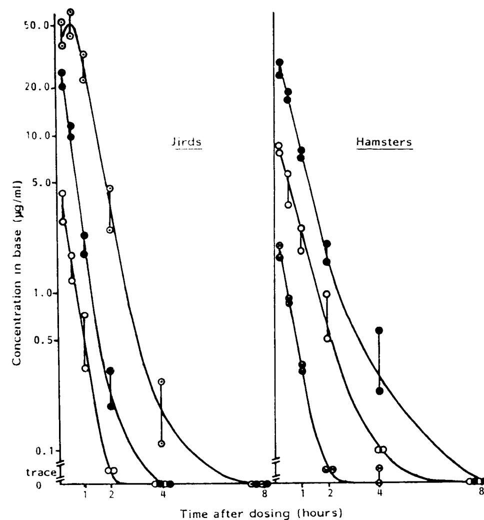

Fig. 1. Relation between dose and plasma level in jirds and hamsters, given single intraperitoneal administration of DEC citrate at 300 mg/kg B.W. (⊙), 100 mg/kg B.W. (●), 30 mg/kg B.W. (o) or 6 mg/kg B.W. (⊗).

When the same dosages of DEC citrate were given to hamsters, the plasma levels elevated higher than in jirds, and the DEC levels in the blood remained 2-4 hours longer in hamsters than in jirds.

Fig. 2 shows the plasma levels of DEC (base) in jirds and hamsters after oral administration of DEC at 100 mg/kg B.W. The plasma level of the drug rose rapidly in both animals, reaching the maximum concentration within 30 minutes. In jirds, DEC disappeared within 4 hours, but a small amount of DEC was found remaining in the blood of hamsters for 8 hours.

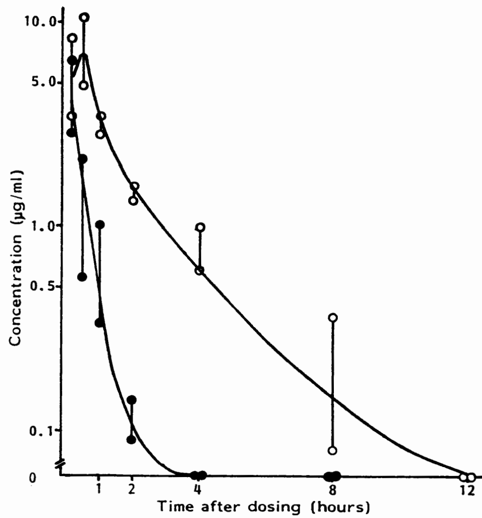

Fig. 2. Plasma level of DEC (base) in jirds (●) and hamsters (○) following single oral administration of DEC citrate at 100 mg/kg B.W.. Note the delayed clearance in hamsters.

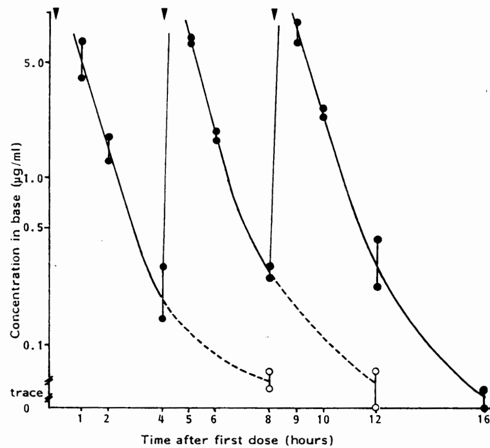

Fig. 3. Plasma level in hamsters dosed intraperitoneally with DEC citrate at 100 mg/kg B.W. every 4 hours. Open circles connected by broken lines show the level in animals to which 2nd or 3rd dose was not given. Arrows denote the time of dosing.

Fig. 3 shows the plasma concentration of DEC (base) in hamsters which were dosed intraperitoneally at 100 mg/kg B.W. every 4 hours. The repeated dose schedule did not bring the accumulation of drug. Fig. 4 shows plasma concentration of DEC (base) in jirds which were given at 300 mg/kg B.W. daily for 5 consecutive days. There was little difference in blood levels of DEC between the first, 3rd and 5th day.

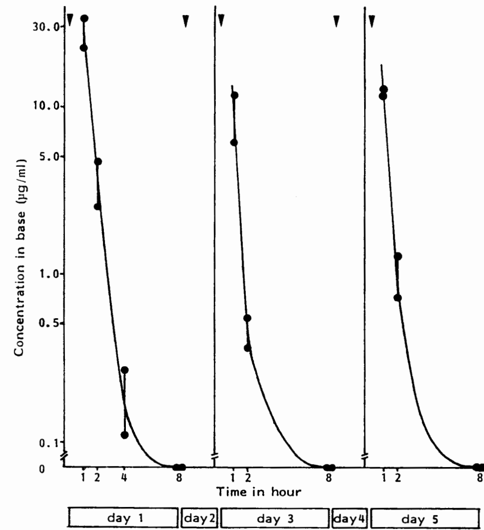

Fig. 4. Plasma level in jirds dosed intraperitoneally with DEC citrate at 300 mg/kg B.W. daily for 5 days. Arrows denote the time of dosing.

Table 1 shows the results of estimations of DEC excreted without metabolic changes into urine and feces in 5 jirds, each of which received a single intraperitoneal dose of DEC at 300 mg/kg B.W. A great majority of unchanged drug was excreted into the 0-12 hour urine, although traces of DEC were still detected in the 36-48 hour urine. DEC was detectable in feces collected during the first 12 hours, but the amount of drug was as low as 3.6-5.8 μg. The total amount of drug excreted was 3.0 to 16.8% (average : 8.1%) of the total dosage administered.

Table 1

Excretion of DEC (unchanged) in urine and feces.

Jird No.	Dosage given* (mg base)		DEC (μg) excreted in hours				
			0-12	12-24	24-36	36-48	Total (% recovery)
1	10.1	urine	275.8	12.2	6.8	2.6	301.0 (3.0)
		feces	3.6	0	0	0	
2	12.2	urine	809.6	25.9	10.4	4.5	856.0 (7.0)
		feces	5.6	0	0	0	
3	11.1	urine	665.4	3.3	9.7	7.6	691.3 (6.2)
		feces	5.3	0	0	0	
4	10.2	urine	1435.9	278.0	1.4	0.6	1720.5 (16.8)
		feces	4.6	0	0	0	
5	8.9	urine	633.9	16.9	1.3	0.4	658.3 (7.4)
		feces	5.8	0	0	0	

*Each animal was given DEC citrate at 300 mg/kg.

DISCUSSION

Since Lubran (1950) described the colorimetric method for the determination of DEC in the blood and urine, several modifications have been developed. In general, however, these spectrophotometric methods have limitations in their sensitivity and specificity.

Allen *et al.*, (1979) reported the new method using gas-liquid chromatograph, which is sensitive and specific for the determination of DEC in biological samples. By modifying their method, the plasma levels of DEC in jirds and hamsters were determined. These animals are suitable experimental hosts for lymphatic-dwelling filarial worms, and have been commonly used in many laboratories. This present data would provide a basis for further studies on the antifilarial activity of DEC, when jirds and hamsters are used as experimental animals. Cats have also been commonly used as a model for chemotherapeutic studies, and the serum levels of DEC in cats by different routes of administration has recently been studied by Hillman *et al.*, (1983).

Effects of DEC vary by different combinations of hosts and filarial worms, and the effectiveness obtained with those animal hosts cannot be assumed to be the same in man (Denham *et al.*, 1978; Hawking, 1979). For instance, in contrast to its powerful action against circulating microfilariae of *Wuchereria bancrofti* or *Brugia malayi* in man, DEC has been reported to be inactive or only slightly active against microfilariae of *B. malayi* or *B. pahangi* in jirds (Denham *et al.*, 1978; Tanaka *et al.*, 1981; Yamashita *et al.*, 1983). The dosage used in their experiments was as much as 200 - 300 mg/kg B.W., which was close to the acute LD_{50} in mice and rats (Hawking, 1979). The present study revealed that in jirds, DEC was eliminated from the blood with surprising rapidity, even if DEC was given at 300 mg/kg B.W. It was only for 1-2 hours after administration when the concentration over 0.8 μg/ml of serum, which was reported to be a minimum effective

concentration against microfilariae of *W. bancrofti* in man (Fujimaki, 1958), was maintained in the jirds. On the other hand, Fujimaki (1958), Rée *et al.*, (1977), and Edwards *et al.*, (1981) reported that DEC was detectable in the blood of patients for 24 hours after a single oral dose. The quick elimination of DEC from blood might be an explanation of relative ineffectiveness of DEC against circulating microfilariae in the jirds, though an impaired phagocytic function of Kupffer's cells of jirds was attributed to the ineffectiveness by Tanaka *et al.*, (1981).

Edwards *et al.*, (1981) reported that the rat was an unsuitable animal in studies of DEC disposition, because the metabolism of DEC in man differs greatly from that of rats. In rats, the excreted drug without metabolic changes accounts for about 15% of the dosage given, and the two major metabolites, 1-ethylcarbamyl-4-methyl-piperazine and diethylcarbamazine-N-oxide, account for about 23% and 50%, respectively (Bangham, 1955; Faulkner and Smith, 1972). In man, DEC was excreted unchanged in the urine to a significant extent, 41-61 % (Edwards *et al.*, 1981). In our study with jirds unchanged drug excreted in the urine accounted for 3-16% (8% on the average) of the given dosage. This value is close to the results of experiments in rats by Faulkner and Smith (1972) and Edwards *et al.*, (1981), though we did not study the amount of N-oxide in the urine. To date there has been no report on the relative antifilarial activity of DEC, DEC-N-oxide and other metabolites. The effect of DEC on filarial worms may differ widely among hosts due to the different metabolism of DEC.

In hamsters, DEC remained in blood 2-4 hours longer than in jirds. When DEC was given orally to hamster, DEC was detected in the blood for 8 hours. If antifilarial activity of DEC depends on its plasma level and duration, a better effect of DEC against microfilariae may be expected in hamsters dosed orally. In our experiment with jirds, oral administration was inferior to the intraperitoneal administration in terms of the concentration and duration. The animals were reported to vomit DEC if given *per os*, and much of the drug would have been lost (Denham *et al.*, 1978; Hawking, 1979). Our jirds might have vomited it out and therefore produced lower blood level of DEC, however, it was difficult to confirm.

SUMMARY

The relation between the dosage and plasma level of diethylcarbamazine (DEC) in jirds and hamsters was examined by gas-liquid chromatography. When the drug was given intraperitoneally to jirds at 100 mg/kg body weight, the plasma level rose rapidly and reached the maximum level (20-25 μg/ml) at 10 minutes and afterwards fell quickly to undetectable level at 4 hours. Even if larger dosage (300 mg/kg) was given, DEC was eliminated completely from the blood circulation within 8 hours.

When the same dosages of DEC were given intraperitoneally, the DEC levels remained 2-4 hours longer in the blood of hamsters than in the blood of jirds. DEC given by stomach intubation at 100 mg/kg remained detectable much longer in hamsters (8 hrs) than in jirds (2 hrs). A repeated doses schedule did not show a tendency for the drug to accumulate. DEC was excreted in the urine and feces, but the total amount of drug excreted without metabolic changes was only about 8% of the given dosage. The majority of unmetabolized DEC was excreted in the urine within 0-12 hours.

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