# THE CHEMOTHERAPEUTIC ACTIVITY OF INJECTED DPT (Ciba-1906)

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Various investigators, T. F. DAVEY (1956), J. Ross INNES et al. (1957), A. M. ALONSO (1958), J. GATE et al. (1958), MUKHERJEE et al. (1958), J. M. B. GARROD (1959), have agreed that DPT exerts an optimal chemotherapeutic effect in leprosy when given in single oral doses of 1.5-3 g./day. Early observations, J. Ross INNES et al. (1957), which revealed that considerable amounts of the drug can be recovered from the faeces, led to the assumption that DPT was absorbed only on a very restricted scale and that this low rate of absorption would possibly impose certain limitations on its therapeutic value. This question was subsequently re-examined in quantitative studies carried out by G. A. ELLARD (1960). Maximum oral absorption occurred in man after a single dose of 1.5 g., with no further increase in response to higher doses. However, the amount of drug absorbed could be greatly enhanced by employing a daily dose of 4.5 g. given in 3 equal fractions at spaced intervals throughout the day. These findings suggest that the restricted absorbability of the drug could partly be overcome by spacing the doses at shorter intervals.

A higher rate of excretion than after single oral doses as well as an increased rate of absorption was demonstrated to occur in animals if the dissolved drug was administered parenterally, SCHMID and TRIPOD (1959). Hence it could be assumed that in man, too, higher concentrations in the body might be attained by parenteral administration than after oral administration. In view of this possibility, a comparison of the chemotherapeutic activity of orally and parenterally administered DPT was undertaken—a comparison which should also shed some light on the effectiveness of DPT injected in suspension.

## Methods

## Suspension of Ciba-1906 for parenteral administration

As DPT is only very sparingly soluble in an aqueous vehicle or in oil, it was administered in the form of a 20% suspension of finely ground substance in peanut oil. If necessary, less concentrated suspensions were prepared by adding appropriate amounts of peanut oil to the stock suspension.

#### Chemotherapeutic investigations

As no suitable method exists so far to produce experimental infections with human leprosy bacilli, the experiments were performed on mice infected with tubercle bacilli, against which the tuberculostatic activity of DPT has been well established, R. L. MAYER *et al.* (1953), P. C. EISMAN *et al.* (1954), and E. A. KANOPKA *et al.* (1954). According to earlier findings based on experiments using tubercle bacilli, oral treatment elicits a maximum therapeutic response if Ciba-1906 is added to the diet in a concentration of 0.05%. The average daily intake of food being approximately 3 g. per mouse, the daily dose of Ciba-1906 thus works out at 70–80 mg./kg., corresponding to a total dose of 1,300–1,600 mg./kg. for the 20 days' duration of the experiment. This dose and mode of administration (standard oral treatment) constitutes the basis for comparison with the effect of injected DPT.

The chemotherapeutic effect is generally either assessed on the basis of the increased survival rate and the median survival time or measured by reference to reduction of the pulmonary lesions. The technique used here for the assessment of pulmonary lesions by measuring the lung density has been described by A. J. CROWLE (1958). The significance of the effects observed was statistically verified by the "sign test", i.e. the values for the lung density of the individual animals were randomly compared in any one group of treated mice with that of the untreated control group or with the group showing a maximum chemotherapeutic response (see Table 1).

Mice weighing 17-20 g. were infected intravenously with a suspension of *M. tuberculosis var. humanus* strain  $Z_3$ , which produces a lethal infection with a peak death rate between the 25th and 30th day. In these experiments, however, the mice were all sacrificed on the 20th day after infection, i.e. at a time when the lung density observed in untreated animals was 0.94 (standard deviation = 0.04).

DPT suspension in varied concentrations and doses was administered subcutaneously to the mice on the same day as the infection, with the exception of a special experiment in which the drug was given 9 days prior to infection.

## Absorption and excretion

The amount DPT absorbed following the injection and the rate of excretion in the urine and faeces were estimated using <sup>35</sup>S labelled DPT. Details regarding the preparation of labelled DPT and the method of calculation employed have already been described by SCHMID and TRIPOD (1959). The radioactivity measured at the site of injection and in the organs or in the faeces and urine was computed as for DPT, i.e. the chemical nature of possible break-down products was studied here.

Conc. DPT in oil suspension	Dose mg./kg.	Mode of administration	Volume ml.	Time of administration	% Reduction in lung involvement	(a)	( <i>b</i> )
5%	800	1×s.c.	0.32	Simultaneously with infection	46%	>5	5
5%	1.600	$1 \times \text{s.c.}$	0.64	Simultaneously with infection	56%	>5	>5
5%	1,600	$4 \times 400$ s.c.	4×0.16	Simultaneously with infection	25%	>5	>5
20%	1,600	$1 \times s.c.$	0.16	Simultaneously with infection	80%	1	>5
	1,300	p.o.		Beginning 1 day after infection*	70%	1	>5
5%	800	$1 \times s.c.$	0.28	9 days before infection	8%	>5	1
5%	1,600	$1 \times s.c.$	0.56	9 days before infection	46%	>5	>5
5%	1,600	$4 \times 400$ s.c.	4×0.14	9 days before infection	17%	>5	1
20%	1,600	$1 \times s.c.$	0.14	9 days before infection	67%	1	>5
	1,300	p.o.		Beginning 9 days before infection*	70%	1	>5

TABLE 1 Chemotherapeutic activity of single subcutaneous doses of Ciba-1906

\* and continued until 20th day after infection.
\*\* (a) Significance of difference as compared with control group.
(b) Significance of difference as compared with group treated orally.

## Results

## 1. Minimum effective dose of injected DPT

The therapeutic effect of a single dose of DPT given on the day of infection is dependent on the dose of the drug and on the volume administered (Table 1). The minimum dose producing an approximately 50% reduction in lung involvement was found to be 800 mg./kg. A dose twice this amount (1,600 mg./kg.), however, is not substantially more effective, unless given in a volume 4 times smaller, i.e. using a 20% instead of a 5% suspension. In this case a pronounced therapeutic effect is observed, an effect even exceeding that of the oral standard treatment. This difference in activity of the same dose of DPT suspension emphasises the extent to which the local absorption of the drug is dependent on the conditions of administration.

#### 2. Duration of activity

If the mice are treated 9 days prior to the infection, the chemotherapeutic effect (Table 1) as compared with that of simultaneous treatment is considerably reduced only in the case of the minimum dose of 800 mg./kg. The higher dose of 1,600 mg./kg., particularly if given in the more highly concentrated suspension, still produces an effect after 9 days which compares well with that of the standard oral treatment. This fact indicates that the dose range between 800 and 1,600 mg./kg. is critical for the maintenance of a therapeutically active drug level.

#### 3. Parenteral absorption and drug concentration in various organs

The amount of DPT ( $^{35}$ S) absorbed on the 1st day, or by the 10th and 20th day after the injection, was determined by reference to the difference between the dose administered and the amount of drug recovered at the site of injection. The resulting curve (Fig. 1) shows an initial increase followed by a rather flat slope. At the same time, it was observed that the liver accumulated a high amount of drug during the first 24 hours, whereas the lung did not participate to the same extent. Not until later, i.e. on the 10th day, when the concentration in the liver had already fallen, did the drug level in the lung attain a value similar to that of the liver. By the 20th day the concentration in both organs had decreased to approximately the same extent.

## 4. Maintenance of the drug level

The excretion of DPT and its possible metabolites was measured in terms of the amount of <sup>35</sup>S recovered in the urine and faeces of the mice, SCHMID (1961). Since losses of material are unavoidable, the amount of 9 mg. DPT which is excreted during the 1st and 2nd





interval of 10 days seems to be definitely superior to the amount of 7 mg. absorbed in the same period of time (Table 2). In view of this negative balance, the drug levels in the organs, after attaining an early maximum, evidently cannot be sustained for a long period. This is also borne out by the declining concentration in the organs.

#### 5. Minimum tuberculostatic concentration and drug level

In experiments with tuberculous mice the target organ for the chemotherapeutic action is acknowledged to be the lung. Hence the drug level attained in this organ after a therapeutically adequate dose of DPT can be related to the minimum drug concentration

#### TABLE 2

Amount of	DPT	$(^{35}S)$	absorbed	and	excreted	by	mice	after	a	single
	S	ubcut	aneous do	ose o	f 1,600 m	g./ł	kg.			

Amount of DPT re-	24 hrs.	On 10th	On 20th
tion expressed as % of amount injected	64%	27%	6%
Total of DPT absorbed	Within first	Within	Within
	24 hours	10 days	20 days
	16 mg.	23 mg.	30 mg.
DPT ( <sup>35</sup> S) excreted* in urine and faeces	Within first day	On 10th day $0.3-0.8$ mg	On 20th day
Total excreted*	0. <del>4</del> -0.0 mg.	Within 10 days >9 mg.	Within 20 days >9 mg.

\* Loss of some material was technically unavoidable.

required to inhibit tubercle bacilli *in vitro*. Although the data available from this work do not indicate the maximum drug levels attained, the concentration of 41 mcg./g. found in the lungs on the 10th day corresponds to a level 10–20 times higher than the (minimum) tuberculostatic concentration of Ciba-1906 *in vitro*, which, according to MAYER *et al.* (1953) is 2–5 mcg./ml. This ratio, however, may be influenced by the interference of body fluids and by metabolic changes which DPT undergoes in the host.

## 6. Toxicity and local tolerability

The possibility of injecting larger amounts of DPT, with presumably increased rates of absorption, called for comparative toxicity studies. In the case of the  $LD_{50}$  for mice, these revealed (Table 3) that no important differences exist between the injected and the oral (gavage) mode of administration.

TABLE 3

Toxicity  $(LD_{50})$  of Ciba-1906, administered subcutaneously and orally to mice.

s.c. (mg./kg.)	p.o. (mg./kg.)			
. 2,400 (±1,060)	2,400 (+900) suspended in water 4,000 (+1,200) suspended in oil			

Only when subtoxic subcutaneous doses are administered (>3,000 mg./kg.) do the signs of intoxication appear earlier when a more dilute suspension (5 or 10%) is used instead of a 20% suspension (5 or 10%) is used instead of a 20% suspension—a fact which reflects the more rapid absorption from depots of larger size.

Rats and mice receiving up to 100 mg. Ciba-1906 (0.5 ml. of 20% suspension) showed an acute inflammatory reaction at the site of the depot 24 hours after the injection. Two weeks later, a non-specific, foreign-body granuloma like reaction developed, with oil droplets visible in the affected tissue.

#### Discussion

It is a generally agreed fact that at present there is still no adequate experimental method for the chemotherapeutic evaluation of leprostatic drugs. The present study, however, is concerned with a problem of a pharmacological nature which does not necessitate a specific infection. Thus any system enabling chemotherapeutically active concentrations of DPT to be measured either directly or indirectly in an animal may be considered suitable for the purpose in question. Both the chemotherapeutic response and analytical determination of <sup>35</sup>S labelled DPT have been used in order to investigate whether parenteral administration of Ciba-1906 can elicit a chemotherapeutic effect comparable to that obtained with oral treatment.

Earlier work by MAYER *et al.* (1953), which has been confirmed in this laboratory, showed that a 0.05% concentration of the drug in the diet was able to produce a maximum chemotherapeutic response in tuberculous mice. This dose and effect was taken by us as a basis of comparison when studying the activity of various doses of injected DPT. Not only was Ciba-1906 active by the subcutaneous route, but it also exerted a prolonged chemotherapeutic effect lasting for at least 9 days. The single dose required to achieve a maximum response under such conditions is necessarily rather high and is comparable to the total oral dose administered in a corresponding experiment of 20 days' duration.

Since the toxicity  $(LD_{50})$  of DPT in mice is almost identical for the subcutaneous and oral routes of administration, the ratio of the dose of a depot injection to the  $LD_{50}$  is of course much smaller than in the case of continuous oral treatment, where the daily intake of DPT is only 1/20 of the total dose.

As in any other type of experiment with drugs forming parenteral depots, the volume of material injected and its colloidal or microcrystalline form are factors of major importance. The results obtained here would suggest that it is advisable to use a high concentration of the drug in order to reduce the initial absorption in favour of a more prolonged, steady degree of absorption to compensate for the rate of excretion or destruction. The respective conditions governing the experiments on mice as described seem to approximate to a state of equilibrium only during an early period of the experiment.

The rate of absorption and excretion of DPT differs widely in various experimental animals, SCHMID and TRIPOD (1959), and it is obvious that in this respect no extrapolation to humans is possible on the basis of the present experiments. However, these experiments in mice do show that parenterally administered DPT is at least as active as the orally administered drug. It has been demonstrated earlier (MAYER, personal communication) that an increased oral dose did not produce a better therapeutic response in mice and that no depot effect could be obtained by oral treatment; it can therefore be assumed that also in other species, especially if oral absorption is restricted as in the case of man, J. Ross INNES et al. (1957) and G. A. ELLARD (1960), the parenterally administered drug will provide higher tissue levels over a certain period of time than where the drug is given orally. The dose required for the treatment of leprosy and the duration of active drug levels achieved by the use of an appropriate concentration of DPT in a suitable vehicle must be determined clinically.

#### Summary

By administering DPT (Ciba-1906) parenterally in the form of a 20% suspension in oil to mice infected with tubercle bacilli, a chemotherapeutic response can be achieved which is better than the maximum effect obtained by perforal treatment. The duration of action of a single injection has been estimated by analysing the chemoprophylactic effect and the rate of absorption and excretion in relation to the concentration of <sup>35</sup>S labelled DPT measured in the liver and the lung.

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