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PLASMA CONCENTRATION CURVES OF N-METHYLPYRIDINIUM-2-ALDOXIME METHANE SULPHONATE (P2S) AFTER INTRAVENOUS, INTRAMUSCULAR AND ORAL ADMINISTRATION IN MAN

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Abstract—The rate of absorption of N-methylpyridinium-2-aldoxime methane sulphonate (P2S) after intramuscular and oral administration has been investigated in man.

It is found that 30 mg per kg body weight can be given intramuscularly without serious pain. The rate of absorption varies considerably, however, peak plasma concentrations averaging 15 μg per ml are reached after 20 min and after 90 min the concentrations are about 9 μg per ml. After 5–10 min most of the patients are expected to have therapeutic plasma levels.

Maximum concentration in plasma after oral administration of 45 mg per kg body weight in gelatin capsules is about 5 μg per ml and a concentration over 3·5 μg per ml is maintained from 60 to 240 min. It is considered that oral administration has little therapeutic effect since the rate of absorption is very slow.

After oral administration of 45 mg per kg body weight, 23 per cent of the dose is excreted in the urine within 4 hr.

THE increased use of anticholinesterases of the organophosphorous type as insecticides, during the last 10 years has caused a great number of cases of fatal poisoning.[1–3]

Formerly, atropine was considered to be the most effective drug for counteracting the toxicity of organophosphorous cholinesterase inhibitors, such as parathion, di*iso*propoxyphosphoryl fluoride (DFP), tetraethyl pyrophosphate (TEPP), etc. Recently it has been demonstrated that certain oximes, when used in combination with atropine, provide more effective therapy than atropine alone for animals exposed to these toxic agents.[4, 5] These new drugs reactivate the phosphorylated cholinesterase, and thus provide a causal therapy.[6, 7] One of the most potent oximes in this respect, N-methylpyridinium-2-aldoxime iodide (PAM), has been used in man both in experimental and in accidental organophosphate poisoning.[2, 8] It is claimed to be safely tolerated in man in intravenous doses of 15–30 mg per kg body weight.[9]

Because of its low solubility in water (5 per cent w/v) PAM has only been administered intravenously. N-methylpyridinium-2-aldoxime methane sulphonate (P2S), which is much more soluble (60 per cent w/v), has been given to man orally and by intramuscular injection.[10]

The aim of the present investigation has been to determine the rate of absorption in man of N-methylpyridinium-2-aldoxime methane sulphonate (P2S) after intramuscular injection, and after oral administration. The concentration of P2S in plasma

was therefore determined at certain interval after administration. No analyses were done on whole blood, since P2S has been shown not to enter the erythrocytes of man or dog.[11]

EXPERIMENTAL

The experiments were carried out on healthy volunteers and on patients in the Ear-nose-throat department of Karolinska sjukhuset.* The patients were treated for minor disorders without any evidence of circulatory, gastrointestinal, renal or hepatic disturbances.

TABLE 1. SENSITIVITY AND STANDARD ERROR OF P2S ANALYSES BY HYDROXYLAMINE DETERMINATION
(Method of Askew *et al.*[13])

Concentration in plasma (μg per ml)	Optical density at 555 mμ Mean and standard error of the mean ($n = 9$)
5·0	0·011 ± 0·001
15·0	0·040 ± 0·002
50·0	0·139 ± 0·004

TABLE 2. SENSITIVITY AND STANDARD ERROR OF P2S ANALYSES BY LIGHT ABSORPTION AT 333 mμ
(Method of Creasy and Green[12])

Concentration (μg per ml)	Optical density at 333 mμ Mean and standard error of the mean ($n = 7$)
4·7*	0·088 ± 0·002
9·3*	0·175 ± 0·004
18·6*	0·344 ± 0·004
24·3†	0·465 ± 0·001
27·9*	0·521 ± 0·004
50·6†	0·964 ± 0·005

* In plasma.
† In water.

P2S (synthesized according to the method described by Creasy and Green[12] was Seitz-filtered, freeze-dryed and stored in sterile flasks.† Before use, the content (2·5 g) was dissolved in sterile pyrogen-free water. This solution had a pH of from 3 to 4.

For oral administration, gelatin capsules were filled with 1 g of crystalline P2S.

Blood samples were taken at intervals via a needle in an antecubital vein and collected in heparinized test tubes. A sample was always taken before administration of P2S and enough blood was taken to enable making double tests.

The plasma was stored at − 20 °C till the following day, when it was analysed.

Two methods have been described which are suitable for the determination of P2S in blood and tissues. One is based on the acid hydrolysis of the oxime to hydroxylamine, which is then determined by a colorimetric method,[13] the other is based on

* The patients were medically supervised by H. Diamant, M.D., to whom my thanks are due.
† Dr. R. Barkman, Military Pharmacy, Stockholm 60, has kindly provided the sterile P2S and the P2S capsules.

the ultra-violet absorption spectrum of P2S.[12, 14] The sensitivity and precision of these two methods were determined and the results are summarized in Tables 1 and 2. As the ultra-violet absorption method was the more sensitive one, it was chosen for this investigation.

Two minor modifications were introduced in the original method. The proportions of zinc sulphate to barium hydroxide had to be changed in order to obtain clear solutions, and it was found more convenient to use heparinized plasma instead of whole blood.

Procedure

Two millitres of heparinized plasma were diluted with 3·3 ml water, and 1 ml 0·2 M $Ba(OH)_2$, 1·5 ml 0·22 M $ZnSO_4$ and 0·2 ml of 20% NaCl were added consecutively. After the addition of each reagent the solution was thoroughly shaken. The mixture was then centrifuged for 20 min at 3000 rev/min. To 3 ml of supernatant, 0·2 ml 5 N NaOH was added, and the light-absorption at 333 mμ was determined in a

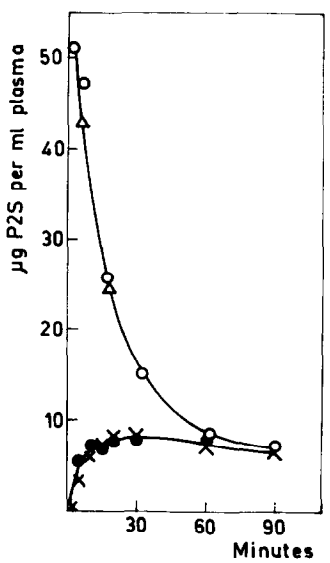

FIG. 1. Plasma concentrations of P2S in man after intravenous and intramuscular injections of 20 mg per kg body weight.
○ Intravenous injection of a 12·5 per cent solution.
△ Intravenous injection of a 25 per cent solution.
× Intramuscular injection of a 12·5 per cent solution.
● Intramuscular injection of a 25 per cent solution.

Beckman DU spectrophotometer in a 1-cm cuvette. The concentration of P2S was read from a calibration curve. The specificity of the method has been studied by Ellin[15] and Ellin and Kondritzer,[14] who found that acid or alkaline hydrolysis of PAM did not produce substances that interfered.

RESULTS

Plasma concentrations of P2S *after intravenous injection*

In preliminary experiments two subjects were injected with P2S (20 mg per kg body weight) by the intravenous route in order to get an idea of the plasma concentrations

after equilibration. The concentrations of P2S in plasma are seen in Fig. 1. The injections caused some mild side reactions such as "dizziness", blurred vision and diplopia, but these symptoms vanished within a few minutes.

Plasma concentrations of P2S *after intramuscular injection*

Two subjects first received 20 mg per kg body weight intramuscularly, in the right gluteal region. The plasma concentrations, as shown in Fig. 1, were found to be almost identical in the two subjects, reaching a peak level of about 8 μg per ml after about 20 min. In one of the subjects a 12·5% solution was used, which caused no pain. The other subject received the same dose in a 25% solution, but this caused some pain at the site of injection.

Ten subjects then received 30 mg P2S per kg body weight, in a 25% solution. Some complained of pain at the site of injection and later of sciatic neuralgia in the leg of

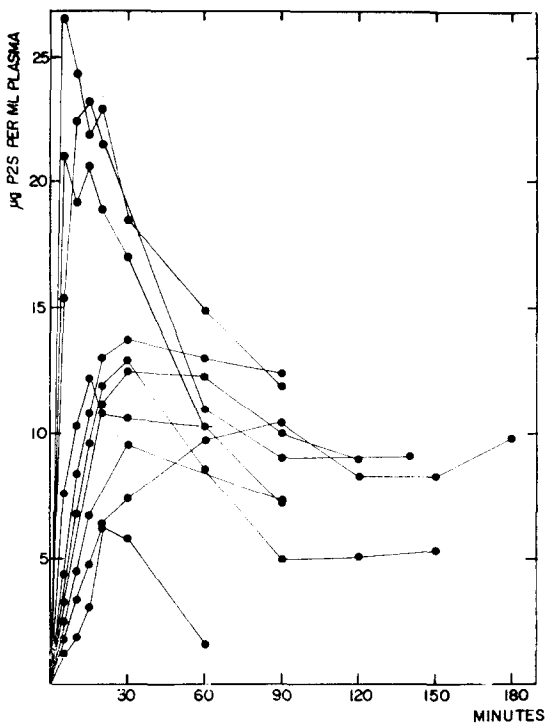

FIG. 2. Plasma concentrations of P2S in man after intramuscular injection of 30 mg per kg body weight.

the injected side, but these symptoms disappeared within a few hours. The plasma concentrations are seen in Fig. 2. A maximum concentration averaging about 15 μg per ml was reached after 20 min and was followed by a progressive fall. The rate of absorption and the maximal plasma levels varied considerably from one subject to another. A plot of the individual results indicated two different patterns of absorption (Fig. 2): one which is fairly rapid with plasma concentrations of about 20 μg per ml after 5 min, and another which is rather slow with plasma concentrations of 10 μg per ml after 20 min.

Plasma levels of P2S *after oral administration*

In these experiments, no food was taken from 5 hr before the start until the end of the experiment. Before the administration of the capsules, 400 ml of water was given orally to facilitate the collection of voided urine samples for the 4 hr period of the experiment.

Three gelatin capsules, each containing 1 g of P2S, were given to each of six subjects, weighing 65–70 kg. The plasma concentrations are shown in Fig. 3. A very slow rise in plasma concentration was observed with a badly defined maximum of from 4 to 5 μg per ml after 2 to 3 hr, followed by a slow decrease.

The amounts of P2S excreted in the urine during the 4 hr after administration averaged 23 per cent of the dose (standard error of the mean $\pm$ 3 per cent).

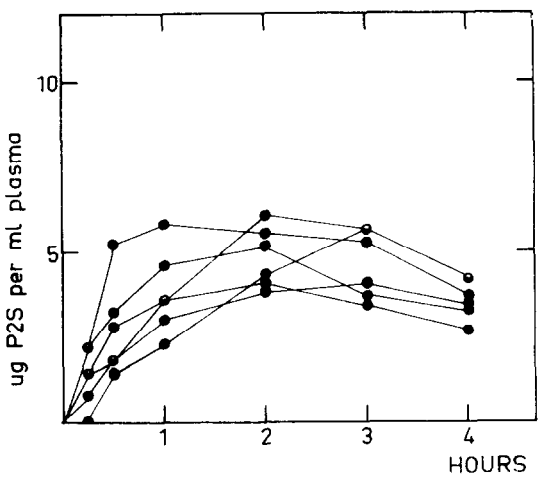

FIG. 3. Plasma concentrations of P2S in man after oral administration of 45 mg per kg body weight in gelatin capsules.

DISCUSSION

The present investigation was carried out in order to find how N-methyl-pyridinium-2-aldoxime methane sulphonate should be administered in case of organophosphate poisoning.

In mice, rats, guinea-pigs and rabbits the LD_{50} of *iso*propoxymethylphosphoryl fluoride (sarin) is raised by multiples of 4·0, 1·6, 38 and 40, respectively, if 30 mg P2S per kg body weight are given intramuscularly in combination with atropine 1 min after poisoning.[16] It was also stated that little is won in therapeutic effect if the dose of P2S was larger.

The results presented in the present paper show that 30 mg P2S per kg body weight can be given intramuscularly to man without serious discomfort or pain. The rate of absorption, however, varies greatly from subject to subject and this is possi bly due to different depths of injection.

Experiments on the anaesthetized cat indicate that plasma levels above 4 μg per ml are needed to counteract the bradycardia and respiratory failure produced by lethal doses of *iso*propoxymethylphosphorylthiocholine.[17] If this figure is valid also in man,

one can assume that therapeutic plasma levels are reached after 5–10 min in most of the cases provided circulatory failure has not yet developed.

The results obtained after oral administration show that a fairly large dose has to be given to obtain effective plasma levels, and these are reached only after a considerable delay. It is then quite evident that oral administration alone is of little therapeutic value as the rate of absorption is too slow. In milder cases or for prophylactic purposes, however, it might be used.

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