Blood Plasma Levels and Elimination of Salts of 2-PAM in Man After Oral Administration

By ALBERT A. KONDRITZER, PETER ZVIRBLIS, A. GOODMAN, and S. H. PAPLANUS

The absorption and elimination of pralidoxime salts after oral administration to human volunteers have been studied at a dosage of 1.5–10 g. of drug for a 70-kg. man. The biological half-life, calculated from blood plasma values and urinary excretion rates, was approximately 1.7 hr. A 10-fold increase in dosage of the pralidoxime salts increased the peak plasma level of oxime 3.5-fold. The average total amount of pralidoxime recovered in the urine was 27 percent. In subjects given the iodide salt, symptoms of iodism were observed; all other clinical tests were negative. Some observations were made on the behavior of a bis-quaternary oxime.

A NUMBER of oximes have been found to reactivate the cholinesterases that have been inactivated by organic esters of phosphoric acid derivatives (1). One quaternary oxime in particular has proved effective in combination with atropine in the treatment of poisoning by some of these toxic chemicals and in reducing their toxicity in various animal species (2). Salts of the oxime, chemically known as 2-hydroxyiminomethyl-1-methylpyridinium chloride, iodide, and methane sulfonate, are commonly referred to as 2-PAMCl, 2-PAMI, and P-2-S, respectively. Pralidoxime (3) is the generic name given to the oxime moiety.

Few studies have been carried out in man of plasma levels after oral administration of salts of 2-PAM. Sundwall (4) reported that the maximum concentration in plasma of man after oral administration of 45 mg./kg. of P-2-S, packed in gelatin capsules, was about 4 mcg./ml. and that a concentration over 3.5 mcg./ml. was maintained from 1 to 4 hr. These results may be compared with peak plasma concentrations, averaging 15 mcg./ml., which were reached 20 min. after an intramuscular injection of 30 mg./kg.

The half-life for 2-PAM iodide in the blood serum of man after its intravenous administration in a dose of 15 mg./kg. has been reported (5) to be 0.9 hr. Most of the injected oxime quickly passed into the urine and no oxime was detected in the spinal fluid 70 min. after its injection.

The present investigation was carried out to measure the concentration of oxime in plasma and urine of man after ingestion of various salts of 2-PAM in order to determine the relation of the amounts ingested to the attainable concentrations and to acquire information concerning rates of excretion.

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MATERIALS AND METHODS

Organic and inorganic salts of 2-PAM, differing in water solubility, were used in this study (6). Adult male volunteers were given aqueous solutions of the 2-PAM salts by mouth; 400 ml. of water was drunk during the hour before and 100 ml. of water each hour for 5 hr. after the ingestion of the drug to insure good hydration and urine collections.

The following information was sought; (*a*) plasma levels of oxime at various times after ingestion, (*b*) rates of disappearance of oxime from blood, (*c*) urinary excretion of oxime, and (*d*) biological half-life of the oxime, calculated from plasma and urine data. The concentrations of the oxime in plasma, urine, and feces were measured by ultraviolet spectrophotometry, based on procedures previously described (7). The oxime salts, dosage, and number of subjects are listed in Table I.

In addition to the 2-PAM salts listed in the table, the bis-quaternary oxime *N,N'*-trimethylenebis-(pyridinium-4-aldoxime) dibromide, commonly referred to as TMB-4 (8), was given to three subjects at a dose of 1 g./70 kg.

Clinical laboratory tests were carried out on the volunteers to uncover indications of any pathologic damage associated with the ingestion of the 2-PAM salts as follows. (*a*) Urinalysis, including microscopic examination, before and the day after the test. (*b*) Blood examination, including hemoglobin, total and differential white blood cells, before and the day after the test. (*c*) Liver function tests, including alkaline phosphatase and serum glutamic-pyruvic transaminase, before and on the morning after the test. (*d*) In addition to the oxime measurements red

TABLE I—SALTS OF 2-PAM AND DOSE GIVEN ORALLY

2-PAM Salt	Mol. Wt.	g. Oxime g. Salt	Dose[a] g. Salt/70-kg. Man		
Chloride	172.6	0.79	1.5 (4)		3.7 (3)
Lactate	226.2	0.61	2 (6)		5 (9)
Dihydrogen phosphate	234.2	0.59	2 (3)		5 (3)
Methane sulfonate	237.3	0.58	2 (6)		5 (4)
Iodide	264.1	0.52	5 (3)	8 (3)	10 (3)

[a] Number of subjects is given in parentheses; the average weight was 72 kg. with a range of 63–84 kg.

cell and plasma cholinesterase activity measurements were made two or three times on separate days before the test, on the morning of the test, 3 and 6 hr. after ingestion of the oxime, and the morning after the test.

During the tests, the volunteers were observed closely for changes in blood pressure, pulse rate, and respiratory rate, and for development of abnormalities in their neurologic status; they were questioned repeatedly concerning their subjective reactions. At regular time intervals after ingestion of the oximes, muscular function was checked by means of a grip dynamometer and the standard step tests.

RESULTS AND DISCUSSION

Blood Plasma—Oxime was found in the blood plasma within 15 min. after oral administration. The concentration rose rapidly, approached a peak at 2–3 hr., and then declined at an approximately first-order rate. The first part of the curve results predominantly from absorption, and at the peak of the curve the rate of absorption is about equal to the rate of elimination; the latter portion of the curve results predominantly from elimination of the oxime. Average oxime absorption-elimination curves for plasma are shown in Figs. 1, 2, and 3. Absorption-elimination curves as a function of dosage of one salt, namely the iodide, are given in Fig. 1. The peak concentration of oxime in plasma increased with the increasing dosages. The time of reaching peak oxime concentration was slightly delayed with increasing dosages, from 2 hr. for the 5-g. dose to 2.5–3 hr. for the 10-g. dose. Evidently absorption of the oxime from the gut is prolonged to some degree with increasing dosage.

Figure 2 compares the rates of absorption and elimination of the lactate and dihydrogen phosphate salts after oral administration. At the dose of 2 g./70-kg. man, the peak plasma concentration obtained was higher with the lactate (6 mcg./ml. after 2 hr.). With the lactate salt a plasma concentration of mcg./ml. was reached in approximately 1.25 hr.

P-2-S and 2-PAMCl are compared in Fig. 3, which shows the plasma molar levels obtained when equimolar amounts of each of the two salts were given at two dosage levels. The results here indicate

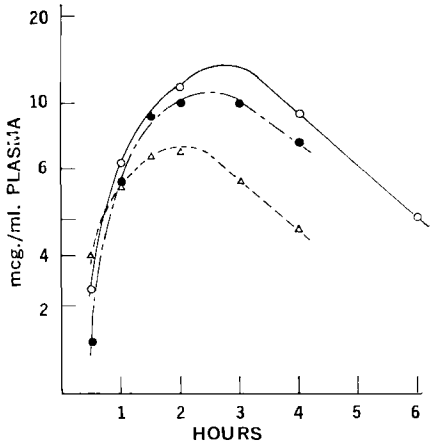

Fig. 1—Plasma concentrations of 2-PAM iodide in man after oral administration of 5, 8, and 10 g./70 kg. of body weight. Key: ●, 8 g.; ○, 10 g.; Δ, 5 g.

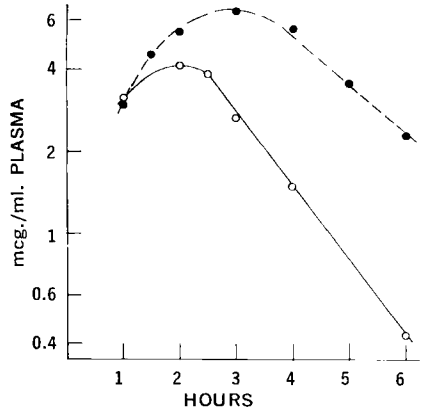

Fig. 2—Plasma concentrations of the lactate and dihydrogen phosphate salts of 2-PAM in man after oral administration. Key: ●, 2 g. lactate; ○, 2 g. phosphate.

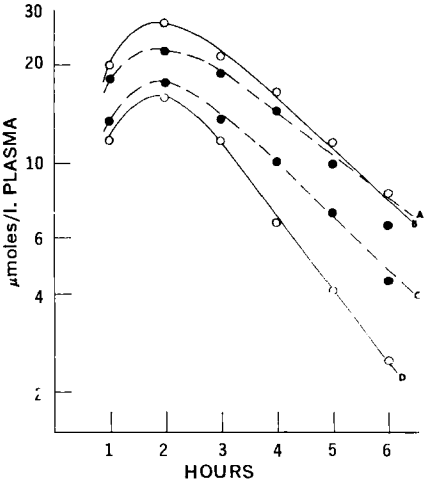

Fig. 3—Plasma concentrations of 2-PAM chloride and 2-PAM methane sulfonate (P-2-S). Key: A, 21.6 mmoles/70 kg. PAM-Cl; B, 21.6 mmoles/70 kg. P-2-S; C, 8.6 mmoles/70 kg. PAM-Cl; D, 8.6 mmoles/70 kg. P-2-S.

clearly that these salts are very similar with respect to absorption into and elimination from blood plasma.

The relationship between peak plasma levels of oxime and oral doses of the various salts may be approximated by an equation of the type

$$Y = kX^n$$

where Y is the oxime concentration in μmoles/l. of plasma, X is the dose in mmoles/70-kg. man, k is a constant, equal to Y when X is 1, n is a constant that describes the change in Y with change in X. The log form of this equation is plotted in Fig. 4. The best values of the constants were calculated by the method of least squares; the calculated value of log k is 0.79 and that of n (slope) is 0.54. Variability among individual results precludes any statement concerning statistically significant differences in peak oxime plasma levels resulting from the various 2-PAM salts. However, within the limits of this variability the estimation can be made that a 10-fold increase in dosage of any of the 2-PAM salts would increase the peak plasma level of oxime 3.5-fold.

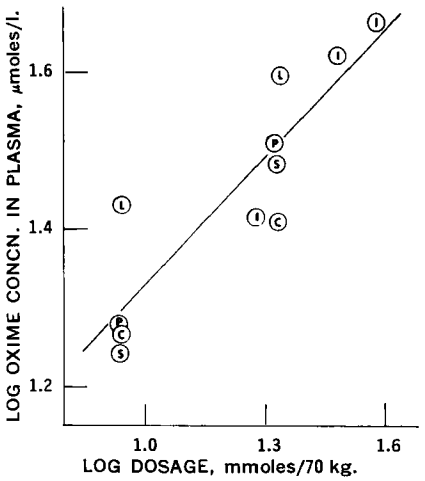

Fig. 4—Relationship between maximum oxime concentration reached in plasma and oral dosage of PAM salts. Key: C, chloride; I, iodide; L, lactate; P, dihydrogen phosphate; and S, methane sulfonate. The equation of the line is log Y = 0.79 + 0.54 log X.

Biological Half-Life—A considerable portion of the plasma concentration curve approaches or resembles the curve for a first-order process or reaction where the decrease in concentration of a reacting substance at a given time, t, is proportional to the concentration present at that time (9). Stated in logarithmic form,

$$\log C = \log C_0 - kt/2.303 \qquad \text{(Eq. 1)}$$

where C is concentration at time t, C_0 is concentration at time zero, t_0, k is the specific rate constant that characterizes the process.

A plot of log C *versus* time, t, gives a straight line; the slope of the line is equal to $-k/2.303$, from which the rate constant, k, is calculated. The time for the plasma concentration to be reduced by one-half may be defined as the biological half-life (10), and is equal to $0.693/k$. This value has been calculated from the plasma data for the 2-PAM salts; the best estimates are given in Table II.

Urinary Excretion—A valuable addition to the literature of urinary excretion equations was that which enabled a specific velocity constant, k, to be derived from the rate of urinary excretion by means of the equation

$$\log dC/dt = kt/2.303 + \log(-kC_0) \qquad \text{(Eq. 2)}$$

Table II—Biological Half-Life of 2-PAM Salts Given Orally in Man

2-PAM Salt	Mean Half-Life (hr.)[a] By Plasma	Mean Half-Life (hr.)[a] By Urine
Chloride	1.7 (7)	1.7 (7)
Lactate	1.7 (9)	1.7 (9)
Phosphate	1.3 (5)	1.5 (5)
Methane sulfonate	1.7 (10)	1.6 (10)
Iodide	2.0 (9)	2.2 (5)

[a] Figures in parentheses indicate number of subjects used.

where, after the attainment of pseudo-steady-state diffusion conditions in the body, C may be defined as the amount of unexcreted substance after a single dose, and C_0 is the apparent maximal amount of excretable substance (11). A plot of the change in C for each equal increment of t is a straight line having the same slope as a plot of Eq. 1. Application of Eq. 2 to urinary excretion data requires a measurement of the drug or substance excreted in a series of reasonably equal time intervals.

Plots illustrating the urinary excretion rates of 2-PAM salts are given in Fig. 5. In Fig. 6 a plot is shown of urinary excretion in which 2-PAMCl and P-2-S are compared; four subjects were used for each salt and all subjects were studied during the same day. The hourly points, in millimoles excreted per hour, were obtained from an average Cartesian coordinate plot for each salt. The excretion rate is approximately the same for both salts. The plateau between 2 and 3 hr. indicates the point where absorption is about equal to excretion of the oxime and agrees with the data on peak oxime levels found in plasma.

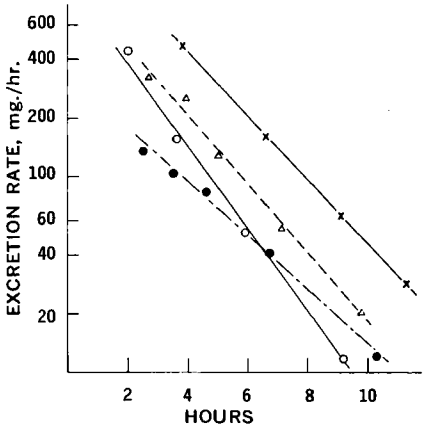

Fig. 5—Urinary excretion rate of salts of 2-PAM in man after oral administration. Key: ×, 10 g. iodide; Δ, 5 g. lactate; ○, 5 g. phosphate; ●, 2 g. P-2-S.

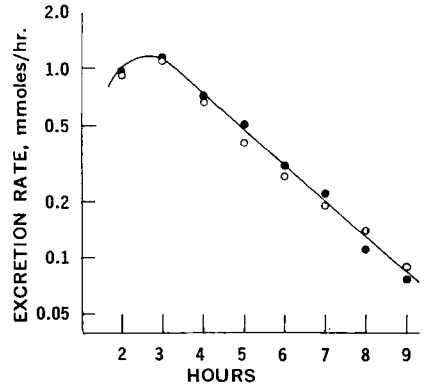

Fig. 6—Comparison of urinary excretion rates of 2-PAMCl and P-2-S following oral administration of 21.6 mmole/70 kg. Points were interpolated from Cartesian coordinate plots of the experimental data and are averages of four subjects for each salt. Key: ○, P-2-S; ●, PAM-Cl.

The concentrations of oxime in urine at various times of excretion were used to calculate the rate constant, k, and biological half-life, $t_{1/2}$ (Table II). Most of the urine samples were reasonably spaced; where the sample or urine collection spacing was too variable, values were interpolated from a smooth Cartesian coordinate plot. The procedure used, of necessity, is not a rigorously correct mathematical application of the equation. However, its use in this manner constitutes, under the conditions of the experiment, a reasonably good approximate evaluation of the total disappearance and excretion rates of the oximes.

An analysis of variance of the data revealed no significant difference at the 95% confidence limit in half-life among the various salts. The mean biological half-life of the five oxime salts was 1.7 hr. with a coefficient of variation of 24%. Statistical comparison of 35 paired replicates showed no significant difference between half-life calculated from plasma data and that calculated from urinary excretion data.

The average total amount of oxime recovered in the urine for the various 2-PAM salts was 27%; the standard deviation of individual recoveries was 9%.

Clinical Findings—With regard to the clinical laboratory tests, the following findings were made: (*a*) no changes were observed in the hematologic status of any of the subjects; liver and kidney functions were also unaffected. (*b*) A 20% drop in both RBC and plasma cholinesterase activity occurred in the men given TMB-4. No change in cholinesterase values occurred after ingestion of the other oxime salts. (*c*) No consistent changes in blood pressure, pulse, or respiratory rate occurred; no effect on either neurologic status or muscular performance was observed. (*d*) No subjective complaints were made by the volunteers, with the exception of those receiving 2-PAM iodide. These subjects had signs and symptoms of iodism: coryza, pharyngeal burning, and painful parotid glands. Two of the 10 subjects receiving 2-PAM iodide had detectable parotid enlargements, and in one subject the swelling was pronounced. These findings appeared to be unrelated to the dosage, inasmuch as the most severe case was a subject who received the lowest dose of 2-PAM iodide (5 g./70 kg.).

General Observations—The amount of oxime found in the feces among 18 subjects, taken at random, was variable, ranging from 5 to 35% of the doses administered. No correlation was observed between the amount found in the feces and either the urinary excretion or the plasma levels. No oxime could be found in the feces of one subject who had taken 2 g./70 kg. of the lactate salt. When this subject was given P-2-S and at another time the dihydrogen phosphate salt, no oxime could be found in the feces. It was later established that this individual harbored intestinal parasites. No studies were carried out to establish a correlation between these observations.

The peak plasma level achieved with the bis-quaternary oxime TMB-4 was approximately 1 mcg./ml., which caused no apparent clinical discomfort; however, all three volunteers suffered a 20–25% reduction in RBC and plasma cholinesterase. This latter result cannot be considered a conclusive characteristic since only three subjects were used.

Recovery of the bis-oxime in the urine over 24 hr. averaged less than 3% for each of three subjects, and the amount found in the feces was about 2% in two subjects. In one *in vitro* experiment 0.1 mg./ml. of 2-PAMI and of TMB-4 each were mixed with aliquots of a 1:3 suspension of a control fecal sample and allowed to remain at room temperature overnight. Recoveries from the samples were 80% for 2-PAMI and 8% for TMB-4, indicating that considerable destruction of TMB-4 may occur in the lower gut.

During the course of these trials the effect of withholding water (dehydration) from the subjects before and during the period of absorption of the oxime salt was studied in three volunteers who were given 5 g. of 2-PAM lactate. The subjects drank no fluids the night before and no water for 8 hr. after ingestion of the material. A significantly lesser amount of oxime was excreted in the urine of these volunteers compared with those subjects who were "hydrated"; no significant differences in blood plasma levels were observed.

Probenecid, which inhibits tubular secretion of substances such as penicillin and phenosulfonphthalein dye and inhibits tubular reabsorption of uric acid, was given in divided doses of 0.5 g. to three volunteers before ingestion of 2 g. of 2-PAM lactate, at the time of ingestion, and 1 hr. after ingestion. The probenecid did not increase or prolong plasma levels of the oxime and did not significantly alter its excretion in urine.

SUMMARY

1. Adult male volunteers were given, orally, aqueous solutions of each of the chloride, iodide, dihydrogen phosphate, methane sulfonate, and lactate salts of pralidoxime in varying amounts. A measurable amount of oxime was found in blood plasma within 15 min.; the concentration rose rapidly, reached a peak at 2–3 hr., and then declined at a rate that approximated a first-order process.

2. The rates of absorption into and elimination from plasma of 2-PAMCl and P-2-S were practically identical.

3. The equation, $\log Y = 0.79 + 0.54 \log X$, gives an approximate relationship between peak plasma levels of oxime (Y) and oral doses (X) of the various salts. Accordingly, a 10-fold increase in dosage of the 2-PAM salts increased the peak plasma level of oxime 3.5-fold.

4. The biological half-life in man of the 2-PAM salts given orally, calculated from blood plasma values and urinary excretion rates, was 1.7 hr., with a coefficient of variation of 24%.

5. The average total amount of 2-PAM recovered in the urine was 27%; the standard derivation of individual recoveries was 9%. Considerably less TMB-4 was excreted *via* the urine; an average of 3% was recovered during 24 hr. among three subjects.

6. Clinical changes observed were (*a*) iodism symptoms in subjects given 2-PAMI and (*b*) a 20% decrease in both RBC and plasma cholinesterase in subjects given 1 g./70 kg. of TMB-4. All other clinical tests were negative.

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Keyphrases

Pralidoxime salts—absorption, elimination
Absorption, elimination rates—pralidoxime salts
Biologic half-life—pralidoxime salts
Pharmacological activity—pralidoxime salts

4-Acetamidophenyl 2,2,2-Trichloroethyl Carbonate

Particle Size Studies in Animals and Man

By LEWIS W. DITTERT*, H. JACK ADAMS†, FRED ALEXANDER, CLIFFORD W. CHONG, THEODORE ELLISON‡, and JOSEPH V. SWINTOSKY*

4-Acetamidophenyl 2,2,2-trichloroethyl carbonate (ATC) was prepared in coarse, regular, and fine particle sizes by sieving and grinding. The powders were found to dissolve in water at significantly different rates in a mechanically stirred system. When administered orally in suspensions to mice, the LD_{50}'s of the ATC powders were as follows: coarse—3340 mg./Kg.; regular—2461 mg./Kg.; and fine—1796 mg./Kg. The three ATC powders produced significantly different blood plasma concentrations of total acetaminophen in mice during the 0- to 2-hr. period after oral administration. In humans, the blood plasma concentration curves produced by oral administration of coarse and regular ATC were nearly identical. However, both had lower peaks and slower rates of decline than those produced by fine particle ATC or acetaminophen. Forty-eight-hour recovery of acetaminophen from human urine indicated that all the ATC powders were as efficiently absorbed as acetaminophen itself. It was concluded that ATC is a true prodrug of acetaminophen and that the acetaminophen blood plasma concentrations produced by orally administered ATC can be controlled to some degree by controlling its particle size.

ONE OF THE MAIN objectives of preparing prodrugs is to influence the dose–time–action profiles of drugs with known pharmacologic activities. The authors' studies with the prodrug carbonate esters of acetaminophen (1, 2) have been directed primarily toward prolonging its duration of action. This is done conveniently when the prodrug is much less soluble in water and has a slower dissolution rate in aqueous fluids than the parent drug. Under these circumstances, the appearance of the parent drug in the body is slowed by the slow dissolution of the prodrug in the gastrointestinal tract. Previous studies have indicated that the dissolution rates of the acetaminophen prodrugs, rather than their rates of hydrolysis, determine the rates at which acetaminophen is released to the tissues following their oral administration (2).

4-Acetamidophenyl 2,2,2-trichloroethyl carbonate (ATC) is much less soluble in water than acetaminophen (1). If the duration of action of ATC is indeed controlled by its dissolution rate in the gastrointestinal tract, then it would be expected that the particle size of ATC powder administered orally might have a pronounced influence on its dose–time–action profile. This paper describes the influence of particle size on oral toxicity of ATC in mice, on the blood plasma concentrations of total acetaminophen it produces in mice and in humans, and on the urinary excretion of acetaminophen after its oral administration to humans.

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† Present address: Astra Pharmaceutical Products, Inc., Worcester, Mass.
‡ Present address: Riker Laboratories, Northridge, CA 91326

EXPERIMENTAL

Preparation of ATC Powders of Various Particle Sizes—ATC was prepared in coarse, regular, and