RENAL TUBULAR SECRETION OF PRALIDOXIME IN MAN

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Pralidoxime chloride is a quaternary ammonium compound used to reactivate organophosphate-inhibited cholinesterase. The drug is rapidly cleared from the plasma by renal tubular secretion, though the mechanism has not been specifically identified. Reduction of pralidoxime clearance rates and prolongation of the biologic half-life after thiamine administration, as compared to those after *p*-aminohippurate (PAH) administration, suggest that pralidoxime is secreted as an organic base. Furthermore, reduced excretion of pralidoxime under conditions of both urine alkalinization and urine acidification implicate an active reabsorption of pralidoxime not heretofore described.

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SUMMARY

Pralidoxime chloride is a quaternary ammonium compound used to reactivate organophosphate-inhibited cholinesterase. The drug is rapidly cleared from the plasma by renal tubular secretion, though the mechanism has not been specifically identified. Reduction of pralidoxime clearance rates and prolongation of the biologic half-life after thiamine administration, as compared to those after *p*-aminohippurate (PAH) administration, suggest that pralidoxime is secreted as an organic base. Furthermore, reduced excretion of pralidoxime under conditions of both urine alkalinization and urine acidification implicate an active reabsorption of pralidoxime not heretofore described.

PREFACE

The work described in this report was authorized under Task No. 1B662706AD2502, Medical Defense Against Chemical Agents, Prophylaxis and Therapy for Lethal Agents. This work was started in November 1971 and completed in April 1973.

The volunteers in these tests are enlisted US Army personnel. These tests are governed by the principles, policies, and rules for medical volunteers as established in AR 70-25 and the Declaration of Helsinki.

Acknowledgment

We wish to thank the nurses and corpsmen of the Clinical Investigation Section for their invaluable assistance in organizing and running these tests; Mr. Andris Kaminskis, Mr. Barry Burkhardt, and Mr. Pat Vicario for their assistance in performing the drug assays; Dr. Samuel A. Cucinell for additional assistance; and Dr. C. Metzler of Upjohn Company for providing our laboratory with the invaluable computer program NONLIN.

CONTENTS

RENAL TUBULAR SECRETION OF PRALIDOXIME IN MAN

I. INTRODUCTION.

Pralidoxime is a quaternary ammonium compound used to reactivate organophosphate-inhibited cholinesterase *in vivo*. It has been established that pralidoxime is rapidly excreted by the kidney with a clearance rate approaching that of *p*-aminohippurate (PAH).[1,2,*] Therefore, it appears that active tubular secretion is the major mechanism for elimination of the drug from the body.[3]

Several studies have attempted to characterize the renal secretory mechanism for pralidoxime elimination, but these studies have been inconclusive. It has been shown, for example, that organic acids such as probenecid[3-5] and salicylates[4] do not interfere with pralidoxime excretion. The effect of organic bases (e.g., nicotinamide) on pralidoxime excretion has been studied,[4] but no conclusive results have been forthcoming.

The frequent description of pralidoxime as a "weak acid"[3,4,6] (on the basis of the dissociation of the aldoxime group to an anion and H+) has only confused the discussion of the ultimate fate of the drug. Since the pK_a (i.e., the pK of dissociation of the "weak acid") of pralidoxime is reported to be 7.8 to 8.0,[7] this compound might be considered as an organic base. The specific mechanism by which the renal tubule handles this drug is of considerable importance, not only in the clinical use of pralidoxime as an adjunct to atropine therapy in organophosphate poisoning, but also in describing the disposition of drugs with a similar chemical structure and similar pharmacological properties.

II. EXPERIMENTATION.

A. Methods.

1. Subjects.

The subjects for these experiments were US Army enlisted personnel who volunteered for testing.[a] Subjects were given a thorough physical examination and routine laboratory screening.[b] All testing procedures and possible drug effects were explained to the volunteers.

2. Materials.

Drugs used in these studies included: pralidoxime chloride,[c] *p*-aminohippurate (pah),[d] thiamine hydrochloride,[e] ammonium chloride,[f] and sodium bicarbonate.[g]

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*Sodium *p*-aminohippurate is a more appropriate name.

[a] These tests were governed by the principles, policies, and rules for medical volunteers as established in Army Regulation 70-25 and the Declaration of Helsinki.

[b] Chest x-ray, electrocardiogram, complete blood count, routine urinalysis, blood urea nitrogen, serum creatinine and 24-hour creatinine clearance, and liver function tests (SGOT, alkaline phosphatase, serum bilirubin).

[c] Protopam[R]; Ayerst Laboratories, New York, New York. Systematic name is 2-formyl-1-methylpyridinium chloride, oxime.

[d] Sodium Aminohippurate; Merck, Sharp and Dohme; West Pointe, Pennsylvania.

[e] Thiamine hydrochloride, U.S.P.

[f] Ammonium chloride, U.S.P.; Mallinckrodt Chemical Works; New York, New York.

[g] Sodium bicarbonate, U.S.P.; Allied Chemical Corporation; Morristown, New Jersey.

B. Procedures.

A total of 22 subjects were studied, each subject being tested once per week under a series of different metabolic conditions. Each of the 22 subjects did not participate in every condition; however, each subject served as his own control for purposes of assessing the effects of any one of the metabolic conditions examined.

The conditions and number of subjects involved were as follows:

1. Pralidoxime, Control.

All 22 subjects received pralidoxime (5 mg/kg, total volume <10 cc, as a rapid intravenous bolus over a 2-minute period) under conditions of forced hydration and bed rest, *control*.

2. Pralidoxime, Acidification.

Eight subjects received pralidoxime (same dose) under conditions of forced hydration and bed rest, one time after 36 hours of ammonium chloride (1 gram every 6 hours, orally, until urine pH was less than 5.0), *acidification*.

3. Pralidoxime, Alkalinization.

The same eight subjects received pralidoxime another time after 24 hours of sodium bicarbonate (1 gram every 4 hours, orally, until urine pH exceeded 7.5), *alkalinization*.

4. Pralidoxime, Organic Base.

Nine subjects* received pralidoxime (same dose) under conditions of forced hydration and bed rest, 20 to 30 minutes after thiamine (200 mg total, intramuscularly), *organic base*.

5. Pralidoxime, Organic Acid.

Eight subjects** received pralidoxime (same dose) under conditions of forced hydration and bed rest, simultaneously with PAH (900 mg total, intravenously), *organic acid*.

6. Pralidoxime, 8- to 12-Hours Fasting and NPO.

Four subjects received pralidoxime (same dose) under conditions of bed rest, after 8 to 12 hours of fasting, *NPO*.

Forced hydration consisted of a light breakfast and 1000 ml of fluids (containing no caffeine) in the 2 hours prior to pralidoxime administration, 250 ml of fluids every 20 to 30 minutes in the first 3 hours after pralidoxime administration, and fluids and meals ad lib thereafter.

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*Five of these subjects also received PAH alone after thiamine administration.

**These eight subjects also received PAH alone under the same experimental conditions.

7. Blood samples; Analysis for Pralidoxime.

Analysis was also carried out in the blood for PAH, where indicated; contents were drawn at 5, 10, 20, 30, 45, 60, 90, 120, and 180 minutes after administration through an indwelling "butterfly" intravenous apparatus, cleared with saline and diluted with heparin after each sampling.

8. Urine Samples; Analysis for Pralidoxime.

These samples were also analyzed for PAH content; they were collected by spontaneous voiding, and the collection for the first 3 hours was separated from that for the subsequent 21 hours. Individual specimens were measured for specific gravity (less than 1.010 in well-hydrated subjects and greater than 1.020 in fasting subjects) and for pH, by pH meter, before being pooled (pH was 6.0 to 7.0 in all *control* subjects).

Pralidoxime content of blood and urine samples was determined by the method of Groff and Ellin.[8] PAH content was determined by the method of Harvey and Brothers.[9]

C. Calculations.

Plasma concentration values for pralidoxime (as well as for PAH) were fit to a biexponential equation using the computer program NONLIN.* This equation describes drug disposition in the two-compartment model, described in detail by Wagner,[10] and discussed with regard to pralidoxime elsewhere.[1,2]

The kinetic parameters reported here include: (1) the half-life values for both the rapid (initial) exponential phase ($t_{1/2}, \alpha$) and the slow (post-equilibrium) exponential phase ($t_{1/2}, \beta$) of the plasma disappearance curve, which describe the rates of drug distribution toward equilibrium and drug elimination after reaching equilibrium, respectively; (2) the volume of distribution in both the central (plasma and plasmalike) compartment (V_1) and the peripheral (tissues unlike-plasma) compartment (V_2); (3) the renal clearance (RC) using the area under the plasma disappearance curve and the urinary excretion rate;[2,11] and (4) the urinary drug recovery in the first 3 hours and in the subsequent 21 hours.

Statistical analysis consisted of comparing the kinetic parameters for each subject under a given metabolic condition to that subject's own control values, using the paired-*t* test.

III. RESULTS.

A. Clinical.

Physiologic measures (blood pressure and heart rate) did not change with pralidoxime administration at 5 mg/kg, even with administration as a rapid intravenous injection (total 10 ml over about 2 minutes). Previous reports of elevations of systolic and diastolic blood pressure were in subjects given higher intravenous doses (20 or more mg/kg) of pralidoxime[4,6,12,13] suggesting that total dose (as well as rate of administration) are the major factors in this physiological change.

*NONLIN was developed by and supplied by C. Metzler at Upjohn Company of Kalamazoo, Michigan.

Other clinical symptoms observed in this study were limited to those known to occur with pralidoxime administration[2,6,12] even though the dose was only 5 mg/kg in all cases. "Blurred vision" or "heaviness in the eyes" were reported by two-thirds of the cases. Diplopia was specifically reported by subjects in approximately 15% of cases of pralidoxime administration, although disconjugate gaze was detected by the physician in only a few of these subjects. Finally, nausea was reported by subjects in four instances (in nearly 80 total pralidoxime administrations), with emesis occurring in two subjects.

Each of these side effects occurred immediately after pralidoxime administration and lasted usually less than 1 minute. No late signs or symptoms could be attributed to pralidoxime. Furthermore, the simultaneous administration of other drugs (e.g. thiamine, PAH, etc.) did not enhance the observed side-effects of pralidoxime.

B. Kinetic.

1. Control.

The overall control values for all 22 subjects are listed in the table. The percent change from control for any of the other metabolic conditions was calculated only for those subjects tested under those conditions; the control mean for each of these subgroups was not statistically different from the overall control group, however. The values reported here are consistent with those previously reported for pralidoxime and PAH under control conditions.

Table. Kinetic Parameters for Pralidoxime – *Control* Values for 22 Subjects

Kinetic parameter	(Units)	Mean ± S.E. (N = 22)
Urinary recovery (% of dose)	3 hours	77.7 ± 1.1
	3-24 hours	7.4 ± 0.9
Renal clearance (ml/min)		612 ± 19
Half-life in plasma (min)	$t_{1/2,\alpha}$	5.4 ± 0.3
	$t_{1/2,\beta}$	75.9 ± 3.6
Volume of distribution (ml/kg)	V_1	232 ± 24
	V_2	502 ± 33

2. Alkalinization (Figure).

Raising the urine pH over 7.5 caused a decrease in the overall excretion of pralidoxime, both in the first 3 hours ($p<0.05$) and in the total 24 hours ($p<0.05$). There was a corresponding decrease of about 15% in the apparent renal clearance rate ($p<0.05$). The slight contraction of the central volume (V_1) ($p>0.10$) and the shortening of the initial phase half-life ($t_{1/2}, \alpha$) ($p<0.05$) suggest that distribution of drug occurred more rapidly than under control conditions. Even though the urinary excretion was reduced, the overall half-life for elimination was not significantly altered.

3. Acidification (Figure).

Lowering the urine pH below 5.0 also caused a decrease in the total amount of pralidoxime excreted, especially in the first 3 hours ($p<0.05$). Again, the apparent renal drug clearance rate was reduced (13%) ($p<0.05$). There were no significant changes in the other kinetic parameters, even though the mean central volume (V_1) did increase by over 20% ($p>0.10$), in contrast to the decrease seen with *alkalinization*.

4. Organic Base (Figure).

Thiamine administration caused a significantly lower urinary excretion of pralidoxime in the first 3 hours ($p<0.02$), although a much larger amount of drug "reappeared" in the urine in the latter collection than was observed under any other condition ($p<0.10$). The renal drug clearance was strikingly reduced in the first 3 hours by 22% ($p<0.001$) and was accompanied by a 25% prolongation in the half-life for the elimination of the drug ($t_{1/2}, \beta$) ($p<0.05$). The volume of distribution of pralidoxime was not altered significantly, suggesting that reduction of renal clearance was not a result of drug redistribution.

5. Organic Acid (Figure).

Simultaneous administration of PAH and pralidoxime did not alter the disposition of pralidoxime to any significant degree. There was a slight decrease in the drug disposition to the peripheral compartment (V_2) ($p>0.10$), but half-life and renal clearance values were essentially unchanged. Overall urinary excretion was not changed, although the mean recovery was slightly higher after PAH administration.

6. NPO (Figure).

Fluid restriction resulted in striking changes in pralidoxime disposition, similar to those previously reported for other conditions of physical stress.[2] It is noteworthy that the overall urinary recovery of pralidoxime did not decrease under these conditions and that the reduction of the renal clearance was due to higher plasma concentration values caused by the contracted central volume (V_1) ($p<0.10$), especially at zero time (i.e., the extrapolated intercept of the biexponential curve).

IV. DISCUSSION.

The results reported here confirm several studies suggesting that pralidoxime is actively secreted into the urine by the renal tubule. The high clearance rates, approaching those of PAH, and the reduction in clearance under conditions of fluid restriction are consistent with the demonstration that renal blood flow is probably the major determinant of excretion rate for this drug.[2]

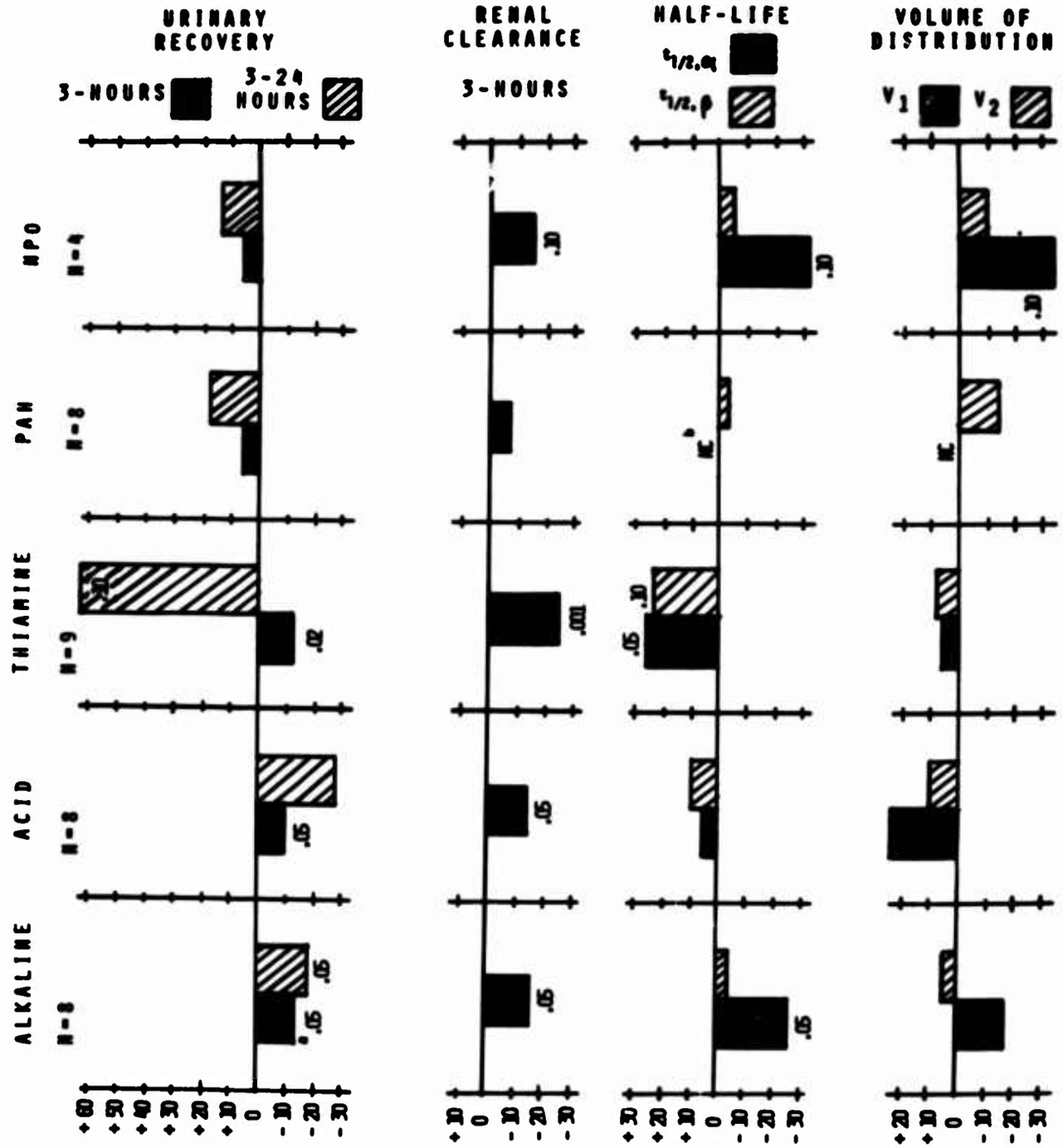

Figure. Kinetic Parameters for Pralidoxime; Percent Change from Control Values under Various Metabolic Conditions

Change from control is expressed as a percentage of the control for each subgroup; the mean for each subgroup was not significantly different from the total group.

[a] p values for the paired t test.
[b] NC denotes "no change."

To date, the study reported by Berglund, *et al.*[3] remains the most detailed investigation into the specific mechanism of the renal tubular handling of pralidoxime; however, that investigation fell short of fully explaining the mechanism. Berglund, *et al.* demonstrated that (1) pralidoxime excretion was pH-dependent between urine pH values of 5.7 to 8.0 with excretion rate decreasing as the pH increased; and (2) pralidoxime excretion was not changed by administration of a weak organic acid (probenecid).

The present studies confirmed that urine alkalinization (pH>7.5) reduced pralidoxime excretion; but these studies demonstrated that more extreme acidification (pH<5.0) also reduced overall pralidoxime excretion. It seems likely that in alkaline urine (where the hybrid resonant form of pralidoxime, the zwitterion-non-ionic form, is more prevalent)[7] non-ionic passive diffusion accounts for a considerable amount of reabsorption of the drug, and a decrease in the apparent clearance rates. In acid urine, however, the situation appears to be more complicated. It may well be that at very low pH values, the quaternary ammonium cation of pralidoxime which is more prevalent is actively reabsorbed. Alternatively, the hyperchloremic acidosis of ammonium chloride administration necessitates the active retention of all cations other than H+, reducing the effective urinary excretion of the drug and the apparent clearance rates.

Active tubular reabsorption of pralidoxime has not been specifically studied, although it may be an important factor affecting measured clearance rates under metabolic conditions such as those in the present study and the study of Berglund, *et al.*[3] This mechanism could be studied using a reabsorption inhibitor, such as phloridzin,* although we were unable to use this potentially toxic compound on the human subjects in these experiments.

Active tubular secretion can be characterized in a similar fashion by inhibiting the known mechanisms for secretion. In the preser.. studies, we chose to use nontoxic competitive inhibitors of organic acid and of organic base secretion. The results strongly suggest that pralidoxime may indeed be handled by the *organic base* secretory mechanism, since clearance rates were markedly reduced by thiamine and only very slightly affected by PAH.

It is well-established that thiamine is secreted as an organic base, and that it competes with other "weak base" compounds for the secretory mechanism.[14] Studies using nicotinamide and pralidoxime were reported by Calesnick and DiPalma,[4] but were inconclusive. In the present studies, it is clear that pretreatment with thiamine strikingly altered the renal clearance of pralidoxime without altering the apparent distribution of the drug. Furthermore, the urinary recovery of pralidoxime demonstrates that drug which was not excreted in the first three hours was ultimately excreted once the inhibitory compound (thiamine) was itself eliminated (i.e., about 2 to 3 hours).[15]

By contrast, the administration of PAH did not alter the excretion of pralidoxime to any significant degree. The slight change in the volume of distribution (peripheral compartment, V_2) suggests that PAH may displace a small amount of pralidoxime from its usual tissue reservoirs; however, this minor alteration did not affect the apparent renal clearance. Previous studies with

*The 1962 C. A. Subject Index favors the spelling Phlorizin. Another spelling, Phlorhizin, is given as is the spelling above, but these are cross references to Phlorizin. Manufacturer's name is Phloridzin.

organic acids, in particular probenecid and salicylates, have shown no alteration in renal pralidoxime clearance. Moreover, other data which we have not included in this report show that PAH clearance is, in turn, not affected to any significant degree by the simultaneous administration of pralidoxime. Therefore, the secretory mechanisms for these two drugs are probably distinct.

For drugs that are rapidly cleared, such as pralidoxime, PAH, etc., it has been demonstrated that renal blood flow changes will alter clearance rates.[2] In the present studies, fluid restriction alone was found to alter clearance rates and volume of distribution; these changes were most likely attributable to decreased renal blood flow. It is not clear what effect the acid urine in fluid-restricted patients, or the altered renal blood flow in patients given ammonium chloride, had on pralidoxime clearance. It is clear, however, that thiamine does not alter renal blood flow (as estimated by the PAH clearance), and we presume that the reduction which thiamine does cause in pralidoxime clearance is a result of direct inhibition.

The results reported here for simultaneous administration of thiamine and pralidoxime suggest a possible role for thiamine in the treatment of victims of organophosphate poisoning. It may well be that the prolonged biologic half-life and reduced renal excretion of pralidoxime after thiamine will enhance the clinical effectiveness of treatment with pralidoxime. Further testing to evaluate this possibility might prove clinically useful.

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