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CONCLUSIONS

PAPP is effective as a pretreatment antidote to cyanide when used by single-dose administration. Further experiments need to be performed to establish a lack of toxicity of PAPP in multiple-dose experiments. Possible chronic hematological and genotoxic effects of PAPP may limit its development in repeated chronic treatment. Combination therapy of a prophylactic sulfur donor with a low dose of a safer prophylactic aminophenone (e.g., PAOP or *p*-aminoheptanoylphenone or their N-hydroxy metabolites) may provide more efficacious protection against cyanide than PAPP. This combination could allow for a reduction in the dosage of MetHb formers that would reduce the chance of unwanted side effects of the employed therapeutic compounds.

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The Pharmacology of *p*-Aminopropiophenone in the Detoxification of Cyanide

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Key Words: Cyanide—Methemoglobin—*p*-Aminopropiophenone—Methemoglobinemia.

The use of cyanide and cyanide-containing compounds as chemical warfare agents has been practiced for centuries (92). Napoleon III proposed the use of cyanide to enhance the effectiveness of his soldier's bayonets during the Franco-Prussian War, and the French used hydrocyanic acid at the World War I battle of Somme (72). During World War II, the United States adopted hydrocyanic acid and cyanogen chloride as a standard or substitute filling in a variety of chemical munitions (68). Hitler employed hydrocyanic acid absorbed onto a dispersible pharmaceutical base (Zyklon B) to exterminate millions as part of the Nazi war effort (74). Recent reports indicated that cyanide-like agents may have been used against inhabitants of the Syrian city of Hama (59), the Kurdish city of Halabja in Iranian-occupied Iraq (50), and during the Iran–Iraq War (4).

There are other sources of cyanide intoxication than military. Depolymerization of nitrile products resulting from fires remain a common source of cyanide intoxication (6). Cyanide from the chemical, mining, paper, photography, textile, agricultural and electroplating industries can occasionally be a source of poisoning (52). Relatively uncommon sources of cyanide intoxication are self-administration or its use as a murder weapon. Cyanogenic glycosides can be found in a wide variety of foods and natural products including clover, almonds, lima beans, and sorghum. Hydrolysis of these compounds also represents a potential source of cyanide intoxication (87). In addition, sodium azide represents a similar toxic agent with additional specific cardiovascular effects (54). This compound, widely used in automobile air bags, may constitute a threat to the driving public.

Even though the cyanides as a group have had a long history as chemicals and poisons,

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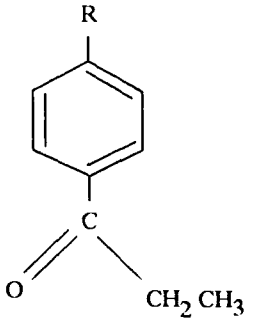
effective antidotes were not developed until the late 19th and early 20th century. In 1888, Pedigo (70) first noted the antidotal action of amyl nitrite. The pioneering work of Chen and co-workers (29–33) led to the development and marketing of the Eli Lilly Cyanide Antidote Kit. This kit consists of ampules of sodium thiosulfate and sodium nitrite and Aspirols of amyl nitrite. Sodium thiosulfate and sodium nitrite must be administered by i.v. infusion, a procedure requiring trained medical personnel. Amyl nitrite, on the other hand, is effective by inhalation, therefore allowing administration by nonmedical personnel. In a battlefield setting, the amyl nitrite ampules are crushed and inserted in the region of the eye lenses of the victim's protective mask (35). Because this therapy is more suitable for use in a battlefield environment, it was adopted as the recommended therapy for cyanide poisoning (38). Although an effective first aid against cyanide, the use of amyl nitrite produced side effects such as marked vasodilation with hypotension, dizziness, and headache in addition to unpredictable levels of methemoglobin (MetHb) (103). These factors, coupled with potential abuse, have caused this drug to be removed from the cyanide antidote kit in the U.S. Army formulary.

Because acute cyanide poisoning continues to constitute a threat for American soldiers in conventional or nonconventional conflicts, there is a need for the development of an effective anticyanide pretreatment. Bright (10) outlined the following criteria for the ideal anticyanide pretreatment: (a) self-administration by p.o. or transdermal routes, (b) long duration of action, (c) low acute and chronic toxicity, and (d) devoid of performance decrement. Additionally, our Institute has recommended that the treatment protect against $2 \times LD_{50}$ cyanide.

Compounds that form MetHb have been shown to be effective pretreatments for cyanide poisoning (31,53,70). *p*-Aminopropiophenone (PAPP) (Fig. 1), a very effective MetHb former (98), has been widely studied as an anticyanide drug (10,16,41,53,64), and PAPP does not produce cardiovascular effects seen with amyl nitrite use.

The Department of Defense's interest in PAPP dates back to World War II. During that time, two active anticyanide research programs were in effect. A U.S. Navy-sponsored program, "Protectives Against War Gases," investigated methemoglobinemia following administration of PAPP. This work was partially contracted out to Parke-Davis & Com-



1, R = NH_2
2, R = NHOH
3, R = NO_2

FIG. 1. Structure of PAPP and its metabolites.

pany (Detroit, MI, U.S.A.) and is summarized in an open literature publication (98). Data related to this project can be found at the Archives Center of the National Museum of American History, Washington, D.C. The U.S. Army's research program on PAPP was, in part, performed in collaboration with Eli Lilly & Company and also resulted in an open literature publication (76).

Recently, the Chemical and Biological Defense Establishment (CBDE) of the United Kingdom has shown active interest in the development of a cyanide pretreatment and it has completed many toxicologic, pharmacologic, pharmacokinetic, and pharmacodynamic studies on PAPP. PAPP was recently re-evaluated by this Institute as part of an active research program for the identification and development of an anticyanide pretreatment drug. This article will provide a historical summary of the current knowledge of PAPP as a cyanide pretreatment.

CHEMISTRY OF PAPP

Synthesis

PAPP was first synthesized in 1900 from acetanilide and propionyl chloride (58). More modern methods of chemical synthesis from aniline derivatives appear in the literature (45). High purity PAPP (melting point of 140°C) is available as a light yellow crystal commercially from Eastman Kodak (Rochester, NY, U.S.A.).

Analytical Methods

For material compound analysis, a potentiometric titration procedure with perchloric acid in acetic acid has been described (97). For analysis in biological fluids, Tepperman and Bodansky (94) demonstrated that PAPP could be determined in the blood and urine by a diazotization of the aromatic amine with nitrous acid and subsequent coupling with N-(1-naphthyl)ethylenediamine · 2HCl. PAPP has been measured in blood using a phosphorimetric method. Both PAPP and *p*-hydroxyaminopropiophenone (PHAPP), a metabolite of PAPP, are phosphorescent in alcohol with excitation/emission wavelengths of 332/449 (62) and 330/450 nm (63), respectively.

MECHANISM OF ACTION AND METABOLISM OF PAPP

Mechanism of Action

The antidotal mechanism of PAPP is thought to be through the formation of MetHb, which has a much higher affinity for cyanide than mitochondrial cytochrome oxidase (3,82), a primary site of action of cyanide (55,102). As cyanide is sequestered within erythrocytes, plasma concentrations and, therefore, intracellular levels of cyanide are reduced. The reduction in intracellular levels of cyanide by PAPP results in the reactivation of cytochrome oxidase and a return towards normal respiration (16,66). The control dissociation constant for the cytochrome–cyanide complex is 5×10^7 (89). These compounds may affect other similar iron-containing proteins such as myoglobin. In this case, myoglobin would be oxidized to metmyoglobin, which combines with cyanide to form cyanometmyoglobin (44). Although this reaction occurs under in vitro conditions (88), it is not clear if this occurs under in vivo conditions.

PAPP is not a direct oxidant of hemoglobin and requires biotransformation to an active metabolite. Attempts by Tepperman and Bodansky (94) to generate MetHb from PAPP in vitro in dogs were unsuccessful. In vitro studies, using liver microsomes, showed that the active metabolite of PAPP was the hydroxylamine derivative, PHAPP (Fig. 1) (42,47, 101).

Once formed, PHAPP is taken up by circulating erythrocytes where a redox cycle, known as the *kreisprozess* (57), takes place (Fig. 2). Various aspects of this scheme were elucidated by Kiese and coworkers using phenylhydroxylamine as a MetHb former. The initial reactions in the cycle involved the oxidation of phenylhydroxylamine to nitrosobenzene (36) with the simultaneous oxidation of heme Fe^{2+} to Fe^{3+}. Graffe et al. (47) isolated both PHAPP and *p*-nitrosopropiophenone (PNPP) from the blood of dogs treated with PAPP. This observation provides strong evidence that PAPP also produced MetHb by the *kreisprozess*. It should be noted that the metabolism of PAPP in rats, dogs, and cynomolgus monkeys differs (104). In rats, PAPP was metabolized by N-acetylation, whereas ring and aliphatic oxidation occurred in dogs. In monkeys, both N-acetylation and oxidation took place. These metabolic differences (56,67) may explain some of the species differences observed when PAPP and related compounds are employed as cyanide antidotes.

The cyclic redox reaction is dependent on active metabolism within the erythrocyte. Intraerythrocytic NADPH, generated from glucose-6-phosphate dehydrogenase, participates in the reduction of PNPP back to PHAPP, which again can oxidize a heme portion of the molecule to MetHb (42). The requirement for reducing equivalents generated from glucose-6-phosphate dehydrogenase showed that the *kreisprozess* did not take place in erythrocytes deficient in this enzyme (99). Further experiments using lysed erythrocytes, which cannot carry out MetHb reduction, showed that the addition of PHAPP resulted only in the stoichiometric conversion of hemoglobin to MetHb without any amplification (37).

Experimental data also indicate that the *kreisprozess* takes place in vivo. Early experiments (94) suggested that a "turnover" reaction was responsible for the large amounts of MetHb formed from a relatively small dose of PAPP. One mole of PAPP can generate 132 mol of MetHb at 75% MetHb and 196 mol at 35% MetHb (94). Additional evidence of recycling becomes apparent when the amount of MetHb formed after the administration of PAPP is compared to that formed by nitrite. Nitrite, unlike PAPP, forms stoichiometric amounts of MetHb directly, without metabolism to an active intermediate. Data provided by Saunders and Heisey (77) showed in cats that PAPP (0.5 mg/kg, 3.4 μmol/kg) or $NaNO_2$ (10 mg/kg, 145 μmol/kg) produced MetHb levels of 1.5 and 1.8%, respectively, after 5 min. (These levels of MetHb should be considered biologically insignificant although larger doses were used in the study.) The important quantitative comparison suggests that, on a molar basis, 43 times less PAPP was needed to generate approximately the same degree of methemoglobinemia. Another interesting observation was that 1 g of MetHb requires 2.9 mg of cyanide for complete formation of cyanoMetHb. More recent studies in sheep also compared the effectiveness of PAPP and nitrite in generating MetHb (19). PAPP, at a dose of 3 mg/kg (20 μmol/kg), or $NaNO_2$, at a dose of 22 mg/kg (318 μmol/kg), produced 36% MetHb after 60 min. Again, on a molar basis, 15 times less PAPP was needed to generate equivalent MetHb levels. These in vivo studies provide

indirect evidence that redox recycling is taking place within erythrocytes. Metabolic products of PAPP act to increase the rate of Hb to MetHb in contrast to the MetHb reductase system, which could decrease the complexation of cyanide.

Structure–Activity Studies

A limited number of studies have been published that examined the ability of different isomers and structural analogues of aminophenones to form MetHb (12,90). Downey (42) found that the *p*-amino derivative was biologically active while *ortho* or *meta* isomers were not. He also showed that aminophenones of different acyl chain lengths (C_2 to C_5) also influenced the formation of MetHb (Fig. 2). With the exception of *p*-aminoacetophenone, the *p*-aminopropio-, butyro-, valeryl-, hexanoyl-, and octanoylphenones all produced significant amounts of MetHb. Furthermore, as the acyl chain length of an aminophenone is increased, the half life of MetHb remaining in the blood also increased. This analysis agrees with the observation that in dogs the half life of *p*-aminooctanoylphenone (PAOP) is extended to approximately 8.5 h from 2.5 h as seen with PAPP (10). An increased half life for formation of MetHb by a compound suggests that the compound will also have an increased half life for protection against cyanide. This pharmacological property could make PAOP a good candidate as prophylactic therapy against cyanide intoxication (80).

The length of the acyl chain also influences the acute toxicity of the aminophenones. Using the data provided by Lanphier et al. (60), the relationship between the lethality in mice and the dose of the aminophenone with acyl chain lengths of C_3 to C_8 were generated. As shown in Fig. 3, the slope of the dose–response relationship for *p*-aminoheptanoylphenone is more shallow than for the other aminophenones examined, suggesting that *p*-aminoheptanoylphenone should be a potentially safer compound as an anticyanide pretreatment than other aminophenones.

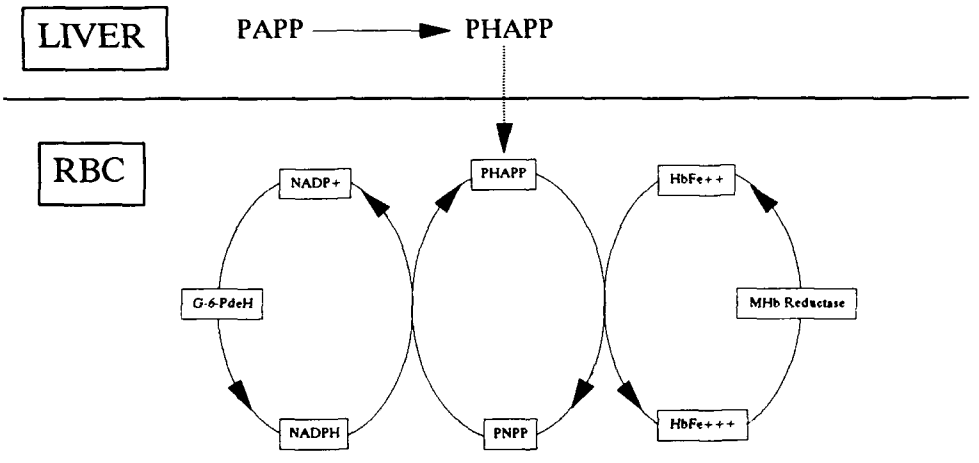


FIG. 2. The Kreisprozess. Intraerythrocytic recycling of PHAPP and *p*-nitrosopropiophenone (PNPP) to bring about the simultaneous oxidation of heme Fe^{2+} to Fe^{3+}. Reaction is dependent upon glucose-6-phosphate dehydrogenase (G-6-PdeH) for generation of reducing equivalents necessary to convert PNPP to PHAPP.

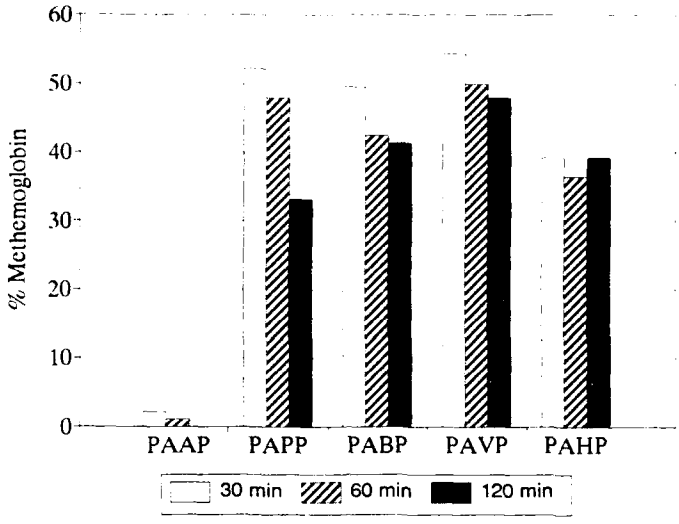


FIG. 3. Effect of acyl chain length on MetHb formation. MetHb formation was measured 30, 60, and 120 min following s.c. administration of PAAP (*p*-aminoacetophenone), PAPP, PABP (*p*-aminobutyrophenone), PAVP (*p*-aminovalerylphenone), and PAHP (*p*-aminohexanoylphenone) at equimolar doses of 27.2, 30, 32.8, 35.6, and 38.4 mg/kg, respectively (42).

PHARMACODYNAMICS OF PAPP

General

PAPP has been shown to be an effective prophylactic treatment for cyanide intoxication in a variety of animal species. Protection from cyanide does not only include death but convulsions as well. On the other hand, the convulsive syndrome of oxygen poisoning was not influenced by PAPP-produced MetHb (71). In addition to its anticyanide action, PAPP will also protect against sulfide toxicity in armadillos, rabbits, and mice (86), azide (1) and cyanogen chloride toxicity (53), and ionizing radiation (37,42,92).

Efficacy in Rodents

The prophylactic efficacy of PAPP was evaluated in mice pretreated i.p. with PAPP at doses of 9.4, 38, or 150 mg/kg at 15 or 60 min before an i.m. challenge with a 2 × LD_{50} dose (5.0–5.6 mg/kg) of NaCN (80). The 24-h mortality was assessed as summarized in Table 1. Pretreatment with PAPP, at doses of 38 or 150 mg/kg, significantly increased survival compared to untreated controls (Fig. 4). The surviving animals were also evaluated for motor incapacitation using the inverted screen test. For the 15-min pretreatment time, none of the surviving mice showed any motor decrement, but for the 60-min pretreatment time, the motor incapacitation observed was not considered dose related. The dose group showing the least amount of performance decrement was the 150 mg/kg dose compared to the 38 mg/kg dose group. Results of a study that compared the motor activities of PAPP and PAOP (15–90 mg/kg) noted that PAPP produced dose-dependent hypoactivity, while PAOP produced dose-dependent hyperactivity (75). This observation suggests that behavioral responses may not be equivalent for all members of this group of compounds.

TABLE 1. *Efficacy of PAPP against 2 × LD_{50} of NaCN in mice*[a]

PAPP (mg/kg)	24-hour survival		Performance	
	15 min	60 min	15 min	60 min
Pos. control[b]	—[c]	10/10	—	8/10
0.0	1/10	0/10	1/1	—
9.38	9/10	0/10	9/9	—
37.5	10/10	9/10	10/10	5/9
150.0	9/10	10/10	9/9	9/10

[a] Animals were pretreated at either 15 or 60 min before NaCN challenge.
[b] The positive control: coadministration of $NaNO_2$ and $Na_2S_2O_3$ at doses of 1,000 and 100 mg/kg, respectively, 60 min before challenge with NaCN.
[c] Means not determined.

Efficacy in Sheep

Burrows (19) studied the efficacy of both PAPP and sodium thiosulfate against oral cyanide intoxication in sheep. In these studies, the maximum allowable time delay for antidote administration was determined. When challenged orally with 2 × LD_{50} of NaCN (7.6 mg/kg), PAPP at a dose of 1 mg/kg was effective when administered 30 min after NaCN challenge. At a higher dose of cyanide (4 × LD_{50}), PAPP (1.5 mg/kg) and sodium thiosulfate (660 mg/kg) were also effective, but treatment must occur within 20 min of the NaCN challenge.

Efficacy in Dogs

Rose et al. (76) investigated the efficacy of PAPP for treatment of cyanide-intoxicated dogs. In these studies, the i.v. LD_{50} for PAPP was found to be 7.15 mg/kg, with LD_1 and

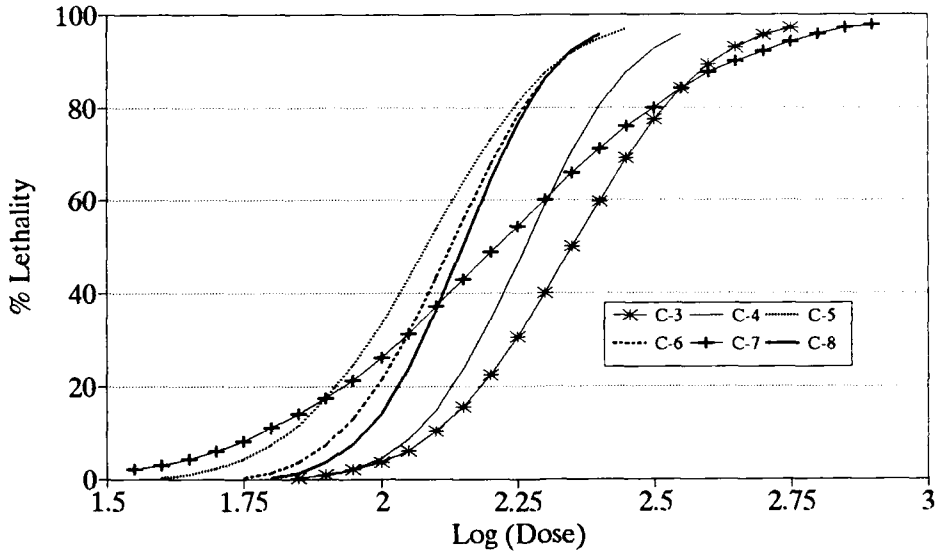


FIG. 4. Effect of acyl chain length on percent lethality. Using published (60) values for LD_{50} and the slope of the probit regression curve, the percent lethality at different doses of phenone was calculated.

LD_{99} values of 2.56 and 19.91 mg/kg, respectively. A dose of PAPP equal to one-tenth of the LD_{50} (0.71 mg/kg) was shown to be optimal; higher doses of PAPP lessened the chances of recovery following cyanide intoxication. For untreated animals, the LD_{50} for NaCN was 5.36 mg/kg, while PAPP, at the 0.71 mg/kg dose, increased the LD_{50} for NaCN to 23.4 mg/kg, a 4.4-fold increase. The efficacy of PAPP could be potentiated significantly with the addition of sodium thiosulfate to the treatment regimen. The coadministration of both PAPP and sodium thiosulfate increased the LD_{50} for NaCN to 105.6 mg/kg, a protective ratio of almost 20.

Other efficacy studies in dogs have been carried out by the CBDE. After treatment with PAPP (1.0 mg/kg p.o.), MetHb levels increased to approximately 8% after 60 min, with a peak of 15.8% after 120 min; i.v. administration of PAPP at a dose of 0.5 mg/kg resulted in a peak MetHb level of 20.6% after 60 min (13). As a prophylactic drug, PAPP, when injected i.v. at a dose of 0.5 mg/kg, protected dogs against supralethal doses (up to 9 mg/kg) of cyanide (16). With the administration of cyanide to dogs in the presence of PAPP, MetHb levels fall very rapidly after the start of the infusion. The decrease in MetHb (apparent conversion to cyanoMetHb) appears to correlate with protection against cyanide by PAPP. The whole blood and plasma levels indicate that most of the cyanide is found in the red blood cells (16).

PHARMACOKINETICS OF PAPP

Guinea Pig Data

Preliminary studies in guinea pigs suggested that subcutaneous or oral administration of PAPP was not effective in producing methemoglobinemia although it was successful in dogs (41). This species difference may reflect metabolic differences, such as the ability to form the N-hydroxy metabolite. Studies in the guinea pig that show the effect of PAPP on cyanide toxicity should be performed. They may shed additional light on the precise role of MetHb for the prevention of cyanide intoxication.

Rabbit Data

Liu and Huang (62,63) compared the pharmacokinetic and pharmacodynamic properties of PAPP and PHAPP in rabbits. A two-compartment open model was used for the modeling of PAPP, whereas one-compartment calculations were performed for PHAPP. After administration of PAPP to rabbits, there was a lag between the peak plasma PAPP levels and the formation of MetHb. Additional experiments showed that administration of PHAPP resulted in higher blood MetHb levels, which peaked faster. The production of MetHb after administration of PAPP was both slower and less when compared to PHAPP. These results were confirmed in studies conducted by Bright et al. (11), who, in unpublished studies, showed that PHAPP, when compared to PAPP, had a faster onset of action and, on an equimolar basis, initiated greater MetHb formation.

Dog Data

A pharmacokinetic profile for PAPP was determined in dogs using a two-compartment model (65). The kinetic data derived from the MetHb profiles were fitted to a nonlinear

least-squares analysis regression program. Dogs, after i.v. treatment with 0.2 to 0.5 mg/kg of PAPP, had peak concentrations of MetHb within 60 min. Profiles were analyzed and the rate constants determined. From the theoretically derived calculations, k_e (a rate constant for elimination of PAPP) was found to be 0.012 min^{-1}, k_1 (a model rate constant) varied between 0.018 and 0.028 mg/kg/min, although k_e ranged from 0.018 to 0.033 min^{-1}. A very good fit was obtained by comparing their model to the experimental data. Since dog and human are very similar with respect to MetHb reductase, this kinetic model would likely to valid in humans as well (15).

Oral dosing of PAPP was studied by using a kinetic model previously developed to describe methemoglobinemia following the i.v. route (14). For certain parameters [i.e., F ($AUC_{p.o.}/AUC_{i.v.} \times 1/dose_{p.o.}$), k_a (rate constant of absorbed PAPP being transformed to a MetHb-forming metabolite of PAPP), and TD (time delay factor)], this model may provide a means to analyze the data kinetically (17). This kinetic model (ISIS) suggests that PAPP was being metabolized at first pass to a nonactive metabolite.

Unlike PAPP, oral dosing of female dogs with PAOP has shown complex pharmacokinetics, making multiple administration of the drug difficult to validate (10). The mechanism of this difference is unclear but it may be due to drug distribution changes because of the greater lipophilicity of PAOP.

Rat Data

Changes in pharmacological parameters in well-controlled rat studies appear to influence responses to these compounds (79). Factors such as stomach acidity, gastrointestinal transit time, and binding of the aminophenones to lipids or proteins need to be appraised. If the longer-chain, more lipophilic aminophenones are going to be employed, these same factors warrant greater consideration.

TOXICOLOGY OF PAPP

Genotoxicity and Mutagenicity Tests

The CBDE completed a comprehensive set of mutagenicity and genotoxicity tests on PAPP. Thorough evaluation of PAPP was warranted because early studies (81) indicated that chronic administration (4 mg/day, 18 months) of PAPP to rats resulted in an increased incidence of tumors (not defined) and carcinomas (impurities of the chemical may contribute to the toxicity). Furthermore, other phenol derivatives [i.e., 4-dimethylaminophenol (DMAP)] of the aminophenones have been shown to be mutagenic (61) and cause muscle necrosis at the injection site and also renal damage (93). The standard battery of tests included the Ames test, micronucleus test, metaphase analysis, forward gene mutation, and unscheduled DNA synthesis.

The mutagenic properties of PAPP were assessed in the Ames test using histidine-dependent auxotrophic mutants of *Salmonella typhimurium* (strains TA 1535, TA 1537, TA 1538, TA 98, TA 100, and TA 1537R+) and incremental concentrations of PAPP (27). Parallel testing in the presence and absence of Aroclor-induced rat liver microsome fraction (S-9) showed that PAPP was not mutagenic. Concentrations examined were consistent with, or above, that which would be administered under in vivo conditions.

Both crude (23) and purified (24) preparations of PAPP were evaluated for toxicity

using the mouse micronucleus test. Whereas the crude preparation yielded negative results, the purified PAPP showed slightly higher micronucleus frequencies in three of the test groups. These values, however, were within either the range of the negative controls or the range of historical controls. This report concluded that PAPP did not induce the formation of micronuclei.

Metaphase analysis of human lymphocytes treated with PAPP (125, 250, 500, or 1000 μg/ml) in the absence and presence of S-9 fraction (25) was performed. Without the S-9 fraction, PAPP gave no significant increases in chromosomal aberrations. In the presence of S-9 fraction, statistically significant chromosomal aberrations were seen. The report concluded that PAPP was not clastogenic in the absence of S-9, but it may be a potential clastogen in the presence of S-9.

The forward gene mutation assay was carried out using L5178Y mouse lymphoma cells treated with PAPP (1.6 to 1,000 μg/ml) with and without S-9 fraction (26). In the absence of the S-9 fraction, PAPP was not mutagenic. However, in the presence of S-9, there were significant increases in both the number of wells with mutant colonies and the mutation frequency. PAPP was therefore shown to be a mutagen in the presence of the S-9 fraction.

The test for unscheduled DNA synthesis in rat liver was performed by the Chemical & Biological Defence Establishment (28). Rats were challenged orally with 25, 100, 250, and 400 mg/kg (80% of LD_{50}). Although deaths occurred in the high-dose groups, which reduced the value of the study, there was no evidence of unscheduled DNA synthesis induced by PAPP.

Acute Toxicity of PAPP

The onset of symptoms following acute PAPP intoxication (Table 2) in mice was very rapid, with convulsions or prostration, cyanosis, and salivation occurring within seconds after i.v. administration (79,91).

Studies by both Vandenbelt et al. (98) and Bodansky and Gutmann (8) correlated the degree of methemoglobinemia produced by PAPP and symptoms. Their findings showed that dogs, treated orally with PAPP, exhibited ataxia at 60% MetHb, prostration and salivation at 75% MetHb, unconsciousness at 85% MetHb, and death at between 87 and 95% MetHb.

The acute toxicity of PAPP in laboratory rodents, dogs, and various species of wildlife is summarized in Table 2. As shown, there are marked differences in the PAPP LD_{50} values, which varied with strain, species (43,78), sex (18), and route of administration (79). These variations in the LD_{50} may be explained by differences in the rate of microsomal metabolism of PAPP to the active compound, PHAPP, and/or the MetHb reductase activity (89). Stolk (89) summarized data from several different investigators in MetHb reductase activities. Reductase activities, normalized relative to human erythrocytes, can be rank ordered from highest to lowest as follows: mouse > rat > (dog ≈ cat), although other rankings are noted (2). The "Smith" rankings are in general agreement with the rank order based on oral LD_{50} values: mouse > rat > dog > cat.

By the oral route, PAPP appears to be most toxic to dogs, cats, bobcats, kit foxes, and coyotes, all of which had LD_{50} values less than 50 mg/kg. Most of the species studied had LD_{50} values in the range of 100 to 500 mg/kg. Both female guinea pigs and female mice appear to have the highest oral tolerance for PAPP, with LD_{50} values of 1,020 and >5,000 mg/kg, respectively.

TABLE 2. *LD_{50} values for PAPP in different species*

Species, sex[a]	Route	LD_{50} (mg/kg)[b]	Reference
Dog, female	i.v.	7.15 (5.22–9.77)	77
Mouse	i.p.	223 (ND)	61
Dog	p.o.	30–50[c]	99
Rat, male	i.p.	55 (ND)	99
Rat (Holtzman), male	i.p.	237 (ND)	44
Rat (Charles River), male	i.p.	85 (ND)	44
Mouse, female, male	i.p.	86 (ND)	46
Coyote	p.o.	5.6 (3.0–10.4)	79
Striped skunk	p.o.	>400	79
Racoon	p.o.	142 (ND)	79
Cat	p.o.	5.6 (3.5–8.9)	79
Bobcat	p.o.	10 (2–20)	79
Kit fox	p.o.	14.1 (ND)	79
Rat, male	p.o.	177 (119–262)	79
Mouse, male	p.o.	233 (186–292)	79
Coturnix quail	p.o.	>316	79
Starling	p.o.	>316	79
Red-winged blackbird	p.o.	133 (ND)	79
Black-billed magpie	p.o.	178 (100–316)	79
Mouse, male	i.v.	145 (82–217)	80
Mouse, female	i.v.	200 (175–310)	80
Mouse, female	p.o.	>5,000	80
Guinea pig, female	p.o.	1,020 (760–1,520)	80
Rat, male	p.o.	475 (89–2,525)	80
Rat, female	p.o.	224 (169–308)	80

[a] The sex of animals is indicated; otherwise, it was unknown.
[b] Values in parentheses represent the 95% confidence intervals. ND = not determined.
[c] Estimated value calculated from data presented in Vandenbelt et al. (98).

Intravenous administration of PAPP was, in all cases, more toxic than the oral route. For dogs, the oral LD_{50} was approximately four to six times greater than the i.v. LD_{50}. The greatest difference was seen with female mice, where there was a 25-fold increase in the oral LD_{50}. Male mice, although the data were compiled from two different laboratories, show a 1.6-fold difference in the LD_{50} values for oral vs. i.v. routes of administration.

Subacute Oral Toxicity Studies

Subacute oral toxicity data in rats (20) and monkeys (21,22) were collected as unpublished studies. Both studies had similar experimental designs, with a 14-day treatment period followed by a 14-day treatment-free period. In both studies, standard hematology (discussed in the section entitled "Hematological Effects of PAPP in Animals"), clinical chemistry, urine analysis, and pathology were evaluated.

The rat [Crl:CD(SD)BR strain] study consisted of four treatment groups, which were dosed daily with 0, 35, 90, or 140 mg/kg for males and 0, 20, 50, or 130 mg/kg for females. Because of different doses used for the two sexes, it is difficult to compare the results with regard to sex. Pertinent histopathological analysis of the spleens revealed a dose-related increase in erythroid hyperplasia, sinusoidal enlargement, erythrophagocytosis, and pigment deposition. Pigment was also evident in the Kupffer cells of the liver

and in the renal proximal tubular epithelial cells of rats in the highest dose group. The pigment, presumably hemosiderin, was still present in the liver, kidney, and spleen of rats in the highest-dose groups at the end of the treatment-free period. The hyperplasia and enlargement, however, had returned to control levels.

The subacute toxicity was studied in cynomolgus monkeys (both sexes) dosed daily with 17, 50, or 150 mg/kg of PAPP. Serum chemistry parameters of the treated animals showed increased LDH (lactate dehydrogenase) levels for the middle-dose group and elevated LDH and GPT (glutamic pyruvic dehydrogenase) levels for the highest-dose group after 4 days of treatment. After 10 days, bilirubin levels were increased in all of the treatment groups, while LDH was elevated in the 50 and 150 mg/kg dose groups. Female animals showed elevated GOT (glutamic–oxaloacetic transaminase) and GPT in the highest dose group. After 28 days, the abnormal serum chemistry values had returned to control levels. In summary, both of these reports concluded that the pathological and histopathological effects seen with PAPP treatment were to be expected from high MetHb concentrations.

Human Studies

There have been clinical studies using PAPP as a potential therapeutic substance. Paulet et al. (69) studied PAPP-induced methemoglobinemia in 51 human volunteer subjects. PAPP, at doses of 50, 80, or 100 mg, was dissolved in water containing 500 mg of lactose and administered orally to the volunteers. After absorption of a 100-mg dose, the maximum blood MetHb levels were 22 ± 14%, with a range of 2 to 48%. After absorption of the 80-mg dose, the maximum amount of MetHb was 13.1 ± 9%, with a range of 0 to 43%. In one volunteer, a dose of 50 mg resulted in a MetHb level of 7%. The maximum MetHb level achieved was highly variable from one subject to another. The concentration of oxyhemoglobin (105), as well as other influences, may affect this variability. Since PAPP was administered as a fixed dose, this variation may be a result of differences in body weights and the contents of the gastric compartments. The maximum MetHb level is usually reached between 1 and 2 h after dosing, with a duration of approximately 4 h.

Clinical signs associated with PAPP-induced methemoglobinemia were also noted by Paulet et al. (69). MetHb levels between approximately 30 and 48% (maximum observed) did not appear to have any adverse effects. Other than bluish lips, no physical, intellectual, or psychological problems were observed. In all subjects, appetite was good and there were no renal problems (urine flow and diuresis were normal). There were no changes in ventilation rate, arterial pressure, or electrocardiogram in 20 subjects, while 2 showed slight changes in AP, AQRS, and AT axes. At very high doses, PAPP was mildly hemolyzing; however, at doses that produced 10 to 30% MetHb, hemolysis was not observed. Cardiac output studied in dogs under light anesthesia showed that MetHb at 58 to 78% caused no significant changes in cardiac output or pulmonary blood gas.

The individual sensitivity to PAPP-induced methemoglobinemia varies substantially. One subject was resistant to MetHb formation with very little MetHb formed after two doses of PAPP (80 and 100 mg). Resistance was confirmed in vitro, where no MetHb was formed after the addition of nitrite to the blood.

Whether such differences are due to alterations in N-hydroxylation, MetHb reductase

activity (90), or some other enzyme deficiency is not clearly understood. The frequency of polymorphism for MetHb reductase in Caucasians is rare and 15 per 20,000 in Eskimos (83). The frequency of occurrence of the glucose-6-phosphate dehydrogenase allele is up to 0.25 (73). By comparison, aminoquinolines (e.g., primaquine), which have been proposed as MetHb-producing cyanide prophylactics (80), may cause acute hemolysis in approximately 10% of black Americans (5,51). Further studies remain to be performed to characterize the influence of race on the capability of the aminophenones to protect against cyanide.

Hematological Effects of PAPP in Animals

Several studies specifically addressed the hematological effects resulting from the administration of PAPP (84,85). Although there was some species variation, the primary hematological effects observed included the appearance of Heinz bodies and hemolytic anemia. Beutler and Mikus (7) studied the subacute effects of PAPP in rats on erythrocyte survival and hematocrit. In their studies, erythrocyte survival using ^{51}Cr-labeled cells and hematocrit was followed in rats treated with PAPP or sodium nitrite. Even though both of these compounds produced essentially the same degree of methemoglobinemia, a loss of erythrocytes was observed only in the PAPP-treated group. Other experiments at a higher dose of PAPP (20 mg/kg) showed an accelerated loss of erythrocytes that was accompanied by substantial hemolytic anemia.

Studies by Scawin et al. (79) also focused on hematological changes following sublethal dosing with PAPP in rodents. In mice and rats, but not guinea pigs, there was an increase in Heinz bodies, which occurred more rapidly and to a greater extent in mice than in rats. Analysis of blood from mice showed a peak Heinz body formation of 85% at 3 days, while rats had approximately 40% Heinz body formation after 7 days. Rats showed significant anemia following sublethal dosing with PAPP, with hematocrits 70% lower than predosing, control values. Although PAPP at 5 mg/kg has been shown to cause hemolysis in rats and Heinz body formation in mice and rats, these toxicities were not observed in guinea pigs (92) or humans (7).

To summarize the rodent studies (7,79,92), pertinent hematological changes include a dose-related decrease in hematocrit and increased mean cell volume, hemoglobin, and white blood cells. During the treatment-free period, there was improvement, but not complete recovery, in the hematological parameters. After 4 weeks, recovery appeared to be complete. Pathological examination revealed enlarged spleens in the majority of animals killed at the end of the treatment period. Hematological findings after 10 days of treatment were decreased hematocrit, hemoglobin levels, and red blood cell count and increased platelet counts in the treated animals. Increased white blood cell counts were evident only in the treated males. After 28 days, hematological parameters had returned to normal control values. Heinz bodies were observed in all of the treated animals, with the highest incidence in the middle-dose group. Similar to the subacute rat studies, the monkeys also showed hemolytic anemia and pigment deposition in the liver and spleen (20).

The relationship between methemoglobinemia and the appearance of Heinz bodies and anemia is not fully understood. In general, the formation of methemoglobin is independent of the hemolytic action of chemicals (34) or Heinz body formation (49). The ob-

served differences in the hemolytic effects of nitrite or PAPP would support this generalization (7). At present, the mechanism by which PAPP produces hemolysis is unknown (85). Other chemicals (i.e., acetanilide) are known to produce methemoglobinemia as well as hemolysis (dogs) (100), but the mechanism is not thought to be due to bone marrow suppression but is common with aminophenyl compounds.

Performance Decrement with Methemoglobinemia

PAPP may be used in a battlefield setting and serious, drug-induced performance decrements, which would place the soldiers at risk, must be minimized or eliminated. Since increased MetHb reduces the capacity of the blood to carry oxygen, a decrease in performance might be expected. Freedman (46) noted an increase in the response time to a conditioned reflex following the administration (s.c.) of PAPP (5–7 mg/kg) to dogs. Human studies (95,96) showed that in four untrained volunteers, working on a bicycle ergometer had little or no apparent effect on oxygenation of working muscle in the presence of 7.5 to 15% MetHb. Two other volunteers, with MetHb levels of 21.7 and 27.1%, did show an increase in blood lactate levels during exercise. This deficiency is particularly obvious at higher degrees of oxygen utilization as measured by the visual rod threshold immediately after exercise (9,96).

The effect of PAPP on swimming performance in guinea pigs was examined by D'Mello and colleagues (39,41). Nonlethal doses of subcutaneous NaCN produced performance decrements within 2 min. At the highest nonlethal dose employed (4 mg/kg), performance did not return to control levels until 64–128 min. PAPP protected the animals against cyanide-induced changes in swimming performance. Recent studies using exercising sheep found that, following treatment with PAPP, the VO_2 (maximal oxygen consumption) was reduced in a dose-dependent manner as the MetHb concentration increased (48).

CONCLUSIONS

PAPP is effective as a pretreatment antidote to cyanide when used by single-dose administration. Further experiments need to be performed to establish a lack of toxicity of PAPP in multiple-dose experiments. Possible chronic hematological and genotoxic effects of PAPP may limit its development in repeated chronic treatment. Combination therapy of a prophylactic sulfur donor with a low dose of a safer prophylactic aminophenone (e.g., PAOP or *p*-aminoheptanoylphenone or their N-hydroxy metabolites) may provide more efficacious protection against cyanide than PAPP. This combination could allow for a reduction in the dosage of MetHb formers that would reduce the chance of unwanted side effects of the employed therapeutic compounds.

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