J. Pharm. Pharmacol. 1997, 49: 282–287
Received August 2, 1996
Accepted October 8, 1996

Pharmacokinetics and Kinetic–Dynamic Modelling of Aminophenones as Methaemoglobin Formers

MARK T. MARINO, MICHAEL R. URQUHART, MICHELLE L. SPERRY, JURGEN VON BREDOW, LARRY D. BROWN, EMIL LIN* AND THOMAS G. BREWER

*Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. and *Department of Pharmacy, School of Pharmacy, University of California, San Francisco, USA*

Abstract

Methaemoglobin, the oxidized form of haemoglobin, can be formed by a variety of agents, most of which act to oxidize haemoglobin directly or indirectly. Cyanide has a higher affinity for methaemoglobin than for mitochondrial cytochromes, making methaemoglobin formation a basis for the treatment of cyanide poisoning. We used the beagle dog model to investigate the relationship between drug concentration and methaemoglobin levels for two candidate anti-cyanide compounds. The compounds studied were the aminophenones *p*-aminopropiophenone (PAPP) and *p*-aminoheptylphenone (PAHP). Both PAPP and PAHP were given as intravenous boluses and as two different oral formulations.

The kinetics of both compounds appeared to follow a three-compartment open model for intravenous bolus administration and a two-compartment open model for oral administration. The first distribution phase seen with the intravenous administration was obscured by the absorption phase during oral administration. Bioavailability for all formulations varied between 20 and 47%. For both compounds there was a delay between the appearance of drug in the plasma and the appearance of methaemoglobin (counter-clockwise hysteresis) which is suggestive of an active metabolite causing methaemoglobin formation. The pharmacodynamics were fit with an effect-compartment kinetic-dynamic model linked to a sigmoid E_{max} pharmacodynamic model. Maximum amounts of methaemoglobin occurred between 2 and 4 h for PAHP and between 1 and 3 h for PAPP. When administered intravenously estimates of EC50 were lower than the estimates of EC50 from oral administration for both compounds. This might be because of oral first-pass inactivation or a 'first-pass' activation through the lungs contributing to the formation of an active metabolite. The phenones as a class appear to have the drug cleared and methaemoglobin return to near baseline within 12 h.

Both compounds seem to produce sufficient methaemoglobin to treat acute cyanide poisoning and to serve as prophylactic agents against acute cyanide poisoning in a military setting.

Cyanide is a rapidly acting poison that reversibly binds to cytochrome aa_3 of the mammalian electron transport chain blocking ATP production (Ballantyne 1987). Cyanide exposure can occur acutely as in workplace exposure or as chronic exposure in tropical ataxic neuropathy (Wilson 1987). A potential use of cyanide is as a chemical warfare weapon or as a terrorist agent. The current medical therapy for acute cyanide poisoning in the USA is the administration of intravenous sodium nitrite then intravenous sodium thiosulphate (Klaassen 1996). Sodium nitrite forms methaemoglobin, the oxidized form of haemoglobin, which binds cyanide. Cyanide has a higher affinity for methaemoglobin than for cytochrome oxidase and methaemoglobin acts as a 'cyanide sink' (Albaum et al 1946). Sodium thiosulphate is given as a sulphur donor to form thiocyanate which is eliminated renally (Schultz 1984). Sodium nitrite administration (10 mL of a 3% solution) produces peak methaemoglobin levels of $10{\cdot}5 \pm 2\%$ occurring at 50 min (range 35–70 min) (Kirk et al. 1993). Many other compounds are useful in treating cyanide toxicity; the majority act via production of methaemoglobin (Marrs 1987). The drug dimethylaminophenol is used in several other countries for the treatment of acute cyanide poisoning (Weger 1990).

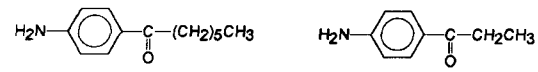
p-Aminopropiophenone (PAPP) has been extensively tested in man to determine the time-course and extent of methaemoglobin formation (Paulett et al 1963). Oral doses of 100 mg kg^{-1} PAPP produced methaemoglobin levels up to 48% without overt clinical toxicity (Paulett et al 1963). PAPP had been studied previously to determine the effect of methaemoglobin on visual performance and exercise in man (Bodansky & Hendley 1946). The effect of methaemoglobin induced by sodium nitrite and PAPP on red cell survival has also been performed in man (Beulter & Mikus 1961). Beulter's study also examined the effect of chronic administration of PAPP at 4-h intervals designed to produce steady-state methaemoglobin levels (Beulter & Mikus 1961). None of the studies looked at simultaneous levels of drug and methaemoglobin in order to optimize the dosing regimen to produce safe and effective levels of methaemoglobin.

The aminophenones are drugs which have been shown to be effective in animal models of cyanide poisoning when given both before and after cyanide exposure (Baskin & Fricke 1992). This is probably because of their ability to produce methaemoglobin rapidly and sustain levels for several hours (Bright & Marrs 1982). The rapidity and extent of methaemoglobin formation should be critical in deciding which drug to use in treating an acute overdose of cyanide and in deciding which dosing schedule to use in order to prevent drug-induced toxicity. This is especially important in the use of these compounds as prophylactic agents for military use.

Although sodium nitrite is the standard of care in the USA it needs to be administered intravenously. Compounds that can

Correspondence: M. T. Marino, Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, USA.

be administered orally could be advantageous for prophylaxis or when intravenous access cannot be obtained and the patient is conscious. In order to estimate the period of effectiveness of these compounds a pharmacokinetic–pharmacodynamic study was undertaken. This study looked at the pharmacokinetics of these drugs, their dynamics (methaemoglobin levels) and the relationship between the two.



PAHP PAPP

FIG. 1. The chemical structures of *p*-aminopropiophenone (PAPP) and *p*-aminoheptylphenone (PAHP).

Methods and Materials

Two aminophenone compounds were tested, *p*-aminopropiophenone (PAPP) and *p*-aminoheptylphenone (PAHP) (Fig. 1). The phenones were obtained from Pharm-Eco labs (Simi Valley, CA). Poly(ethylene glycol) (PEG 200) and 2% carmellose were obtained from Sigma (St Louis, MO). All other chemicals used were commercially obtained and of HPLC grade.

Formulations

The PEG 200 solutions for the PAHP experiments were prepared daily before dosing the animals. All other formulations, which included the PEG 200 solution of PAPP and 2% carmellose suspension for both PAHP and PAPP, were prepared at the University of Iowa, Department of Pharmacy and shipped overnight before each day's experiment. The PEG 200 solutions were used for intravenous administration. The PAPP suspension and solution contained 1 mg mL^{-1} 2% carmellose and PEG 200, respectively. The PAHP suspension and solution contained 10 mg mL^{-1} 2% carmellose and PEG 200, respectively.

Animals

Male beagle dogs, 9–16 kg, between 6 and 37 months old were obtained from Hazelton Research Products (VA). They were housed in 4 × 10 foot runs; the temperature was 22 ± 2°C and the humidity 50 ± 20%. Animals were given measured amounts of Purina dog chow and had free access to water. All animals were examined by members of the supporting veterinary staff before their use in the protocols. Animal care and husbandry for all studies described herein conformed to the standards outlined in the Guide for the Care and Use of Laboratory Animals, DHHS Publication (NIH) No. 86–23. All studies were humanely conducted survival experiments. Male beagle dogs were selected for several reasons. Beagle dogs and man seem to have similar drug-elicited methaemoglobin responses (Stolk & Smith 1966). Beagle dogs also enable easier dosing and obtaining of multiple samples.

Dosing and blood analysis

The doses were 0·4 mg kg^{-1} PAPP and 6 mg kg^{-1} PAHP. Oral doses were given by gavage. Intravenous doses were given over 10 min via the cephalic vein. Doses were selected to produce at least 10% methaemoglobin at peak response. This was based on previously published work with PAPP (Bright et al 1987) and small pilot studies performed at the Walter Reed Army Institute of Research. All animals were fasted overnight before each study, with free access to water, and were allowed regular meals 4 h after dosing. Blood samples (3–5 mL) for measurement of drug levels and methaemoglobin were taken from the cephalic vein opposite the injection site, or from a saphenous vein, with a heparinized syringe (0·1 mL of 10 000 units bovine lung heparin per 5 mL blood). Methaemoglobin levels were measured within 30 min of blood sampling with the sample kept on wet ice. The remaining blood sample was centrifuged and the plasma separated and frozen at −30°C until drug analysis could be performed.

Drug analysis

Drug analysis for the two phenones was performed by high-performance liquid chromatography (HPLC). Two closely related methods were employed, one for each compound. Dog plasma samples (0·5 mL) were analysed for PAPP using an Beckman ODS 5 μm, 25 cm × 4·6 mm column, WISP model 710B injector, LC-600 Shimadzu pump and a Kratos Spectroflow 773 UV detector set at 316 nm. The mobile phase was 1:4, v/v, acetonitrile–water containing 0·15% H_3PO4. Samples were extracted from plasma treated with 50 μL 1 M NaOH into 3 mL of methyl *t*-butyl ether; the organic layer was separated, evaporated and redissolved in mobile phase before separation by HPLC. The peak-height ratio of PAPP/internal standard (3-[*o*-methoxyphenoxy]-1,2-propanediol) was calculated for each sample from the measured peak heights obtained by HPLC. The retention time of PAPP was 10·7 min, that of internal standard 8·5 min. The 4·04–404 ng mL^{-1} standard curve was calculated by y^{-1} weighted least squares regression. The linearity of the curve was at least 0·99 (r^2) for PAPP. The lower limit of quantitation for PAPP was 4·04 ng mL^{-1} in plasma (CV = 5·7%, n = 6). Mean CVs for inter-day control concentrations (n = 12) were less than 10%. Recovery was between 87 and 91%. No sample degradation was found when samples were kept for up to 6 months at both −20°C and −70°C. For drug concentrations greater than 485 ng mL^{-1} the plasma was diluted appropriately.

The PAHP method is identical to the above method except that the mobile phase was 1:1, v/v, acetonitrile-water containing 0·15% H_3PO_4. The flow rate was 1·3 mL min^{-1}. The peak height ratio of PAHP to internal standard (*p*-aminooctylphenone, PAOP) was calculated for each sample from the measured peak heights obtained by HPLC. The retention time of PAHP was 10·2 min, that of internal standard was 16·5 min. Linearity of the curve was at least 0·99 (r^2) for PAHP. The lower limit of quantification was 4·08 ng mL^{-1} (CV = 2·6%, n = 6) and the standard curve range was 4·08–816 ng mL^{-1}. Mean CVs for inter-day control concentrations (n = 6) were less than 10%. No sample degradation was found in samples kept up to 6 months at both −20°C and −70°C (Lin 1993).

Kinetic analysis

Initial kinetic parameter estimates were obtained using Rstrip (MicroMath Scientific Software, Salt Lake City, UT, USA). MKMODEL (Biosoft, Cambridge, UK) was used for the final

kinetic parameter estimation and the pharmacokinetic–pharmacodynamic (PK–PD) fitting. The kinetic data were fit to the two equations below for oral dosing and intravenous dosing (where F is bioavailability, V_c is the volume of the central compartment, K_a is the absorption rate constant, K_e is the elimination rate constant, K_α, K_β and K_γ are the disposition rate constants, and K_{21} and K_{31} are the inter-compartmental transfer rates from the second and third compartments into the central compartments respectively). The two- or three-compartment model that fit the data best was determined by the log likelihood, the Schwartz criterion (Holford 1994), the visual fit of the data, 95% confidence intervals for each estimated parameter and residual analysis.

Oral dosing

$$C_{oral} = \frac{K_a \times F \times Dose}{V_c} \times \left[\frac{(K_{21} - K_a) \times e^{-K_\alpha t}}{(K_\alpha - K_a) \times (K_\beta - K_a)} + \frac{(K_{21} - K_\alpha) \times e^{-K_a t}}{(K_a - K_\alpha) \times (K_\beta - K_\alpha)} + \frac{(K_{21} - K_\beta) \times e^{-K_\beta t}}{(K_\alpha - K_\beta) \times (K_\alpha - K_\beta)} \right] \quad (1)$$

Intravenous dosing

$$C_{iv} = \frac{Dose}{V_c} \times \left[\frac{(K_{21} - K_\alpha) \times (K_{31} - K_\alpha) \times e^{-K_\alpha t}}{(K_\beta - K_\alpha) \times (K_\gamma - K_\alpha)} + \frac{(K_{21} - K_\beta) \times (K_{31} - K_\beta) \times e^{-K_\beta t}}{(K_\alpha - K_\beta) \times (K_\gamma - K_\beta)} + \frac{(K_{21} - K_\gamma) \times (K_{31} - K_\gamma) \times e^{-K_\gamma t}}{(K_\alpha - K_\gamma) \times (K_\alpha - K_\gamma)} \right] \quad (2)$$

Methaemoglobin analysis

Methaemoglobin was measured on a CoOximeter (Radiometer, Copenhagen, Denmark). The CoOximeter has a lower limit of detection of 0·1% methaemoglobin with a 10% CV.

Kinetic–dynamic analysis

Kinetic–dynamic analysis was done on MKMODEL using a linked effect compartment with a sigmoid E_{max} effect model. E_{max} was fixed at 100% for all models. For the effect site concentration and the effect the models used were as follows (where all symbols as used above and K_{eo} is the rate constant out of the effect compartment, EC50 is the concentration of drug in the effect compartment that gives the half maximum response and n is the hill coefficient).

Oral dosing-effect compartment

$$C_{e\,(oral)} = \frac{Dose \times F \times K_a \times K_{eo}}{V_c} \times \left[\frac{(K_{21} - K_\alpha)) \times e^{-K_\alpha t}}{(K_\beta - K_\alpha) \times (K_a - K_\alpha) \times (K_{eo} - K_\alpha)} + \frac{(K_{21} - K_\beta) \times e^{-K_\beta t}}{(K_\alpha - K_\beta) \times (K_\alpha - K_\beta) \times (K_{eo} - K_\beta)} + \frac{(K_{21} - K_\alpha) \times e^{-K_a t}}{(K_\alpha - K_a) \times (K_\beta - K_\alpha) \times (K_{eo} - K_a)} + \frac{(K_{21} - K_{eo}) \times e^{-K_{eo} t}}{(K_\alpha - K_{eo}) \times (K_\beta - K_{eo}) \times (K_a - K_{eo})} \right] \quad (3)$$

Intravenous dosing–effect compartment

$$C_{e\,(iv)} = \frac{Dose \times K_{eo}}{V_c} \times \left[\frac{(K_{21} - K_\alpha) \times (K_{31} - K_\alpha) \times e^{-K_\alpha t}}{(K_\beta - K_\alpha) \times (K_\gamma - K_\alpha) \times (K_{eo} - K_\alpha)} + \frac{(K_{21} - K_\beta) \times (K_{31} - K_\beta) \times e^{-K_\beta t}}{(K_\alpha - K_\beta) \times (K_\gamma - K_\beta) \times (K_{eo} - K_\beta)} + \frac{(K_{21} - K_\gamma) \times (K_{31} - K_\gamma) \times e^{-K_\gamma t}}{(K_\alpha - K_\gamma) \times (K_\beta - K_\gamma) \times (K_{eo} - K_\gamma)} + \frac{(K_{21} - K_{eo}) \times (K_{31} - K_{eo}) \times e^{-K_{eo} t}}{(K_\alpha - K_{eo}) \times (K_\beta - K_{eo}) \times (K_\gamma - K_{eo})} \right] \quad (4)$$

Concentration–effect relationship

$$Effect = \frac{E_{max} \times C_e^n}{EC_{50}{}^n + C_e^n} \quad (5)$$

Experimental design

After an overnight fast animals had 20G intravenous catheters placed in both the cephalic veins or saphenous vein as appropriate. Animals then had blood sampled for measurement of baseline values. Oral doses were either given by capsule or gavage as indicated. Intravenous infusion was given over 10 min. Time 0 was counted as the time of oral administration or the end of the infusion. Samples were taken at 0, 3, 5, 10, 15, 30, 45 and 60 min, then hourly until 12 h, then daily until the methaemoglobin levels were less than 5%.

Results

Pharmacokinetics

The pharmacokinetic data of the phenones are shown in Tables 1 and 2 for PAHP and PAPP, respectively. The pharmacokinetics for both compounds were best fit to a three-compartment model for intravenous dosing and a two-compartment model with first order absorption for oral administration. Bioavailability for PAPP was $32 \pm 10\%$ and the volume of the central compartment (V_c) was $0{\cdot}89 \pm 0{\cdot}26$ L kg^{-1}. The initial distribution phase of the intravenous formulation was rapid (average $t\frac{1}{2} = 2{\cdot}7$ min) with slower intermediate and terminal elimination phases. Bioavailability for PAHP was $50 \pm 8\%$ and the volume of the central compartment (V_c) was $2{\cdot}4 \pm 0{\cdot}57$ L kg^{-1}. PAHP also had a rapid distribution phase (average $t\frac{1}{2} = 1{\cdot}5$ min) with slower intermediate and terminal elimination phases. Fig. 2 shows blood levels and methaemoglobin % for PAHP given intravenously to one animal.

Pharmacodynamics

The potency of PAPP, defined as the peak methaemoglobin divided by the dose kg^{-1} given intravenously, was 32–37%/(mg kg^{-1}) and the time until maximum methaemoglobin was 60-90 min. PAHP had a potency of 5·7–6·8%/(mg kg^{-1}) with time until methaemoglobin maximum being 180–270 min. Both compounds produced methaemoglobin levels of 5% or greater within 30 min. PAPP sustained levels of 5% or greater for 145-230 min and PAHP sustained levels of 5% or greater for 645–710 min. Significant reductions in methaemoglobin production occurred with oral dosing with both the PEG 200 solution or the carmellose suspension, compared with intra-

Table 1. Pharmacokinetic and pharmacodynamic parameter estimates for *p*-aminoheptylphenone.

	Intravenous	Oral suspension	Oral solution
Volume of central compartment (L kg^{-1})	2·35 ± 0·57	—	—
Volume of central compartment divided by bioavailability (L kg^{-1})	—	11·11 ± 7·00	5·03 ± 3·57
Disposition rate constant in first distribution phase (min^{-1})	0·456 ± 0·511	0·038 ± 0·017	0·027 ± 0·040
Disposition rate constant in second distribution phase (min^{-1})	0·030 ± 0·021	0·017 ± 0·007	0·032 ± 0·027
Disposition rate constant in terminal elimination phase (min^{-1})	0·002 ± 0·001	0·002 ± 0·001	0·001 ± 0·001
Rate constant for transfer from second compartment into central compartment (min^{-1})	0·009 ± 0·006	0·005 ± 0·003	0·003 ± 0·003
Rate constant for transfer from third compartment into central compartment (min^{-1})	0·390 ± 0·510	—	—
Concentration giving half maximum response (50% methaemoglobin) (ng mL^{-1})	347 ± 75	663 ± 138	611 ± 201
Rate constant out of the effect compartment (min^{-1})	0·004 ± 0·001	0·013 ± 0·013	0·014 ± 0·003
Hill coefficient	2·92 ± 0·74	1·31 ± 0·08	1·42 ± 0·37

In all experiments dose was 6 mg kg^{-1}. Values given are mean ± s.d. (n = 6, intravenous; n = 8 oral suspension and oral solution).

Table 2. Pharmacokinetic and pharmacodynamic parameter estimates for *p*-aminopropiophenone.

	Intravenous	Oral suspension	Oral solution
Volume of central compartment (L kg^{-1})	0·891 ± 0·259	—	—
Volume of central compartment divided by bioavailability (L kg^{-1})	—	2·72 ± 0·68	2·69 ± 0·54
Disposition rate constant in first distribution phase (min^{-1})	0·260 ± 0·180	0·104 ± 0·063	0·106 ± 0·093
Disposition rate constant in second distribution phase (min^{-1})	0·016 ± 0·005	0·021 ± 0·003	0·026 ± 0·008
Disposition rate constant in terminal elimination phase (min^{-1})	0·002 ± 0·002	0·004 ± 0·003	0·005 ± 0·003
Rate constant for transfer from second compartment into central compartment (min^{-1})	0·003 ± 0·003	0·003 ± 0·003	0·005 ± 0·004
Rate constant for transfer from third compartment into central compartment (min^{-1})	0·097 ± 0·003	—	—
Concentration giving half maximum response (50% methaemoglobin) (ng mL^{-1})	356 ± 82	1098 ± 445	700 ± 287
Rate constant out of the effect compartment (min^{-1})	0·014 ± 0·002	0·048 ± 0·015	0·034 ± 0·008
Hill coefficient	1·17 ± 0·08	0·88 ± 0·09	0·97 ± 0·17

In all experiments dose was 0·4 mg kg^{-1}. Values given are mean ± s.d. (n = 6, intravenous; n = 8 oral suspension and oral solution).

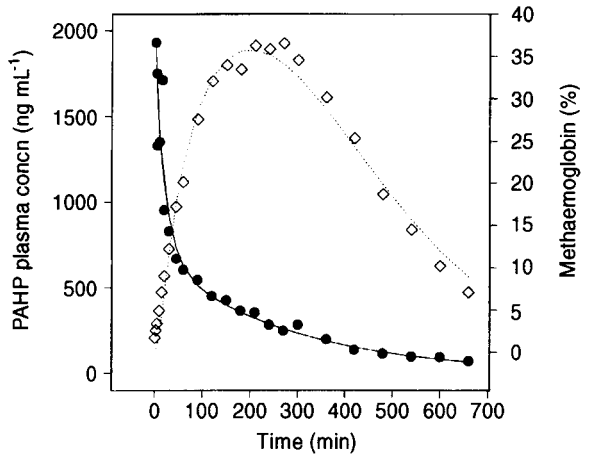


FIG 2. Pharmacokinetic profile and methaemoglobin profile of PAHP when given intravenously: ●, measured plasma concentration; ◇, measured methaemoglobin values. The fit of the data is represented by the solid lines. Concentration is in ng mL^{-1}, methaemoglobin is in percent and time is in min.

venous dosing. Overall the carmellose formulations had slightly lower methaemoglobin response than the PEG 200 solutions which might be secondary to their lower bioavailability.

Kinetic–dynamic models

Simultaneous kinetic–dynamic fitting of the drug concentration data and the methaemoglobin data was performed using MKMODEL. The appropriate pharmacokinetic model (two- or three-compartment, bolus or first order input) was linked to an effect site where drug concentrations were related to effect (methaemoglobin %) by a sigmoid E_{max} model (Sheiner et al 1979). EC50 values (concentration at the effect site giving half maximum response, i.e. 50% methaemoglobin), Hill factors (a constant which determines the slope of the concentration–effect curve) and K_{eo} values (the rate constant governing the disappearance of the drug from the effect compartment) are all given in Tables 1 and 2. In general the dynamic response for both phenones closely paralleled their drug-concentration profile. The dynamic parameter estimates for PAPP were EC50 values of 356, 700, and 1098 ng mL^{-1}, K_{eo} values of 0·014, 0·034 and 0·048 min^{-1} and Hill coefficients of 1.17, 0·97 and 0·88, respectively, for the intravenous, solution and suspension formulations. The dynamic parameter estimates for PAHP were EC50 values of 347, 611 and 663 ng mL^{-1}, K_{eo} values of 0·0041, 0·0143 and 0·0132 min^{-1} and Hill coefficients of 2·92, 1·42 and 1·31, respectively, for the intravenous, solution and suspension formulations. It is of note that the EC50 values for the intravenous formulations were significantly less than those for the oral formulations (analysis of variance with Student-Newman-Keuls comparisons $P < 0·05$ except for the PAPP intravenous and oral solution which did not reach statistical significance). The EC50 values indicate that for any plasma concentration of drug at steady state the route of administration will influence the methaemoglobin level, with intravenous administration giving higher methaemoglobin levels than oral administration.

Discussion

One class of methaemoglobin forming drugs was explored in our experimental work. These compounds are indirect formers of methaemoglobin in that they do not form methaemoglobin in-vitro but do form it in-vivo, indicating that a metabolite might be the active species (Graffe et al 1964). On the basis of the UV spectra obtained from blood samples taken from animals given PAPP it has been suggested that the metabolite is *p*-hydroxyaminopropiophenone (Graffe et al 1964). Further work has provided strong supporting evidence that the metabolite is a *p*-hydroxy compound (Marrs et al 1991). We have studied the relationship between the parent compound and the effect because the parent is easily quantitated and stable. The phenones are, in general, compounds with relatively short half-lives (1–3 h). Both PAHP and PAPP were modelled with three and two compartments respectively and had extremely short distribution half-lives. Oral absorption for both of the compounds was relatively rapid. Although both PAHP and PAPP produced methaemoglobin rapidly (within 30 min) peak methaemoglobin values lagged behind peak plasma drug levels by at least 60 min. This might reflect drug distribution into the red blood cells, drug metabolism to an active metabolite, or a 'build-up' in the red blood cells of a minimum effective concentration.

Although elucidating which mechanism is responsible for the delay is not possible from the data in these experiments it is possible to model the kinetic–dynamic relationship with an effect compartment linked to the central compartment (Sheiner et al 1979). PAHP produced a longer duration of methaemoglobinaemia, which probably reflects the drug's longer half-life, but strict comparisons can not be made as to duration of methaemoglobin as it is both a function of the drug's elimination half-life, the drug's intrinsic effect on haemoglobin and the dose given. A larger dose will both prolong the methaemoglobinaemia and also raise the methaemoglobin to higher levels. The utility of kinetic–dynamic modelling is the ability to predict the effects of dosages different from those used in the study. Using the mean values of the pharmacokinetic parameter estimates simulations were run for the PAHP suspension. An initial dose of 5 mg kg^{-1} followed by a dose of 3 mg kg^{-1} given every 6 h was simulated to predict PAHP plasma concentrations and percent methaemoglobin. The peak value of methaemoglobin was 13% with a trough of 5% and a mean of 9% (Fig. 3). For this compound a profile with less peak-to-trough variation would require a more frequent dosing interval or the development of a zero-order or slow-release formulation. The estimates of the EC50s enables predictions of steady-state concentration effect. In this study the EC50s were different for intravenous and oral routes of administration, suggesting that effect is dependent on route of administration. The EC50s for both PAPP and PAHP are higher for oral administration than for intravenous administration. Because they might act via a metabolite, a plausible hypothesis is that oral first-pass inactivation or intravenous lung first-pass activation occurs. In animal models the lungs can be a primary site of metabolism for basic amine drugs (Roth 1985). This can be a significant effect—up to 75% of an intravenous dose of propranolol can be removed on first passage through the pulmonary vasculature (Geddes et al 1979). Early work showed that eviscerated rats given an intravenous dose of PAPP produced higher levels of methaemoglobin than did control rats (Tepperman et al 1946). This suggests both hepatic inactivation and lung activation because the parent compound is inactive. Most compounds, though, are inactivated via a first pass effect (Pond & Tozer 1984).

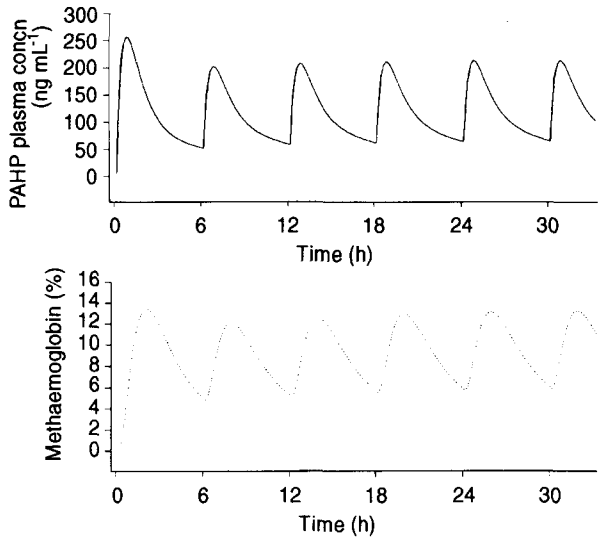


FIG. 3. Simulated pharmacokinetic profile and methaemoglobin profile of PAHP when given as an initial dose of 5 mg kg^{-1} oral suspension then a maintenance dose of 3 mg kg^{-1} oral suspension every 6 h. The simulated PAHP plasma concentration is represented in the top graph and the simulated percent methaemoglobin is represented in the bottom graph.

The potential metabolic pathways and cytochromes responsible for the metabolism of these compounds are important for several reasons. It would be interesting to know if any special population might not produce methaemoglobin or if any group is more likely to produce toxic levels of methaemoglobin. This is especially important in the military use of these compounds because they might be used in a large group of patients simultaneously without the benefit of post-marketing safety data. If the primary organ responsible for the metabolism of phenones is the lung, then CYP450 classes IIB, IIE and IVB might be involved in bioactivation (Alvares & Pratt 1990). This work needs to be confirmed in further studies and the role of the lung in phenone metabolism should also be explored. When the compound has been metabolized to the active form it must enter the red blood cell to exert its effect. The proposed oxidative pathway of haemoglobin to methaemoglobin is outlined in Fig. 4. The oxidation of the phenones to the hydroxylamines is consistent with results obtained by several other groups (Graffe et al 1964; Marrs et al 1991). The one-electron transfer characteristic of iron interactions would act to form a hydronitroxide radical, as has been demonstrated by several investigators (Stolze & Nohl 1989, 1990). The further decomposition products of the hydronitroxide radical have not been characterized, although the nitroso compound suggested by the kreisprozess is consistent with this mechanism. The phenones have a very short time of effect compared with other methaemoglobin-forming compounds (Marino et al 1993). A prolonged infusion experiment could be used to test whether the intrinsic ability of red blood cells to maintain the

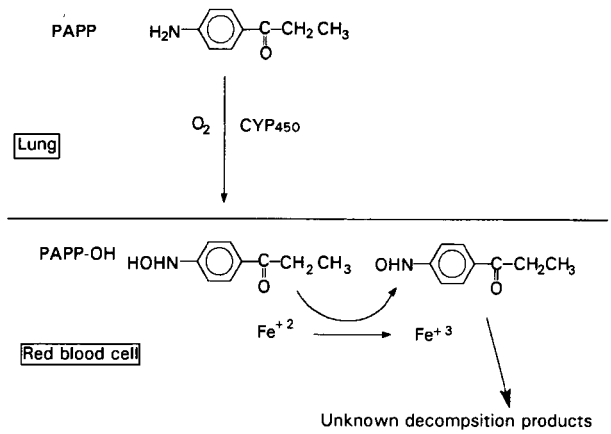


FIG. 4. Proposed metabolic pathway and mechanism of action of the phenones as methaemoglobin formers. Metabolism of PAPP to PAP-P(OH) occurs in the lung and the PAPP(OH) is then transferred to the red blood cell where it is reduced in the process of oxidizing haemoglobin from the Fe^{2+} state to F^{3+}.

redox cycle or the elimination of the compound limits the maintenance of methaemoglobin.

The production of methaemoglobin from these compounds is equivalent to or better than that from sodium nitrite (Kirk et al 1993). A potential advantage would be that these compounds can be administered orally, which could be useful as a prophylactic for exposure or for a patient without intravenous access who maintains consciousness. An intramuscular preparation would also be of great use in treatment of mass casualties.

Whereas both of these compounds have been studied previously for their ability to form methaemoglobin (Lanphier et al 1947) this is the first report of the compounds' simultaneous pharmacokinetics and pharmacodynamics. The understanding of the kinetic–dynamic relationship might aid the design of effective and safe dosing regimens.

Acknowledgements

The authors wish to thank Ms Kimberly Allow, Mr John Hardin, Sgt Andrew Kimbrow and Mr Antonio J. Aguilar for assistance with the animal studies. The authors also wish to thank Dr Douglas Flannagan from the University of Iowa for providing the formulated compounds.

References

Albaum, H. G., Tepperman, J., Bodansky, O. (1946) A spectrophotometric study of the competition of methemoglobin and cytochrome oxidase for cyanide in vitro. J. Biol. Chem. 163: 641–647

Alvares, A. P., Pratt, W. B. (1990) Pathways of drug metabolism. In: Pratt, W. B., Taylor, P. (eds) Principles of Drug Action. The Basis of Pharmacology. Churchill Livingstone, New York, pp 365–422

Ballantyne, B. (1987) Toxicology of cyanides. In: Ballantyne, B., Marrs, T. C. (eds) Clinical and Experimental Toxicology of Cyanides, Wright, Bristol, pp 41–127

Baskin, S. I., Fricke, R. F. (1992) The pharmacology of *p*-aminopropiophenone in the detoxification of cyanide. Cardiovasc. Drug Rev. 10: 358–375

Beulter, E., Mikus, B. J. (1961) The effect of sodium nitrite and *para*-aminopropiophenone administration on blood methemoglobin levels and red blood cell survival. Blood 18: 455–467

Bodansky, O., Hendley, C. D. (1946) Effect of methemoglobinemia on the visual threshold at sea level, at high altitudes, and after exercise. J. Clin. Invest. 25: 717–722

Bright, J. E., Marrs, T. C. (1982) A comparison of the methemoglobin-inducing activity of moderate oral doses of 4-dimethylaminophenol and *p*-aminopropiophenone. Toxicol. Lett. 13: 81–86

Bright, J. E., Woodman, A. C., Wood, S. G., Marrs, T. C. (1987) Sex differences in the production of methaemoglobinaemia by 4-aminopropiophenone. Xenobiotica 17: 79–83

Geddes, D. M., Nesbitt, K., Traill, T., Blackburn, J. P. (1979) First-pass uptake of ^{14}C-propranolol by the lung. Thorax. 34: 810–813

Graffe, W., Kiese, M., Rauscher, E. (1964) The formation in vivo of *p*-hydroxyaminopropiophenone from *p*-aminopropiophenone and its action in-vivo and in-vitro. Naunyn Schmiedeberg's Arch. Exp. Pathol. Pharmacol. 249: 168–175

Holford, N. (1994) MKMODEL: A Quantitative Modelling System for Pharmacologists, Version 5, Biosoft, Ferguson, MO

Kirk, M. A., Gerace, R., Kulig, K. W. (1993) Cyanide and methemoglobin kinetics in smoke inhalation victims treated with the cyanide antidote kit. Ann. Emerg. Med. 22: 1413–1418

Klaassen, C. D. (1996) Nonmetallic environmental toxicants: air pollutants, solvents and vapors, and pesticides. In: Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P. (eds) The Pharmacological Basis of Therapeutics, 9th edn. Pergamon Press, New York, pp 1673–1696

Lanphier, F. H., Jenny, E. H., Pfeiffer, C. C. (1947) The use of the oximeter to study the potency of *p*-aminophenones as methemoglobin-forming drugs (abstract). Fed. Proc. 6: 348

Lin, E. (1993) High performance liquid chromatography of p-aminopropiophenone, *p*-aminoheptylphenone, and *p*-aminooctylphenone. Contract 17-92-C-2028 US Army Medical Research and Development Command, University of California, San Francisco

Marino, M. T., Peggins, J. O., Brown, L. D., Urquhart, M. R., Brewer, T. G. (1993) Pharmacokinetics and kinetic-dynamic modelling of an 8-aminoquinoline candidate anticyanide and antimalarial drug (WR242511). Drug. Metab. Disp. 22: 358–366

Marrs, T. C. (1987) The choice of cyanide antidotes. In: Ballantyne, B., Marrs, T. C. (eds) Clinical and Experimental Toxicology of Cyanides. Wright, Bristol, pp 383–402

Marrs, T. C., Inns, R. H., Bright, J. E., Wood, S. G. (1991) The formation of methaemoglobin by 4-aminopropiophenone (PAPP) and 4-(N-hydroxy)aminophenone. Human. Exp. Tox. 10: 183–188

Paulett, G., Aubertin, X., Laurens, L., Bourrelier, J. (1963) De l'action methemoglobinisante de la paraaminopriopiophenone chez l'homme, avec un complement chez le chien. Arch. Int. Pharmacodyn. 142: 35–51

Pond, S. M., Tozer, T. N. (1984) First-pass elimination: basic concepts and clinical consequences. Clin. Pharmacokin. 9: 1–25

Roth, R. A. (1985) Biochemistry, physiology and drug metabolism-implications regarding the role of the lungs in drug disposition. Clin. Physiol. Biochem. 3: 66–79

Schultz, V. (1984) Clinical pharmacokinetics of nitroprusside, cyanide, thiosulfate, and thiocyanate. Clin. Pharmacol. 9: 239–251

Sheiner, L. B., Stanski, D. R., Vozeh, S., Miller, R. D., Ham, J. (1979) Simultaneous modelling of pharmacokinetics and pharmacodynamics: application to *d*-tubocurarine. Clin. Pharmacol. Ther. 25: 358–371

Stolk, J. M., Smith, R. P. (1966) Species differences in methemoglobin reductase activity. Biochem. Pharmacol. 15: 343–351

Stolze, K., Nohl, H. (1989) Detection of free radicals as intermediates in the methemoglobin formation from oxyhemoglobin induced by hydroxylamine. Biochem. Pharmacol. 38: 3055–3059

Stolze, K., Nohl, H. (1990) EPR studies on the oxidation of hydroxyurea to paramagnetic compounds by oxyhemoglobin. Biochem. Pharmacol. 40: 799–802

Tepperman, J., Bodansky, O., Jandorf, B. J. (1946) The effect of *para*-aminopropiophenone-induced methemoglobinemia on oxygenation of working muscle in human subjects. Am. J. Physiol. 146: 702–709

Weger, N. P. (1990) Treatment of cyanide poisoning with 4-dimethylaminophenol (DMAP)-experimental and clinical overview. Middle East J. Anesthesiol. 10: 389–412

Wilson, J. (1987) Cyanide in human disease. In: Ballantyne, B., Marrs, T. C. (eds) Clinical and Experimental Toxicology of Cyanides. Wright, Bristol, pp 292–311