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Effects and Biotransformation of 4-Dimethylaminophenol in Man and Dog

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Abstract. The cyanide antidote 4-dimethylaminophenol · HCl (DMAP) was administered orally, i.v., or i.m. to man and dog. Ferrihemoglobin formation and changes of several parameters in human blood were investigated to obtain information on damage to liver, kidney, muscle, and red blood cells; in addition, the metabolism of DMAP was studied.

In dogs, the initial rate of ferrihemoglobin production (DMAP, 3.25 mg/kg i.v. or i.m., 15 mg/kg orally) amounted to 28%, 3.5%, and 2% of the total hemoglobin per min; the corresponding values for man were 9%, 2%, and 2% per min. The dogs behaved normally while CPK increased after i.m. injection.

In man, only i.m. injection of DMAP (3.25 mg/kg) was followed by increases in LDH, GOT, and CPK of 110, 260, and 490%, resp.; while total bilirubin, conjugated bilirubin, and iron concentration rose by 270, 120, and 50%, respectively. Bilirubin and iron concentration increased also after DMAP i.v. (3.25 mg/kg) or when it was taken orally (600 or 900 mg). The lactate concentration was not influenced while the pyruvate concentration increased by 50%. DMAP produced hemolysis in vitro. Generally, the values determined in vivo approached the starting level within 1 week. Intramuscular injection of DMAP induced reversible subjective and objective symptoms, e.g., local pain, swollen buttock, fever reaction. The urine showed no pathological changes. About 54% of DMAP taken orally was excreted as metabolites in the urine, 41% as glucuronide, 7% as sulfate, and 6% as thioethers. After i.v. administration the total of metabolites was somewhat higher, and the thioether proportion was 15%. The results indicate that DMAP is readily absorbed after oral administration but undergoes significant first pass effect in the liver. Therefore, the 4-fold i.v. dose must be administered orally to achieve the same ferrihemoglobin formation.

Key words: 4-Dimethylaminophenol – Ferrihemoglobin – Metabolism

Introduction

For a number of years, 4-dimethylaminophenol · HCl (DMAP) has been used as a potent antidote against poisoning by cyanide. The compound is known to be effective not only in animals (Klimmek et al. 1979b, 1982) but also in humans (Daunderer, unpublished results). The rapid onset of cyanide poisoning and its high lethality require that the patient receives the antidote as soon as possible. In emergency cases intravenous injection of DMAP may be impracticable when the qualified medical staff is absent; consequently, some other effective route of administration for use by first aid personnel would be desirable.

Therefore, we investigated the kinetics of ferrihemoglobin formation and other effects due to intravenous (i.v.), intramuscular (i.m.) or oral administration of DMAP, firstly in dogs and then in man.

Our interest focused also on several biochemical parameters in human blood to obtain information on damage to liver, kidney, muscle, and red blood cells; the parameters were found previously to be unaltered in dogs (Klimmek et al. 1979a).

Since ferrihemoglobin formation after oral administration of DMAP was significantly lower than by the parenteral routes, it was of interest to investigate the bioavailability of oral DMAP. Thus the present study deals also with the metabolic fate of DMAP in connection with various routes of administration.

Materials and Methods

Experiments with Dogs. In the preliminary experiments with animals male beagles of mean body weight 13.9 ± 0.5 kg were used. DMAP was given at 3.5 or 15 mg/kg with some normal diet. For this purpose the substance was dissolved in phosphate buffer to obtain pH 7 and encapsulated in gelatine. A 5% solution of DMAP was used for i. m. injections (see below). Hemoglobin and ferrihemoglobin were determined in venous blood.

Pressure transducers (Hansen S & W) were used to record blood pressure in the femoral artery and blood flow was measured using electromagnetic flow probes (Statham). Heart rate was determined by a frequency gauge from the ECG, respiratory minute volume by a pneumotachometer (Hellige).

Experiments with Human Volunteers. Twenty male and three female healthy volunteers (20–30 years; mean body weight: 74.8 ± 2.2 kg) took part in this study for 1 week. Their written consent was obtained after the experimental procedures had been fully explained.

The subjects were given 4-dimethylaminophenol · HCl (DMAP) in three different preparations for i.v. (5% solution), i.m. (12.5% solution), or oral use (300-mg tablets). The products were kindly given by Dr. Franz Köhler-Chemie, Alsbach, FRG.

DMAP was given at 3.25 mg/kg i.v. and 3.5 mg/kg i.m., the latter being injected deep in the right buttock muscles. In the oral study, the subjects received 300, 600, or 900 mg DMAP, which was taken with water.

A cannula was inserted in the antecubital vein and blood samples were taken at regular intervals. Parameters of clinical chemistry were determined with Biochemica Test Combinations (Boehringer, Mannheim, FRG). Ferrihemoglobin was measured by the increase in absorbance at 546 nm after addition of KCN to 0.1 ml blood in 10 ml distilled water. The hemolysate was buffered with 1 ml 0.2 M phosphate buffer, pH 6.6. Total hemoglobin was determined as ferrihemoglobin after oxidation of ferrohemoglobin with potassium hexacyanoferrate (III).

To examine the influence of DMAP on the osmotic resistance of human red cells ACD-blood was obtained from a transfusion center. Whole blood was centrifuged; the supernatant and buffy layer were removed and the packed red cells washed three times with phopshate-buffered saline (PBS: NaCl 145 mmol/l; phosphate buffer, 15.7 mmol/l, pH 7.4) The washed cells were suspended in PBS after addition of glucose (5 mmol/l) and hemoglobin concentration was adjusted to 15 g/100 ml. Ten milliliters of the suspension was placed in several Erlenmeyer flasks and incubated with DMAP at various concentrations (0.1, 0.5, 1 mmol/l) while being shaken gently at 37° C under air for 20 h. Suspensions without DMAP served as controls.

0.1-ml samples were added to 10 ml distilled water (to give 100% hemolysis) and to 10 ml of the following sodium chloride solutions: 0.1, 0.3, 0.5, 0.7, 0.9 g/100 ml of distilled water. After 7 min, at room temperature, the suspension was centrifuged at 5,000 rpm. Extracellular hemoglobin was determined as described above.

Immediately before and 4, 24, 48, and 168 h after the administration of DMAP, a drop of fresh blood was stained with 1% nile blue sulfate (Kiese and Seipelt 1942/43) and examined for Heinz bodies.

The urine was collected in three portions: for 24 h before (blank), 24 h after and from 24 to 48 h after the administration of DMAP. Samples were cooled at 4° C and examined for nitrite, pH, proteins, glucose, ketones, urobilinogen, bilirubin, and hemoglobin using the Combur-8-Test (Boehringer, Mannheim, FRG). Remaining samples were ultra-filtered (0.22 μm, Millipore) and kept frozen at −24° C until analysis of DMAP metabolites was carried out.

For analysis of DMAP metabolites, 5 ml of heparinized blood was separated into the washed red cell and the plasma fraction, deproteinized by trichloroacetic acid (TCA), and extracted with ether to reduce the content of TCA. The entire procedure was performed at 4° C immediately after venipuncture.

4-Dimethylaminophenol hydrochloride (DMAP) (U^{14}C-phenyl) of specific activity 8.5 μCi/μmol was synthesized by Farbwerke Hoechst, Frankfurt, FRG. Radiochemical purity was greater than 98%. *(^{14}C-4-Dimethylaminophenyl-glucuronide (DMAP-glucuronide)* was isolated from rat hepatocytes incubated with (^{14}C)-DMAP as reported by Szinicz and Weger (1980). After deproteinization with TCA, the supernatant was chromatographed on Sephadex LH 20 and DE_{52}-cellulose according to Eyer and Gaber (1978). Radiochemical purity was greater than 98% as determined by high pressure liquid chromatography (HPLC).

(^{14}C)-4-Dimethylaminophenyl-sulfate (DMAP-sulfate), S,S,S-(2-dimethylamino-5-hydroxy-1,3,4-(^{14}C)-phenylene)-tris-glutathione (tris-(GS)-DMAP), and S,S,S,-(2-dimethylamino-5-hydroxy-1,3,4-(^{14}C)-phenylene)-tris-cysteine (tris-(Cys)-DMAP) were prepared as described elsewhere (Eyer and Gaber 1978). The specific activities ranged between 0.27 and 0.55 μCi/μmol. Radiochemical purities were greater than 97% (HPLC).

Metabolites of DMAP in urine, plasma, and red cells were determined by HPLC using an ALC/GPC 244 chromatograph (Waters Associates, Milford, Mass., USA) equipped with a UV detector and a data module integrator (Waters). DMAP-glucuronide and DMAP-sulfate were determined at a wavelength of 254 nm and tris-(GS)-DMAP at 280 nm.

DMAP-Sulfate was separated by paired ion chromatography on μ-Bondapak C-18 (4 mm ID × 30 cm, Waters) with 30% (v/v) methanol and 70% 2 mM tetrabutyl ammonium phosphate, pH 6.5 (PIC A-reagent, Waters). DMAP-sulfate was eluted at 25 ml (flow rate 2 ml/min).

DMAP-Glucuronide was separated by paired ion chromatography on RP-8 (4 mm ID × 25 cm, Hewlett Packard). With 30% (v/v) methanol and 5 mM tetrabutyl ammonium phosphate, pH 7.3, DMAP-glucuronide was eluted at 10 ml (flow rate 2 ml/min).

Tris-(GS)-DMAP was separated on μ-Bondapak-Phenyl (4 mm ID × 30 cm, Waters) by elution with 5% (v/v) methanol and 95% 50 mM phosphoric acid adjusted to pH 2.2 with NaOH (retention vol. 6 ml, flow rate 1 ml/min). Under these conditions DMAP-glucuronide and DMAP-sulfate were eluted by 3.8 and 4.7 ml, respectively.

Calibration of HPLC analyses was made by comparing the peak areas of the authentic standards (see above). In addition, sample and standards were mixed and applied to HPLC to ensure correct addition of the respective peak areas. All determinations were carried out at least in duplicate, the deviations being in the 5% range.

The sum of *DMAP-thioethers* in urine was determined as DMAP liberated upon treatment with Raney nickel (Eyer and Gaber 1978). To determine recoveries during the whole procedure, labelled tris-(Cys)-DMAP, the major constituent of the urinary DMAP-thioethers in man (Jancso et al. 1981)

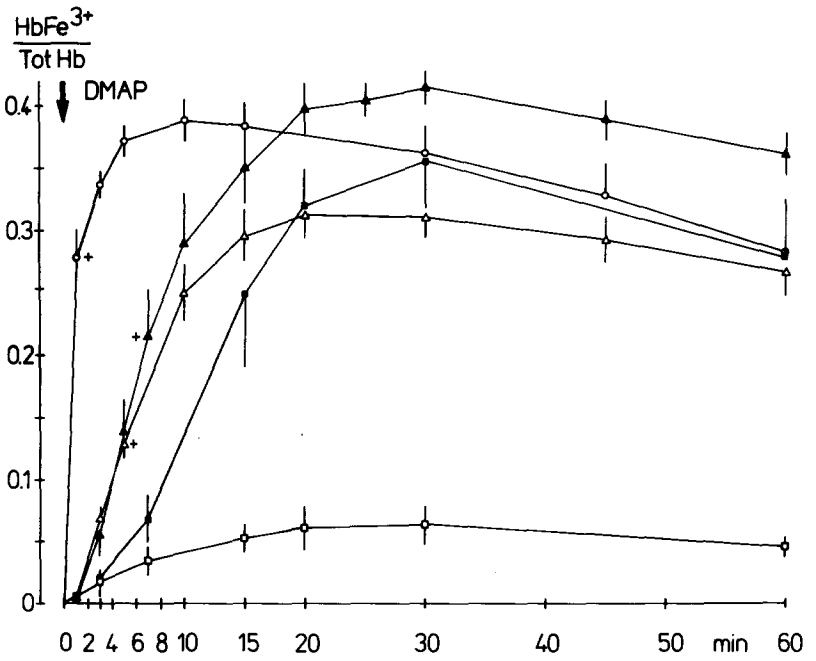

Fig. 1. Ferrihemoglobin formation by DMAP in dogs after i.v., i.m., or oral administration: (○———○) 3.25 mg/kg i.v., $n = 8$; (□———□) 3.25 mg/kg orally, $n = 4$; (■———■) 15 mg/kg orally, $n = 4$; (△———△) 2.5 mg/kg i.m., $n = 4$; (▲———▲) 3.25 mg/kg i.m., $n = 6$; mean ± SE. + End point for the calculation of initial ferrihemoglobin formation; cf. Discussion

and dog (Eyer and Gaber 1978) was added to urine samples, and DMAP liberated was determined by isotope dilution (Oliverio and Guarino 1971). Usually, about 70% of DMAP was liberated from the thioether linkage. As reported previously (Jancso et al. 1981) DMAP was not liberated from DMAP-glucuronide or DMAP-sulfate under these conditions. Radioactivity was measured in Bray's solution using a Packard Tricarb 2660 scintillation spectrophotometer. All results were corrected for recovery (external standardization) and background radiation.

Significance was estimated at the 5% level by Student's *t*-test. The average data are shown as mean ± standard error (SE).

Results

A. Experiments with Dogs

Figure 1 shows the influence of the route of administration on the ferrihemoglobin formation by DMAP in conscious dogs. After i.v. injection (3.25 mg/kg) the maximum venous ferrihemoglobin content was reached within 5–10 min and was found to be 38.8 ± 1.7% of the total hemoglobin. Intramuscular injection of DMAP (3.25 mg/kg) led to a maximum ferrihemoglobin content of 41.6 ± 1.3% at 30 min; 2.5 mg/kg i.m. increased the ferrihemoglobin content to 31.3 ± 1.8% at 20 min. The lag phase that preceded the rapid increase lasted about 1 min. After oral administration of DMAP (3.25 or 15 mg/kg) the ferrihemoglobin content increased to 6.3 ± 1.6% and 35.7 ± 3.6%, respectively.

In the experiments that were performed on anesthetized dogs i.m. injection of DMAP (5 mg/kg; $n = 3$) was not followed by significant changes in mean arterial blood pressure and blood flow, venous pressure, heart rate, respiratory minute volume, respiratory rate, or electrocardiogram.

The conscious dogs behaved quite normally and did not show any sign of inflammation at the site of injection while the CPK activity increased to the sevenfold from 20.1 ± 2.4 to 147.1 ± 27.3 U/l by 24 h; 7 days later the values had returned to the starting level.

B. Experiments with Human Volunteers

1. Ferrihemoglobin Formation and Changes in Erythrocytes

DMAP i.v. (3.25 mg/kg). Three subjects were injected with this dose. By 15 min, the ferrihemoglobin content increased to 34.7 ± 3% of the total hemoglobin, which itself did not change (Fig. 2). The number of reticulocytes was initially 1.39 ± 0.005%; counts of the smears gave maxima of 2.9 and 3.7% after 7 days and 2.5% after 3 days, respectively. Heinz bodies were not seen.

DMAP i.m. In the first trial, three different doses (1, 2, 3.5 mg/kg) of the i.v. preparation of DMAP were injected with 1 ml (20 mg) Lidocain (Xylocain, Astra Chemicals). The increase in the ferrihemoglobin content (Fig. 2) was measurable after a delay of 2–4 min and showed dose-dependent maxima of 8.2, 18.7, and 29.1% at 45 min and the corresponding half maxima at 12, 9, and 9 min, respectively.

In a second trial, the i.m. preparation of DMAP was injected. The ferrihemoglobin content increased in four subjects to 1.7 ± 0.7, 2.2 ± 0.7, 6.7 ± 0.8%, and 29.7 ± 0.2% at 3, 5, 10, and 60 min, respectively (Fig. 2). In the fifth subject, the ferrihemoglobin content was 1.1% at 10 min, 2.2% at 30 min, and 7.5% at 60 min. Total hemoglobin was constant in all subjects and Heinz bodies were not detected.

Oral DMAP. The ferrihemoglobin content (Fig. 2) began to increase after a lag time of 5 min. Raising the DMAP dose from 300 to 600 or 900 mg did not shorten the time lag. The ingestion of 300 mg DMAP led to the formation of 1.0 ± 0.5, 5.1 ± 1.3, and 10.7 ± 2% ferrihemoglobin at 5, 10, and 15 min, respectively. A maximum of 12 ± 1.4% was reached at 30 min. The corresponding ferrihemoglobin values after ingestion of higher doses of DMAP were 2.2 ± 1.2, 8.2 ± 1.9, and 15.4 ± 2.7% for 600 mg (Fig. 2) and 0.9 ± 0.4, 9.1 ± 3.6, and 19.6 ± 6.7% for 900 mg (Fig. 2) at the given times. After 30 min, maxima of 19.3 ± 2.2% and 27.4 ± 3.2%, respectively, were measured. Total hemoglobin did not change and Heinz bodies were not observed.

DMAP in vitro. The experiments were carried out to test human erythrocytes for osmotic resistance. The percentage hemolysis did not differ from control for an

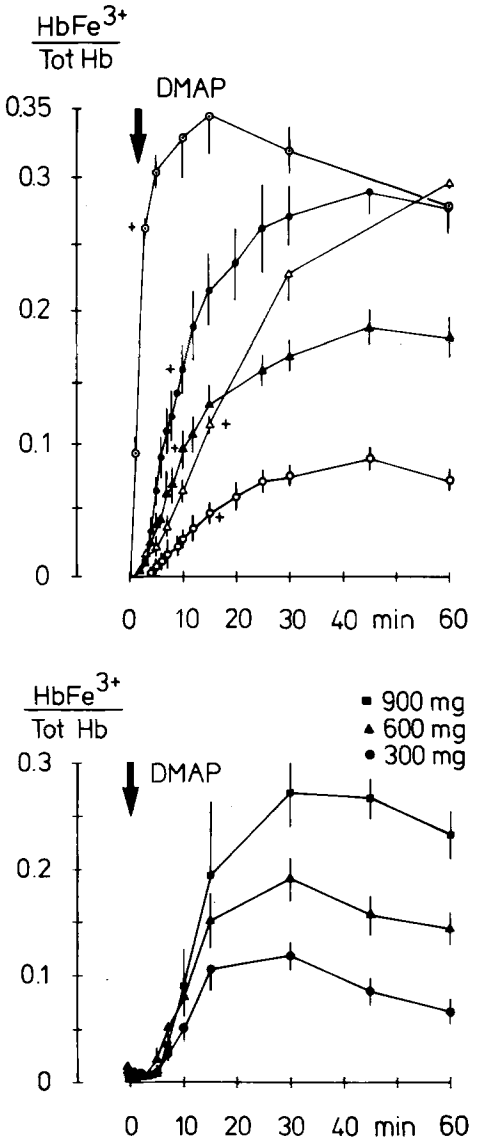

Fig. 2. Ferrihemoglobin formation by DMAP in human volunteers. *Upper part:* Intravenous and i. m. administration. Preparation for i.v. use: (⊙———⊙) 3.25 mg/kg i.v., $n = 3$; (○———○) 1 mg/kg i.m., $n = 6$; (▲———▲) 2 mg/kg i.m., $n = 6$; (●———●) 3.5 mg/kg i.m., $n = 6$. Preparation for i.m. use: (△———△) 3.5 mg/kg i. m., $n = 4$. *Lower part:* Oral administration, $n = 5$; mean ± SE. + End point for the calculation of initial ferrihemoglobin formation; cf. Discussion

incubation period of less than 20 h and a DMAP concentration of 1 mmol/l. At 20 h, the hemolysis with 1 mM DMAP was 14.5 ± 6.9% compared with the control value of 2.7 ± 0.4% when determined in 0.7% NaCl solution ($n = 4$) and 4.9 ± 1.4% compared with 1.4 ± 0.1% when determined in 0.9% saline ($n = 4$).

Table 1. Changes in the concentrations of iron and bilirubin in human serum after injection of DMAP, 3.25 mg/kg i.v. Mean ± SE; $n = 3$

Time (h)	Serum iron (μmol/l)	Total bilirubin (μmol/l)	Conjugated bilirubin (μmol/l)
− 24	12.4 ± 2.2	5.6 ± 0.9	1.4 ± 0.7
0	13.4 ± 2.1	5.8 ± 1.1	2.0 ± 0.3
1	17.8 ± 2.1	7.4 ± 1.2	2.3 ± 0.9
4	29.6 ± 4.9	9.5 ± 0.8	2.0 ± 0.3
24	40.1 ± 7.1	13.9 ± 3.5	–
48	14.6 ± 2.8	10.7 ± 1.1	5.6 ± 2.1
168	21.1 ± 0.8	11.2 ± 2.4	3.3 ± 0.6

Table 2. Changes in the concentrations of iron and bilirubin and in the activities of CPK, LDH, and GOT in human serum after injection of DMAP, 3.5 mg/kg i.m. Mean ± SE; $n = 4$

Time (h)	Serum iron (μmol/l)	Total bilirubin (μmol/l)	Conjugated bilirubin (μmol/l)	CPK (U/l)	LDH (U/l)	GOT (U/l)
− 24	19.8±3.7	12.5±1.4	4.9±1.0	93.8±24.0	160.8±23.8	13.4±0.9
0	23.3±1.9	12.3±0.2	4.7±0.3	75.0±19.8	166.0±14.0	10.4±1.2
1	22.8±3.0	13.3±0.6	5.1±0.4	78.6±18.2	163.6±12.5	12.2±1.0
4	35.6±2.5	22.4±2.2	8.1±0.6	205.8±49.6	261.0±35.7	23.7±2.3
24	13.1±6.3	45.7±4.2	9.6±0.5	441.4±93.0	328.8±32.5	54.4±1.6
48	8.9±2.5	25.0±2.8	10.5±1.0	183.6±52.4	347.0±32.9	37.0±5.9
168	18.8±1.5	12.3±0.5	5.4±0.3	131.8±66.2	215.0±23.0	16.2±1.5

2. Changes in Blood Serum

DMAP i.v. The lactate concentration in serum (1.6 ± 0.22 mmol/l) remained constant while the pyruvate concentration increased from 0.061 ± 0.004 to 0.093 ± 0.006 mmol/l by 5 min. Thereby the ratio of [lactate]/[pyruvate] diminished by 26% from 24.9 ± 1.4 to 18.5 ± 1.8; the ratio was 23.2 ± 2.4 after 60 min.

No change was observed in the activities of CPK, GOT, GPT, γ-GT, or LDH. Total bilirubin increased by 140%, conjugated bilirubin by 180%, and the iron concentration by 200%. The data are summarized in Table 1.

DMAP i.m. Total bilirubin increased by 270% by 24 h and then declined rapidly while conjugated bilirubin rose by 120% and the iron concentration by 50%. The activities of CPK, LDH, and GOT rose by 490%, 110%, and 260%, respectively; the values were still elevated after 7 days. The concentration of lactate and the activities of GPT and γ-GT remained unchanged. Table 2 shows the experimental data.

Oral DMAP. Total bilirubin and conjugated bilirubin did not change significantly when 300 mg DMAP was taken orally but the iron concentration increased by 25% from 16.8 ± 1.2 to 21.0 ± 2.7 μmol/l by 4 h.

After an oral dose of 600 mg DMAP total bilirubin and conjugated bilirubin rose by 60% and 160%, respectively, the iron concentration by 50% (Table 3).

After an oral dose of 900 mg DMAP total and conjugated bilirubin increased by 170% and 80% respectively, the iron concentration by 60%. The values for these parameters were still elevated by 7 days (Table 3).

Oral doses of DMAP had no influence on lactate concentration and on most of the enzyme activities measured; only a transient, statistically insignificant increase in LDH activity from 154.3 ± 4.6 to 172.8 ± 14.3 U/l at 4 h was found.

3. Changes in Urine

The volumes of urine excreted over 24 h before and after the administration of DMAP were equal and were independent of the dose administered. There was no indication of pathological urinary excretion, in particular of conjugated bilirubin, urobilinogen, erythrocytes, hemoglobin, or proteins.

4. Subjective and Objective Symptoms

DMAP i.v. After 4 h one subject complained of a mild headache. Two subjects complained of severe headache after 4 h; 6–7 days later they were affected by phlebitis of the antecubital vein where the DMAP had been injected.

DMAP i.m. The general well-being of the subjects was followed after 5–10 min by awareness of a slight pressure felt at the site of injection, slowly growing in intensity and finally leading to complaints of a more or less severe pain in the affected thigh. In the evening about 10 h after the injection shivering, sweating, and fever occurred and four out of five subjects had difficulty in moving about. Only one subject, the same one whose ferrihemoglobin formation was strikingly low, rejected any analgesic offered (acetylsalicylic acid or pentazocine) because he felt well except for a mild pressure-like sensation at the site of injection; he differed also in that he was overweight. In the morning or at midday of the following day the pain and fever sensations had disappeared and the upper part of the buttock was greatly swollen. In some cases the sclerae showed a yellowish color. After 1 week local symptoms had disappeared. Weeks and months later the subjects had no complaints.

Oral DMAP. The subjects who had received 300 mg DMAP were well except for one who felt a mild intraocular pressure and slight fatigue lasting about 3 h.

After ingestion of 600 mg DMAP the condition of the subjects was characterized by a feeling of inner calm, slowed mental activity, and slight

Table 3. Changes in the concentrations of iron and bilirubin in the serum of five subjects taking DMAP orally, 600 mg or 900 mg. Mean ± SE

Time (h)	Serum iron (μmol/l)		Total bilirubin (μmol/l)		Conjugated bilirubin (μmol/l)	
	600 mg	900 mg	600 mg	900 mg	600 mg	900 mg
− 24	17.4 ± 2.4	19.8 ± 2.1	10.1 ± 0.9	15.2 ± 2.8	3.7 ± 0.8	4.5 ± 0.6
0	21.9 ± 3.5	20.9 ± 2.4	12.9 ± 0.7	14.0 ± 2.1	4.4 ± 0.3	5.7 ± 0.7
1	24.1 ± 2.8	24.2 ± 3.0	13.3 ± 1.3	16.0 ± 2.3	6.6 ± 1.0	7.5 ± 1.5
4	27.5 ± 2.7	28.1 ± 1.7	15.6 ± 1.0	17.1 ± 2.7	6.3 ± 1.0	7.0 ± 1.1
24	33.0 ± 9.0	32.9 ± 4.0	20.5 ± 3.7	37.5 ± 8.6	7.7 ± 1.6	10.2 ± 1.2
48	18.4 ± 2.4	30.0 ± 3.0	20.0 ± 3.8	28.9 ± 5.4	11.2 ± 2.8	9.4 ± 1.8
168	25.1 ± 4.1	32.9 ± 4.3	14.7 ± 0.8	27.1 ± 5.7	5.0 ± 0.9	7.3 ± 1.9

Table 4. Metabolites of DMAP (% of the dose administered in human urine collected over 24 h after oral administration of different doses. Mean ± 1.96 SE (95% confidence limit)

Dose (mmol)	DMAP-glucuronide	DMAP-sulfate	DMAP-thioethers	Total metabolites	*n*
1.73 p.o.	41.5 ± 9.8	8.7 ± 2.6	3.6 ± 1.1	53.6 ± 11.4	5
3.46 p.o.	41.7 ± 8.3	6.3 ± 1.0	7.3 ± 4.8	55.4 ± 10.9	5
5.19 p.o.	40.5 ± 9.9	7.0 ± 3.7	5.2 ± 0.9	52.6 ± 12.2	5

Table 5. Metabolites of DMAP (% of the dose administered in human urine collected over 24 h after i.v. injection of 3.25 mg/kg. Data from Jancso et al. (1981)[a]

Subject	Body wt (kg)	Urine vol (ml)	DMAP-glucuronide	DMAP-sulfate	DMAP-thioethers	Total
1	85	2,740	39.4	10.4	27.0	77.8
2	80	2,615	27.0	12.3	18.2	57.5
3	72	935	28.7	10.7	19.4	58.8
[a] $n = 6$	75.7±3.3	1,615±225.2	40.7±4.3	11.6±1.6	15.3±1.8	67.6±4.1

tiredness over 4 h. Nine-hundred milligrams DMAP was tolerated by one subject without any symptoms. Four subjects felt slightly dizzy and dull, a mild pressure in the forehead or ear, and buzzing in the ears; one person saw sparks.

5. Metabolites of DMAP in Blood and Urine

The pattern of metabolites in the urine collected over 24 h from the subjects that received three different oral doses of DMAP is shown in Table 4. On average 54% of the DMAP administered in the three groups was excreted as

Table 6. Metabolites of DMAP (nmol/ml) in human blood 15 min after i.v. injection of 3.25 mg/kg

Subject	Metabolites in plasma			Metabolites in red cells
	DMAP-glucuronide	DMAP-Sulfate	Tris-(GS)-DMAP	Tris-(GS)-DMAP
1	4.8	3.1	2.5	36
2	4.6	6.2	0.7	39
3	6.0	4.7	0.8	22
Dog[a] $n = 1$	4.8	22	2.7	26

[a] Data from Eyer and Gaber (1978)

metabolites; approximately 41% consisted of DMAP-glucuronide, 7% of DMAP-sulfate, and 6% of DMAP-thioethers. When DMAP was given i.v. the total of the metabolites recovered in the urine was somewhat higher and the proportion of thioethers increased significantly (Table 5). In Table 6 the profile of DMAP metabolites in the blood of three subjects is compared with the data obtained from a dog (Eyer and Gaber 1978).

Discussion

The antidotal activity of a ferrihemoglobin forming agent in acute cyanide poisoning is dependent essentially on the initial oxidation rate of the heme iron in vivo. On the other hand, ferrihemoglobin formation should not exceed some 30% of total hemoglobin in order to avoid insufficient oxygen transport because of lower oxygen capacity. These requirements are met by DMAP. 3.25 mg DMAP/kg administered i.v. produces about 30% ferrihemoglobin within 10 min, the initial oxidation rate being 9%/min, so that three lethal i.v. doses of cyanide (LD 0.026 mmol/kg; Paulet 1957) may be detoxified within 1 min.

The disadvantage of i.m. or oral administration of DMAP is the delayed onset of ferrihemoglobin formation. A lag phase of 2–3 min between i.m. injection or oral ingestion of DMAP and the beginning of ferrihemoglobin production as found in man is certainly negligible in comparison with the much longer time that is lost awaiting treatment or when the physician himself fails in injecting the antidote i.v.

Allowing for the statistical deviation, increases in the ferrihemoglobin content (Figs. 1 and 2) of man and dog seem to be linear from the end of the lag phase to the cross-marked (+) points where the curves are beginning to flatten. With equieffective doses of DMAP which produce about 30% ferrihemoglobin, i.e., 3.25 mg/kg i.v., 3.5 mg/kg i.m., and 12 mg orally, the corresponding initial rates of ferrihemoglobin formation were 9%/min, 2%/min, and 2%/min. Obviously, the preparation of DMAP for i.m. use contained a constituent that inhibited absorption from the muscle in comparison with the i.v.

preparation since the initial rate of ferrihemoglobin formation (0.9%/min) was lower by about one half.

In dogs, the initial rate of ferrihemoglobin production due to i.v. or i.m. injection of DMAP was about three times (28%/min) and twice (3.5%/min) as high as in man while being equal (2%/min) after oral administration of comparable doses.

The adverse effects of DMAP were severe only after i.m. injection. The administration of DMAP by this route apparently resulted in aseptic necrosis of muscular tissue as indicated by local pain, fever, and elevated activities of CPK, LDH, and GOT. Muscular necrosis may be due to a direct action of the phenolic compound itself or may be mediated by ferrimyoglobin formation (unpublished results) such as to inhibit the oxygen transfer from blood to myofibrils. The local irritation observed at the site where DMAP was injected intravenously is in accord with this view.

The other less harmful adverse effects may be accounted for by impaired oxygen supply that may result from lowered oxygen capacity. It is doubtful whether a viable injection was given to the subject who showed a painless response and a very low ferrihemoglobin formation; possibly the antidote was deposited in adipose tissue.

The liver function seems not to be affected by DMAP since the activities of the liver-specific enzymes GPT and γ-GT remained unchanged.

The changes in bilirubin and iron concentration are indicative of a life-span shortening action of DMAP on erythrocytes that takes about 1 day to develop. Since the hemolysis that was found in the test for osmotic resistance was maximal after about the same time as in vivo one may assume a common lytic mechanism under in vitro and in vivo conditions.

Release of myoglobin and iron from necrotic muscle may have contributed to the increase in bilirubin and iron concentration after i.m. injection of DMAP. This increase amounted to only 0.1–0.4% of the hemoglobin equivalents of normal blood in the different groups but the degree of hemolysis was sufficient to stimulate erythropoiesis as demonstrated by the reticulocyte counts.

Impairment of membrane stability may be due to lowered content of reduced glutathione in erythrocytes caused by thioether formation with an electrophilic DMAP intermediate (Eyer et al. 1976, 1978) or to a direct action of this intermediate with membrane proteins. Shortening of life span has been reported also for canine erythrocytes to which ^{14}C-labelled DMAP had been bound covalently (Eyer and Gaber 1978).

The lack of Heinz bodies supports the view that ferrihemoglobin production alone is not sufficient for Heinz body formation (Beutler 1969; Kiese 1974).

Uptake of bilirubin into tissue occurs only when the serum bilirubin level exceeds the threshold value of 34 μmol/l (20 mg/l). Subsequently, the subjects who were injected with DMAP i.m. showed yellow sclerae whereas scleric jaundice was not seen in all the other cases.

It is not surprising that bilirubin was not detected in the urine because bilirubin-glucuronide does normally not appear in the urine until its plasma level exceeds 26 μmol/l; the maximal concentration of conjugated bilirubin, however,

was always much lower. The actual serum iron concentrations with maxima ranging between 30 and 40 μmol/l were not high enough to exhaust the iron binding capacity of transferrin (70–90 μmol/l).

The decrease in the serum lactate/pyruvate ratio (26%) and the constancy of the lactate concentration after i.v. injection of the antidote in humans are the same as found with dogs (Klimmek et al. 1979). Reduction of ferrihemoglobin by lactate (Wendel 1931) and diminution of the conversion of pyruvate to lactate brought about by the oxidation of NADH in the presence of oxidized DMAP (Klimmek et al. 1979) may account for the tansient increase in the pyruvate concentration.

Determination of urinary metabolites of DMAP after its administration to man has already been reported elsewhere (Jancso et al. 1981). At that time, all metabolites were determined by the direct isotope dilution technique requiring column chromatography, enzymatic or Raney nickel cleavage and thin layer chromatography for each assay. This time consuming procedure was replaced by HPLC using paired ion chromatography. The normal urinary constituents of the blank urines did not absorb at 254 nm wavelength during the elution period of DMAP-glucuronide and DMAP-sulfate.

The partition of the metabolic fractions as shown in Table 4 indicates that the formation of glucuronide, sulfate, and thioethers is likely to operate within the linear range of Michaelis-Menten kinetics.

Incomplete absorption of DMAP and its partial degradation in the alkaline medium of the intestinal tract may account for the diminution of total metabolite recovery after oral administration in comparison with i.v. injection; DMAP rapidly autoxidizes at pH values above 7 and is transformed to a variety of degradation products (Eyer et al. 1974).

Rapid thioether formation with DMAP has been shown to occur in red cells where DMAP is oxidized by oxyhemoglobin to the corresponding quinonimine, which yields adducts with red cell glutathione (Eyer and Kiese 1976). One of these, tris-(GS)-DMAP, slowly penetrates the red cell membrane and is excreted in the urine mainly as a tris-cysteinyl derivative, tris-(Cys)-DMAP. Eyer and Gaber (1978) have shown that this pathway is used by dogs after i.v. injection of tris-(GS)-DMAP. The compound is presumed to be broken down in the γ-glutamyl cycle of the kidney (Meister 1973) so that cysteinyl glycine and cysteinyl derivatives arise.

From the amount of tris-(GS)-DMAP in the red cells of dogs and the amount of thioethers excreted with their urine Eyer and Gaber (1978) estimated that virtually all thioethers found in the urine must have originated from the red cells. This conclusion is supported by the fact that the thioether excretion is negligible when the formation of glutathionyl thioethers is slight, as previously shown for rats where DMAP is bound preferentially to the abundant SH-groups of rat hemoglobins (Eyer and Kampffmeyer 1978).

Table 6 shows that the tris-(GS)-DMAP concentrations in blood were similar in man and dog and that also the proportions of DMAP-thioethers as found in the urine samples were equal to one another. Since the reaction of oxidized DMAP with intracellular glutathione is very fast and tris-(GS)-DMAP penetrates the red cell membrane with an apparent half-life of 1 h (Eyer 1978),

the average amount of tris-(GS)-DMAP at the time t_0 may be estimated at about 38 nmol/ml of whole blood. Hence, by assuming a total blood weight of $^1/_{12}$ of the body weight, from the 18.8 μmol DMAP/kg injected some 3.2 μmol tris-(GS)-DMAP/kg, i.e., 17% must have been formed in the red cells. Thus the DMAP thioethers excreted with the human urine (15.3 ± 1.8% of the dose; Table 2) are likely to originate mainly from red cells; this underlines the importance of red cells in DMAP metabolism in man.

On the other hand, the liver has been shown to conjugate DMAP rapidly to DMAP-glucuronide and DMAP-sulfate. Up to 0.1 mM DMAP can be conjugated nearly quantitatively during protein-free single pass perfusion of rat liver (Eyer and Kampffmeyer 1978). Correspondingly, oral administration of DMAP could entail a significant first pass effect. The lower proportion of DMAP-thioethers after oral administration of DMAP compared with i.v. injection (Table 4 and 5) seems to confirm this assumption.

Only few seconds are available for the reaction of DMAP with red cells after intestinal absorption because it is rapidly extracted from the portal blood and conjugated by the liver. Subsequently, maximum ferrihemoglobin formation was considerably reduced when DMAP was given orally. Thus more than three times the i.v. dose must be administered orally to man and more than four times the i.v. dose to dogs to achieve the same effect in erythrocytes.

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