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Short Communications

Ferrihemoglobin and Kidney Lesions in Rats Produced by 4-Aminophenol or 4-Dimethylaminophenol

M. Kiese, L. Szinicz, N. Thiel and N. Weger

Pharmakologisches Institut der Universität München, München, FRG

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Abstract. 4-Dimethylaminophenol hydrochloride (DMAP), 20 mg/kg i.v., was found to oxidize in rats as much as 50 % of the hemoglobin to ferrihemoglobin but did not cause kidney lesions. 4-Aminophenol hydrochloride, 400 mg/kg i.v., oxidized only 25 % of the hemoglobin and produced large tubular necroses. In highly toxic doses only, e.g., twice the LD_{50}, DMAP also produced tubular necroses.

Key words: 4-Dimethylaminophenol — 4-aminophenol — Ferrihemoglobin — Kidney lesions.

Zusammenfassung. 4-Dimethylaminophenolhydrochlorid (DMAP) 20 mg/kg i.v., oxidierte in Ratten 50 % des Hämoglobins zu Ferrihämoglobin, verursachte aber in dieser Dosis keine Nierenschäden. 4-Aminophenolhydrochlorid, 400 mg/kg i.v., oxidierte nur 25 % des Hämoglobin, bewirkte aber ausgedehnte Nekrosen der Nierentubuli. Nur in höchst toxischen Dosen, etwa der zweifachen LD_{50}, bewirkte auch DMAP Nekrosen der Tubuli.

Schlüsselwörter: 4-Dimethylaminophenol — 4-Aminophenol — Ferrihämoglobin — Nierenschäden.

Kiese *et al.* (1966, 1969) have shown that 4-dimethylaminophenol after intravenous injection rapidly produces limited amounts of ferrihemoglobin and that it is superior to nitrite in the treatment of cyanide poisoning. Since 4-aminophenol after i.v. injection has been found to produce kidney lesions (Hinsberg and Treupel, 1894; Green *et al.*, 1969; Calder *et al.*, 1971), it was of interest whether DMAP also causes kidney lesions in doses which produce therapeutic amounts of ferrihemoglobin.

Materials and Methods

Male Sprague-Dawley rats weighing 150 to 200 g were used. They were fed Altromin standard diet and water ad lib. In experiments on the effect of low sodium or high sodium on kidney lesions the rats were fed low sodium Altromin diet and water ad lib or the same diet with 60 g sodium chloride per kg and 0.9 % sodium chloride solution ad lib. The control animals received the same diet with 6 g sodium chloride per kg and water ad lib.

Blood for the determination of ferrihemoglobin was obtained by cutting off the tip of the tail, or from the blood vessels when the animals were sacrificed by decapitation. Ferrihemoglobin was measured by the increase in absorbance at 550 nm after the addition of potassium cyanide (Kiese, 1959).

For the collection of urine rats were kept individually in metabolic cages for 48 hrs. Combi-Uristix (Ames) were used to detect increased glucose content of urine.

Abbreviations used: DMAP = 4-Dimethylaminophenol hydrochloride, AP = 4-Aminophenol hydrochloride.

At various intervals after the administration of DMAP or AP, animals were sacrificed and samples of kidneys, livers, hearts, spleens, and lungs prepared for microscopic examination.

Tissues were fixed in 3% formaldehyde and studied with standard histologic techniques.

Results

Acute Toxicity of 4-Dimethylaminophenol

Five groups of 10 rats each were injected i.v. with various doses of DMAP. Animals which did not survive the respective dose died within 7 to 40 min after injection of DMAP. Only one animal died 4 days after injection. The LD_{50} was calculated according to van der Waerden (1940) to be 57 ± 1[1] mg/kg. The LD_{50} after intraperitoneal injection of DMAP was found to be 90 ± 1[1] mg/kg.

Formation of Ferrihemoglobin

The data presented in Fig. 1 show that DMAP, 20 mg/kg i.v., rapidly oxidized half the hemoglobin in rats. The maximum of the ferrihemoglobin concentration was observed between 10 and 20 min after i.v. injection. Then the ferrihemoglobin concentration quickly decreased. AP produced ferrihemoglobin more slowly and was much less effective than DMAP. Intravenous injection of AP, 400 mg/kg, raised the ferrihemoglobin concentration only half as high as DMAP, 20 mg/kg. 20 min after i.v. injections of AP, 250 mg/kg, or 100 mg/kg, the ferrihemoglobin concentration was found increased by 15.4 and 3.8% of the total hemoglobin, respectively.

Microscopic Examination of the Kidneys

For the study of the effect on the kidneys 30 rats were injected i.v. once with DMAP or AP. After 1, 2, 3, 7, and 14 days 6 animals were sacrified and the tissues microscopically examined. Intravenous injection of DMAP, 100 mg/kg, i.e., nearly

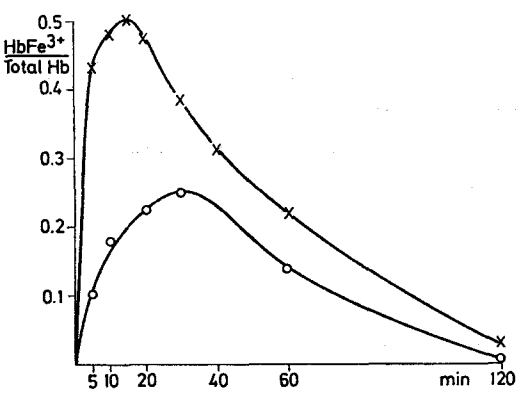

Fig. 1. Formation of ferrihemoglobin in rats by 4-dimethylaminophenol hydrochloride or 4-aminophenol hydrochloride. ○ 4-Aminophenol hydrochloride, 400 mg/kg i.v.; 6 experiments. × 4-Dimethylaminophenol hydrochloride, 20 mg/kg i.v. injected in 5 sec; 4 experiments

[1] SEM.

twice the LD_{50}, which oxidized about 60% of the hemoglobin to ferrihemoglobin, caused renal damage in the rats. Moderate to large necrosis of the convoluted tubules with no or only a few inflammatory cells was found 24 hrs after injection. The glomeruli were not affected, nor was papillary damage found.

The amount of inflammatory cells increased up to the 7th day postinjection. 4 days after injection some tubules were found to be coated with a new flat basophilic epithelium. 1 week after injection the regeneration of the tubules was nearly complete, and 2 weeks after the injection no signs of tubular damage were detectable, except increased amounts of inflammatory cells.

No tubular necrosis was found after injection of DMAP, 30 mg/kg. The only difference from the findings in control animals was a slight increase in inflammatory cells, 1 and 2 weeks after the injection of DMAP.

The i.v. injection of AP, 400 mg/kg, which transformed only 25% of the hemoglobin to ferrihemoglobin, caused large bandshaped necroses of the convoluted tubules in the deepest cortical zone. 1 and 2 days after injection only a few inflammatory cells were found. Henle's loops and collecting tubules were filled with eosinophilic debris. 4 days after injection regeneration of tubular epithelium was observed, but it was still incomplete 2 weeks after injection. The inflammatory reaction was most severe 1 and 2 weeks after injection.

Lower doses of AP caused less severe renal damage. After 250 mg/kg microscopic findings in the kidneys were similar to those described above with DMAP, 100 mg/kg. Tubular necrosis was also found after i.v. injection of AP, 100 mg/kg. Regeneration was complete after 2 weeks.

High or low sodium diet did not noticeably affect the nephrotoxicity of DMAP or AP.

No pathologic changes were found in livers, hearts, or spleens. Changes in the lungs were not more frequent than in the control animals.

Glucose was found in the urine of all of 10 rats i.v. injected with DMAP, 50 mg/kg. Only 5 of 10 rats injected with AP, 250 mg/kg, showed increased glucose content of urine.

Discussion

The ferrihemoglobin-forming activity of DMAP in rats was found to be higher than in mice and rabbits and much lower than in dogs and cats, as determined by Kiese and Weger (1969). As in these earlier experiments, the net ferrihemoglobin formation is in inverse proportion to the rate of ferrihemoglobin reduction which is found between the rates of mice and rabbits on the one hand and dogs and cats on the other.

Our experiments with AP confirm the results of Green *et al.* (1969) and Calder *et al.* (1971, 1975). The latter studied a variety of aminophenols and their derivatives and found large differences in nephrotoxicity. 4-Methylaminophenol in a dose as low as 12 mg/kg produced necrosis of the distal third of all proximal convoluted tubules. This seems to be an effect even stronger than the effect of 4-dimethylaminophenol, 100 mg/kg, in our experiments. Calder *et al.* (1971, 1975) give no data on the ferrihemoglobin formation by 4-methylaminophenol. Therefore, no comparison of nephrotoxicity on the basis of ferrihemoglobin-forming activity is

possible. But there is no doubt that the nephrotoxicity of 4-dimethylaminophenol is much lower than that of 4-methylaminophenol. Compared on the basis of ferrihemoglobin-forming activity the nephrotoxicity of DMAP is much lower than that of 4-aminophenol. A dose of DMAP 20 mg/kg, which quickly oxidizes 50% of the hemoglobin and therefore would be very effective in the treatment of cyanide poisoning did not cause any tubular necrosis in the kidneys. In species where DMAP produces therapeutically effective ferrihemoglobin concentrations with much smaller doses, in dogs, cats, and humans, no kidney damage is to be expected. This has been confirmed by as yet unpublished experiments on the chronic toxicity of DMAP in dogs. Intravenous injections twice a week for 15 weeks of DMAP, 3 mg/kg, which oxidized 30 to 40% of the hemoglobin, did not produce any kidney lesions.

The mechanism of the glucosuria observed after a large dose of DMAP, 50 mg/kg, needs further investigation.

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Prof. Dr. Manfred Kiese
Pharmakologisches Institut der Universität
D-8000 München
Nussbaumstr. 26
Federal Republic of Germany