URAEMIC SYMPTOMS AND COMPLICATIONS INDUCED IN DOGS CHRONICALLY INTOXICATED WITH CREATININE AND METHYLGUANIDINE


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In a previous study it was found that guanidines, creatinine and creatine increase the amount of haemolysis which occurs spontaneously in normal blood during incubation at 37°C (Giovannetti et al., 1968b). It was found subsequently that methylguanidine accounts for almost all the monosubstituted guanidines which accumulate in chronic uraemia (Giovannetti et al., 1968a).

To check whether methylguanidine and creatinine induce haemolysis also ‘in vivo’ and/or cause other uraemic symptoms, 18 mono-nephrectomized mongrel dogs were intoxicated with methylguanidine, 7 with creatinine and 6 controls receiving saline were studied.

MATERIALS AND METHODS

Creatinine (Erba, S.p.A., Milan) was injected every 8 hours at single doses of 300 mg/kg body weight. This induced plasma creatinine concentrations ranging from about 35 to about 15 mg%. After 14–18 days of intoxication dogs were killed with an intravenous injection of pentobarbital.

The intoxication with methylguanidine was started by injecting subcutaneously 30 mg/kg body weight of methylguanidine hydrochloride (Schuchardt, Munich) every 8 hours. After 4–5 days the frequency of the injections was increased to 4 per day and, after the next 4–5 days, it was increased again to 5 injections per day. The plasma concentrations of methylguanidine induced in dogs by these doses were of 1.0–1.5 mg% after 20–30 minutes from the injection. They decreased rapidly in 20–30 minutes and then very slowly during the following hours. Before the next injection they were 0.3–0.6 mg%.

All the animals intoxicated with methylguanidine refused drink and food after the first days of intoxication while the urine output remained constant or even increased. In order to prevent a salt and water depletion and to supply calories, they received daily intravenous injections of 70–90 ml of a modified Ringer solution containing 15 g% of dextrose. Multivitamin commercial products were also injected daily. All the dogs intoxicated with methylguanidine died spontaneously after 8–19 days of intoxication.

Anaemia was estimated on the basis of the red cell mass which was calculated from the haematocrit and the plasma volume measured with the radioiodated albumin. This parameter was selected in order to avoid possible mistakes due to overhydration or dehydration.

Standard procedures were used to measure the erythroid iron turnover and the red cell survival was determined as previously described (Giovannetti et al., 1966).

The motor nerve conduction velocity was measured by using a standard electromyograph machine which had a synchronized stimulator and oscilloscope. The results were photographed for measurements.

Plasma creatinine was determined with an Autoanalyzer and methylguanidine with the method described by Yatzidis et al. (1966).
RESULTS

The body weight of the animals receiving methylguanidine decreased every day by about 1.4% of the basal value, starting from the beginning of intoxication when they were still eating and in the cases whose survival was long enough cachexia became evident during the last days of life.

Fig. 1. The per cent changes in the red cell mass which occurred in dogs intoxicated with creatinine and methylguanidine (black circles). The behaviour of the controls is indicated by the white circles.

Fig. 2. The behaviour of the erythroid iron turnover and of the red cell survival in the controls, in the dogs intoxicated with creatinine and in those intoxicated with methylguanidine.
URAEMIC SYMPTOMS IN DOGS INTOXICATED WITH CREATININE AND METHYLMGUANIDINE

Since the daily per cent decrease in the body weight which occurs in dog during the first days of starvation is about the half of that of these animals (which on the other hand, received about 40 calories per kg body weight as dextrose), we may conclude that methylguanidine exerts a strong catabolic effect.

Anaemia developed both in the creatinine and in the methylguanidine group, being, however, much more rapidly progressive in the latter (Fig. 1). The erythroid iron turnover was reduced severely only in the animals intoxicated with methylguanidine, but the red cell survival was severely shortened also in those receiving creatinine (Fig. 2).

In the animals intoxicated with methylguanidine the spleen was pale and contracted and the Peres stain for iron revealed large haemosiderin deposits in it. These were also present, though less pronounced, in the spleen of the animals intoxicated with creatinine.

Fig. 3. Multiple ulcerations in the stomach of a dog which died after 15 days of methylguanidine intoxication.

Fig. 4. The microscopic appearance of a gastric ulceration in a dog intoxicated with methylguanidine. Haematoxylin and eosin.

221
Fig. 5. Electromyograms of the plantar muscle obtained in a dog before (above) and after 7 days of intoxication with methylguanidine. On the left is shown the proximal stimulation (sciatic nerve at the trochanter); on the right is shown the distal stimulation (posterior tibial nerve at the distal insertion of the Achilles tendon). The latency (between the stimulation artefact and the beginning of the muscle potential) of the basal state is indicated by the arrows and its increase, after intoxication, is well evident.

Fig. 6. Longitudinal sections of the posterior tibial nerve of a dog intoxicated with methylguanidine and which died after 15 days. Right: axis cylinders appear swollen, fragmented and interrupted (Glees stain). Left: foci of demyelinization (osmic acid stain).
Diarrhoea was present in almost all the 18 dogs intoxicated with methylguanidine; in 5 melaena occurred and in 2 of these also haematemesis was observed.

Necropsy revealed the presence of single or multiple ulcerations in the stomach (11 cases) (Fig. 3) or in the duodenum (2 cases). Haemorrhages in the mucosa and submucosa were also found in the stomach (3 cases) and in the duodenum (3 cases).

The histological examination revealed that the ulcerations affected only the mucosa (Fig. 4). In 2 animals which had developed melaena and which had blood in the bowel at the necropsy, no lesions were found which might account for these findings. An ileo-caecal intussusception was found in 3 dogs and in 12 a fatty degeneration of the liver was detected with patchy areas of centrilobular necrosis in 3.

In the creatinine group no digestive symptoms were noted; however, necropsy revealed a severe congestion of the duodenal mucosa in all the 5 cases examined. In 2 of them small ulcerations were also detected in the stomach and in the duodenum.

Severe nervous symptoms appeared in all the animals intoxicated with methylguanidine. The early stage of intoxication was characterized by ataxia, incoordination, hypertonia, fibrillary tremors, clonic twitching and generalized convulsions. In the late stage drowsiness appeared which might be considered as a semicomatous state in 3 cases. In 4 animals a paresis occurred which was particularly evident in the hind legs.

Similar results were obtained by Mason et al. (1937) who intoxicated dogs acutely with guanidine and methylguanidine at various doses.

The existence of pruritus was suggested by the fact that dogs continuously scratched their muzzles.

The motor nerve conduction velocity was measured in the hind legs of 9 dogs and in 7 it was found to be reduced. The increase in the latency (between the stimulus and the muscle potential) was much higher in the distal nerves (+61.5%) than in the proximal ones (+37.7%) (Fig. 5). This demonstrates that the function of distal nerves was more impaired as has been observed in the human uremic neuropathy (Versaci et al., 1964).

The histological examination revealed that peripheral nerves, whose conduction velocity had been found to be reduced, were affected by severe regressive changes. Axis cylinders were swollen, fragmented and disintegrated and foci of secondary demyelination were present (Fig. 6).

The histological examination of the central nervous system revealed also degenerative changes in the fibres of the spinal cord and of the cerebellar and cerebral cortex.

Stertorous breathing and cough were common symptoms during the late stage of intoxication with methylguanidine. Macroscopic examination of the lungs revealed severe congestion and a bronchopneumonia in 3 cases. Areas of oedema and alveolar haemorrhages were also detected at the histological examination.

It is noteworthy that these findings were present in areas of the lungs apparently not affected by infectious complications.

Tachycardia was common in the animals intoxicated with methylguanidine; in one dog an atrial fibrillation and in another an atrio-ventricular dissociation was clinically diagnosed. The histological examination of the myocardium revealed focal degeneration of the fibres.

DISCUSSION

The clinical and pathological abnormalities which appear in dogs intoxicated with methylguanidine closely resemble those of uraemia. Hypercatabolism, haemolysis and reduced red cell production, acute ulcerations and haemorrhages in the stomach and duodenum, neurological disturbances with degeneration of the fibres in the central nervous system, peripheral neuropathy, pruritus, arrhythmias and myocardial degeneration, congestion, oedema and haemorrhages in the lung are well-known symptoms and complications of uraemia and all appear in dogs intoxicated with methylguanidine.
Also creatinine induces symptoms, such as haemolysis and gastro-duodenitis, which are typical of uraemia.

Since creatinine and methylguanidine accumulate in renal insufficiency, the conclusion is justified that both these metabolites, but chiefly the latter, play an important role in the genesis of the uraemic syndrome.

The fact that practically all the organs are damaged in the animals intoxicated with methylguanidine is well explained by the strong enzymatic inhibition which is exerted by this metabolite.

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REFERENCES


