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ORIGINAL ARTICLES

CLINICAL SIGNS, RADIOLOGY AND TISSUE LEAD DISTRIBUTION OF DOGS ADMINISTERED A MIXTURE OF LEAD CHLORIDE, LEAD BROMIDE AND LEAD SULPHATE

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SUMMARY Eight-month-old dogs maintained on a high-fat-low-calcium diet were administered a mixture of lead chloride, lead bromide and lead sulphate for prolonged periods at 4 different dose levels. Dogs on high levels of lead showed marked weight loss and gastrointestinal symptoms followed by death. Two dogs on low lead levels developed neurological signs. Radiological investigations showed radiopaque particles in 27 per cent of abdominal radiographs and 'lead lines' in the distal radius of 3 dogs. Highest tissue lead levels were found in bones followed by liver and kidney, brain and spinal cord.

Introduction

Lead in petrol is the major source of pollution in the United States (Beliles 1975). During recent years much attention has been given to the study of chronic lead exposure in animals. Most of the experimental studies have used lead acetate or lead carbonate for producing the toxicity but neither of these substances are significant constituents of exhaust emission of internal combustion engines using leaded petrol.

The purpose of this paper is to report on the clinical observations, radiological investigations and tissue distribution of lead in dogs which were exposed for prolonged periods to a lead salt mixture at different dose rates.

The lead salt mixture consisted of lead chloride‡, lead bromide‡, and lead sulphate‡ in 1:1:2 proportions respectively. These lead compounds in approximately similar proportions are the major byproducts in the exhaust emission of internal combustion engines which utilise leaded petrol (Bloom 1979 personal communication).

Materials and Methods

Animals and Diet

Eleven Kelpie-cross dogs of 8 months of age were obtained for the purpose of this experiment. Prior to the commencement of the experiment, the dogs were vaccinated against canine distemper, canine hepatitis, parvovirus infection (using inactivated feline enteritis vaccine) and were dewormed (Praziquantel§ and Pyrantel Pamoate‖). The dogs were then divided into 5 groups of 2 dogs, except group 5 which had 3 dogs. All the dogs were maintained on a high-fat-low-calcium diet which consisted of minced meat scraps and fat. Each dog received 500 g of this diet per day in a stainless steel container. The diet was analysed and found to contain 35% to 45% fat and less than 0.1% calcium (dry weight basis). After a period of stabilisation on this diet (7 days), the 5 groups of dogs were administered the lead salt mixture at the dose rate of 0, 5, 15, 30 and 60 mg per kilogram body weight per day (Table 1). The lead salt mixture was weighed into gelatin capsules and was administered orally. The control group (group 4) was given 2 mg sodium chloride* (containing less than 0.005% lead) per day in gelatin capsules. The dogs were weighed once a week and the lead dose for the week was calculated on this weight.

Samples

Blood samples (7.5 ml) were drawn from the jugular vein of each dog. 5 ml of this blood was placed in plastic tubes containing heparin (for lead analysis) and the remainder was placed in EDTA (for routine hematology). Samples were collected daily for the first 3 days and then weekly for the remainder of the experimental period (21 weeks). Routine hematology was carried out using standard laboratory methods.

Radiographs were taken of the right foreleg (A-P view) and of the lateral abdomen (which also included the lumbar vertebrae) of each dog prior to the commencement of the experiment. The radiographs were repeated approximately every 30 days to detect lead particles in the gastrointestinal tract and to monitor changes in the bones. Radiographs of all the dogs were taken at the same time, except in the case of the dogs that died within the first 30 days in which case the radiographs of the right foreleg were taken after death.

A complete post mortem examination of all dogs was carried out immediately after death. Samples of liver, kidney, right distal radius, fourth lumbar vertebra, cerebrum (frontal lobe) and lumbar spinal cord were frozen for subsequent determination of lead content.

Representative tissues of most organs including whole brain were preserved in 10% buffered formalin for histopathological examination. The pathological findings will be published elsewhere.

Chemical Analysis

All chemical analyses were performed at the Victorian Department of Agriculture, Division of Agricultural Chemistry

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§ Bayer Australia Ltd., Botany, New South Wales
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* May and Baker Ltd., Dagenham, United Kingdom

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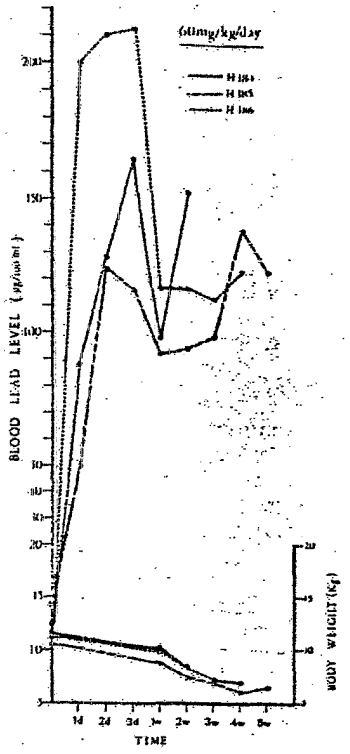

Figure 5. Body weights (kg) and blood lead levels (μg/dl) of dogs administered lead at 60 mg per kilogram body weight per day.

3 days. The level then dropped markedly for the next 2 weeks and then gradually increased again (Figures 3 and 5).

In group 4 (control dogs), the blood lead level increased slightly and remained above 35 μg/dl in dog H182 for approximately 9 weeks (Figure 4). Dog H183 was killed by euthanasia on day 58 since by this time more than half the experimental dogs were dead.

Hematology

Routine hematological findings indicated a slight drop in the packed cell volume in the dogs fed lead. Early in the course of the experiment, nucleated red blood cells and basophilic stippling were visible in all dogs dosed with lead, but this was not a regular feature of blood smears from these dogs. No nucleated red blood cells or basophilic stippling were seen in the blood from the control dogs.

Radiology

Table 2 shows the radiological findings in the lead administered dogs. Lead particles were detected in the gastrointestinal tract in only 4

TABLE 2

Monthly Radiographic Examination of Abdomens of Lead-Administered Dogs

Dog No.	Dosage	No. of X-rays Taken	No. Showing Lead Particles
H 176	5 mg/kg/day	–	–
H 177	5 mg/kg/day	6	1
H 178	15 mg/kg/day	–	–
H 179	15 mg/kg/day	3	2
H 180	30 mg/kg/day	2	0
H 181	30 mg/kg/day	2	1
H 184	60 mg/kg/day	–	–
H 185	60 mg/kg/day	2	0
H 186	60 mg/kg/day	–	–
Total (9 dogs)		15	4 (27%)

abdominal radiographs (27% of the total radiographs of the dogs fed lead).

'Lead lines' (radiopaque lines) were detected in the right distal radius of 3 dogs (one each from groups 2, 3 and 5) and were not seen in the lumbar vertebral bodies of any dogs. The 'lead lines' were visible in the first series of radiographs which were taken on day 30 of the experiment. At this time they were rather faint and could only be appreciated when the radiographs were compared to the pretreatment films. These lines became progressively denser in subsequent radiographs (Figure 6B). Radiopaque particles in the alimentary tract or 'lead lines' were not seen in the radiographs of the control dogs.

Tissue Lead Levels

Tissues from the dogs administerd lead showed higher levels of lead than did the tissues of the control dogs (Table 1). Generally the highest lead levels were seen in the distal radius, followed by the lumbar vertebra, the bones of the calvarium, liver and kidney, cerebrum and spinal cord. The dogs showing 'lead lines' on their radiographs had the highest lead levels (over 400 ppm) in the distal radius and dogs that developed neurological signs had high lead levels (over 3 ppm) in the cerebrum.

Discussion

Anorexia, weight loss, vomiting and diarrhoea as seen in the dogs dosed with lead have been reported in accidental and experimental cases of lead poisoning (Zook *et al* 1969; Stowe *et al* 1973; Stowe and Vandevelde 1979; Hamir 1981). In the low dose group (5 mg/kg), vomiting and diarrhoea were not observed. Thompson (1979) and Mitema *et al* (1980), who used lead carbonate

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ment. The efficacy of our experimental procedure in producing nervous signs thus campares favourably with that achieved by previous workers. This suggests that the simple diet of meat scraps and fat imparted a significant predisposition to lead encephalopathy. However, our results also emphasize that the development of the neurological signs is favoured by the use of low levels of lead (5 to 15 mg/kg).

A blood lead level of 35 μg/dl is considered as the upper normal limit for dogs (Zook 1978) and levels of 60 μg/dl or more are diagnostic of lead poisoning (Zook and Carpenter 1977). In our study blood lead levels increased to above 120 μg/dl in all lead administered dogs. The increase was dependent on the dose level and the duration of exposure to lead. This was also observed by Thompson (1969) and Mitema *et al* (1980) who used lead carbonate and lead acetate respectively to produce lead poisoning in dogs. However, in our study, the rise in blood lead concentration in dogs dosed with lead was steeper and of greater magnitude than was found by these workers (Figures 1, 2, 3 and 5). This suggests that either the lead salts used were more readily absorbed from gut than are lead acetate and lead carbonate or that the high-fat-low-calcium diet was conducive to increased lead absorption. Our view is that the experimental diet played a major role in increasing lead absorption since Barttrop and Khoo (1976) have demonstrated that diets deficient in minerals or containing a high proportion of dietary fat increased lead absorption from the gut of laboratory animals by 20 and 7 fold respectively.

The slight increase in the blood lead level of the control dogs between the first and the 13th week of experiment was attributed to the control dogs coming in contact with dogs administered lead during routine experimental procedures (weighing, radiography and sample collection) since an extensive search aimed at detecting any possible source of contamination during collection, storage, handling and analysis of blood failed to reveal any source of lead contamination. The assumption that the control dogs acquired lead from the dogs administered lead appears to be valid since after the tenth week of the experiment, only one dog fed lead remained and soon after that the blood lead level of the remaining control dog (H182) decreased markedly (Figure 4).

Radiological investigations of dogs suspected of having lead poisoning for detection of lead particles in gastrointestinal tract and for detection of 'lead lines' in long bones has been recommended in the standard veterinary texts (Oehme 1975; Buck and Hoskin 1979) and in published work (Zook *et al* 1969). Zook *et al* (1969) however, recommended further investigations on the usefulness of detection of 'lead lines' as diagnostic aid in cases of lead toxicity.

Results of our radiological investigations were not particularly rewarding since only 27 per cent of abdominal radiographs showed lead particles in the alimentary tract and only 3 dogs (33 per cent of lead-administered dogs) had 'lead lines' in the radius. These findings are however comparable to those of Zook *et al* (1969) who found radiopaque particles in 32 per cent and 'lead lines' in 39 per cent of cases of accidental lead poisonings.

In the present experiment, the animals were maintained on a high-fat-low-calcium diet and the lead was administered in a powdered form. Since all 3 factors, high dietary fat, low dietary calcium and small particle size of lead are known to increase lead absorption (Barttrop and Khoo 1976; Barttrop and Meek 1979), it is probable that a high proportion of the administered lead was absorbed from the intestines and this may have contributed to negative radiological findings in a large proportion of the abdominal radiographs.

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