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Experimental fluoride poisoning in Icelandic sheep

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SUMMARY

Fluoride was administered as an aqueous solution of sodium fluoride *per os*, five days a week for 20 weeks, to three groups of four 6–8 months old ewe lambs. The first group (A) received 5mg/kg, the second group (B) 10 mg/kg and the third group (C) 15 mg/kg. A fourth group of four lambs (group D) served as controls and was administered water instead of fluoride. Once a week each animal was examined and weighed and blood samples collected for the analysis of fluoride, calcium, magnesium, hemoglobin, hematocrit, γ-glutamyl transferase, bilirubin, glucose and creatinine. At the end of the experiment the animals were killed and necropsied.

Diarrhea, loss of appetite and general prostration were the most overt signs of acute fluoride poisoning in this study. These symptoms were seen in groups B and C, but were absent in group A. At necropsy no gross pathological changes were seen in the inner organs, bones or teeth or any pathological abnormalities observed by histological examination. No changes were seen in the plasma levels of the substances determined in blood or plasma except for fluoride levels that rose steadily during the experiment. The pharmacokinetics of fluoride was seemingly linear at these doses as judged by a linear relationship between dose and the concentration of fluoride in plasma and mandibles, respectively. Plasma levels of fluoride above 860 ng/ml were always associated with toxicity and it was concluded that plasma levels markedly above 500 ng/ml after a short time exposure should be considered potientially toxic. Even though dental fluorosis is a reliable sign of chronic exposure it is occuring too late to be of preventive value.

Key words: floride poisoning, oral administration, sheep.

YFIRLIT

Rannsóknir á flúoreitrun í íslensku sauðfé

Tólf 6–8 mánaða gömlum gimbrum var gefið inn tiltekið magn af flúoríði í formi natríumflúoríðs í vatnslausn fimm daga vikunnar í 20 vikur. Var þeim skipt í þrjá jafnstóra hópa og fékk fyrsti hópurinn 5 mg/kg (hópur A), annar 10 mg/kg (hópur B) og sá þriðji 15 mg/kg (hópur C). Sem viðmiðunarhópur

voru notaðar fjórar gimbrar, jafngamlar, sem ekki fengu flúoríð en voru meðhöndlaðar að öðru leyti eins og hinar. Vikulega var féð skoðað og vegið og blóðsýni tekin til ákvörðunar á flúoríði, kalsíum, magnesíum, hemóglóbíni, hematókrít, γ-glútamýltransferasa, bilirúbíni, glúkósa og kreatíníni. Að lokinni tilraun var lömbunum slátrað og gerð á þeim meinafræðileg rannsókn.

Vanþrif, lystarleysi og skita voru augljósustu einkenni flúoreitrunar í þessari rannsókn. Þau komu fram í hópum B og C, en varð ekki vart í hópi A. Við krufningu fundust engar marktækar breytingar í innri líffærum, beinum eða tönnum. Engin marktækur munur var heldur á niðurstöðutölum blóðmeinafræðilegra og meinefnafræðilegra rannsókna, öðrum en þéttni flúoríðs í plasma, en hún fór stöðugt hækkandi á meðan á rannsókninni stóð. Línulegt samband var á milli skammta og þéttni flúoríðs í plasma við lok tilraunar annars vegar og þéttni flúoríðs í kjálkum hins vegar. Einkenna um flúoreitrun varð ætíð vart þegar þéttni þess í plasma var umfram 860 ng/ml. Niðurstöður rannsóknarinnar benda til þess að flúoreitrun sé yfirvofandi fari þéttni þess í plasma verulega yfir 500 ng/ml. Enda þótt gaddur í tönnum sé gott einkenni um síðkomna flúoreitrun er hann þó of síðkominn til þess að hafa forvarnargildi.

INTRODUCTION

Fluoride is a potentially toxic element in man and animals. It is ubiquitously distributed in the environment. Occasionally industrial pollution or volcanic activity have contributed toxic amounts to the environment which has resulted in outbreaks of fluoride toxicosis (fluorosis) in farm animals. This has repeatedly been observed in Icelandic sheep when pastures and drinking water have become contaminated with fluoride from volcanic eruptions. The propagation and symptoms of fluorosis in sheep caused by the eruptions of Hekla in 1947 and 1970 has been investigated by Sigurðsson and Pálsson (1957) and Georgsson and Pétursson (1972).

Limited information seems to be available on the relationship between blood plasma fluoride levels and acute toxicity of fluoride in the sheep. Milhaud *et al.* (1985) administered up to 4 mg/kg daily *per os* of fluoride to lambs for 9 weeks without observing any signs of toxicity. Plasma levels of fluoride were apparently dose related and rose steadily during the experiment and reached a maximum of 0.75 µg/ml in the lambs receiving the highest dose. In a study with a single dose of fluoride administered *per os* to adult sheep Kessabi *et al.* (1985) observed disturbances of kidney and liver function at dose levels of 28.5 and 38 mg/kg, respectively. The corresponding maximum plasma levels of fluoride were 4700 and 7200 ng/ml, respectively. Lower doses had little or no effect. It can not be concluded from these studies which plasma levels of fluoride are associated with toxicity when sheep is temporarily exposed to water or forage containing excessive amounts of fluoride. The purpose of the present study was therefore to investigate the relationship between plasma levels of fluoride and possible signs of toxicity when fluoride is administered to sheep over a period of several weeks.

MATERIALS AND METHODS

Sixteen 6–8 months old ewe lambs of the Icelandic breed, weighing 24–40 kg, were used in the experiment. They were housed in well ventilated stables and given water and hay *ad libitum* and were acclimatized to the stables for ten days before the start of the experiment. All the lambs were considered healthy on the basis of a general physical examination.

Four groups of four lambs each were randomly selected (groups A, B, C and D). Three of the groups were administered fluoride in the form of sodium fluoride solution *per os*, five days a week for 20 weeks. The dose administered to the first group (group A) was 5 mg/kg, the second (group B) 10 mg/kg, and the third (group C) 15 mg/kg. The fourth group (control group; group D) was administered water instead of the fluoride solution. Once a week each animal was examined and weighed and blood samples collected for the

analysis of fluoride, calcium, magnesium, hemoglobin, hematocrit, γ-glutamyl transferase, bilirubin, glucose and creatinine. Blood samples were collected from a jugular vein just prior to the administration of fluoride. Samples used for the determination of fluoride were collected in polyethylene tubes previously rinsed with 35% perchloric acid and distilled water. They were centrifuged and the plasma used for fluoride determination.

Fluoride in plasma was determined with a fluoride selective electrode as previously described (Kristinsson *et al.*, 1986ab). The results were expressed as ng fluoride per ml of plasma.

At the end of the experiment the animals were anesthetized with pentobarbital (Vetanarcol®) and killed by decapitation. Samples were taken from the liver, kidneys, lungs and intestines for histopathological examination. The mandibles were taken for determination of fluoride in the bone. The animals were necropsied and tissue samples taken for light microscopy and fixed in 10% formaldehyde.

At the beginning of the experiment samples were taken from hay and water for determination of fluoride. Fluoride was determined in hay, water and mandibles as previously described (Dozinel and Sigg, 1976; Kristinsson *et al.*, 1991).

Area under plasma concentration vs time curves was calculated for each animal with the trapezoidal rule. Analysis of variance was used for the statistical comparison of the groups. Linear regression analysis was used for testing the relationship between dose and the levels of fluoride in blood or bone. P value of <0.05 was considered statistically significant.

RESULTS

In the first week of the experiment the lambs receiving 10 mg/kg (group B) and 15 mg/kg (group C) lost appetite and suffered from diarrhea, and general prostration. The symptoms started on day two and became gradually worse during that week. The administration of fluoride to these lambs was therefore discontinued from day 7 to day 14. After that fluoride administration was resumed without any other signs of toxicity than mild diarrhea and loss of appetite. At day 3 the fluoride concentration in group B was in the range 867–1215 ng/ml (average 968 ng/ml) and 1305–3635 ng/ml (average 2267 ng/ml) in group C. No apparent signs of toxicity were observed in the control group or the group receiving 5 mg/kg. One lamb in group C, receiving 15 mg/kg succumbed on day 4 due to accidental administration of fluoride into the lungs.

The results of the weighing experiment are shown in Figure 1. In the beginning the mean weight of the four groups was not statistically different. Lambs in all four groups lost weight during the first two weeks. At the end of the experiment the lambs in the control group (D) had on an average gained 14.2 kg in weight, and the lambs in groups A, B, and C, 9.9, 2.6 and 1.5 kg, respectively. In groups B and C the mean weight at the end of the experiment was not statistically different from that in the beginning. Weight gain was not statistically different

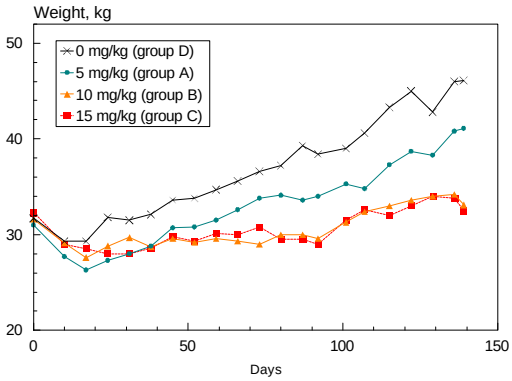

Figure 1. Changes in weight of the lambs during the experiment. Each point on the curve represents the mean weight of the lambs in the respective group.
1. mynd. Breytingar á þyngd lambanna meðan á tilrauninni stóð. Sérhver punktur á ferlinum táknar meðalþyngd lamba í hverjum hópi fyrir sig.

between groups A (5 mg/kg) and D (control animals).

No significant changes were seen in the blood levels of calcium, magnesium, hemoglobin, hematocrit, γ-glutamyl transferase, bilirubin, glucose or creatinine during the experiment.

Plasma fluoride levels on days 0, 24, 52, 87, and 129 are shown in Figure 2. On day 129 the plasma fluoride levels in group A were in the range 720–840 ng/ml, 950–2000 in group B, 2000–2150 in group C and 47–66 in group D (the control group). The area

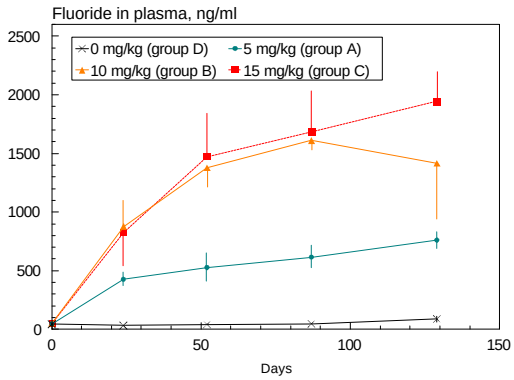

Figure 2. Plasma fluoride levels on days 0, 24, 52, 87 and 129. The vertical bars represent the standard deviation.

2. mynd. Þéttni flúoríðs í plasma 0., 24., 52., 87. og 129. degi. Lóðréttu línurnar sýna staðalfrávik.

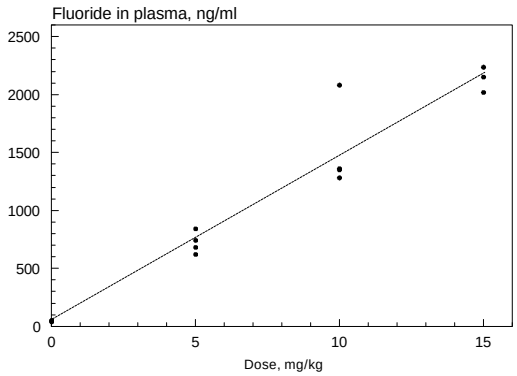

Figure 3. The relationship between dose and fluoride in plasma on day 129.

3. mynd. Samband skammta og þéttni flúoríðs í plasma á 129. degi.

under the plasma concentration curves in the lambs receiving fluoride was on an average 56 709 ng·days/ml in group A, 143 005 ng·days/ml in group B and 158 751 ng·days/ml in group C. The areas under the plasma concentration curves were not significantly different for groups B and C. As shown in Figure 3 there was a linear relationship between dose and fluoride levels in plasma on day 129 (the last measured fluoride levels). Linear relationship was also found between dose and fluoride levels on day 129 in mandibles in group A, B and C (Figure 4). Samples of mandibles from group D were accidentally lost and were therefore not available for analysis. The concentration of fluoride in the hay was 0.6 mg/kg and <0.05 mg/l in the drinking water.

At necropsy no gross pathological changes were seen in the inner organs, bones or teeth. No pathological abnormalities were observed by histological examination.

DISCUSSION

Diarrhea, loss of appetite and general prostration as seen in groups B and C were the most overt signs of acute fluoride poisoning in this study. Even though these symptoms became milder with time or even disappeared,

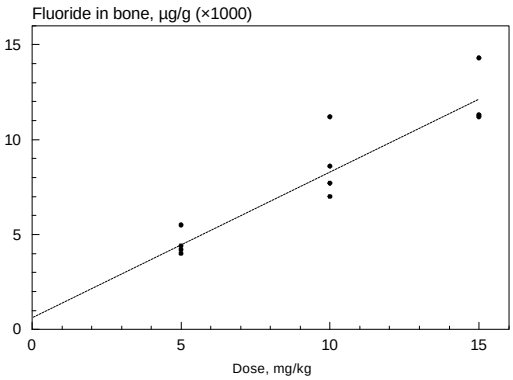

Figure 4. The relationship between dose and fluoride in mandibles at the end of the experiment.

4. mynd. Samband skammta og þéttni flúoríðs í kjálkum við lok tilraunar.

these lambs thrived poorly and did not gain significantly in weight during the experiment (Figure 1). It is not clear whether it was entirely due to loss of appetite or whether malabsorption was a contributing factor. The diarrhea at least indicates some disturbances in the gastrointestinal function. In spite of this no pathological changes were seen in the digestive tract at autopsy, or in other inner organs. Similar gastrointestinal symptoms were seen in the single dose study of Kessabi *et al.* (1985) and in sheep exposed to volcanic ashes during the eruptions of Hekla in 1947 (Sigurðsson and Pálsson, 1957) and 1970 (Georgsson and Pétursson, 1972). Since no signs of acute poisoning were seen in group A, which received a dose of 5 mg/kg, the lowest dose causing acute poisoning in this experiment seemed to lie somewhere in the interval 5–10 mg/kg. However, it must be kept in mind that the fluoride was administered five days a week only and the limit of toxicity for a daily dose might therefore be closer to 5 mg/kg than 10 mg/kg. In a study by Milhaud *et al.* (1985) no signs of toxicity were seen in lambs receiving 4 mg/kg fluoride daily for a period of 9 weeks and this seemingly confirms the assumption that the limit of toxicity for a daily dose is not lower than 4–5 mg/kg. Accordingly plasma levels of fluoride associated with toxicity must lie above the curve shown in Figure 2 for group A. On day 24 the plasma concentration in this group was in the range 377–506 ng/ml. Plasma levels markedly above 500 ng/ml after a short time exposure should therefore be considered potientially toxic. This is substantiated by the fact that plasma levels above 860 ng/ml as found in groups B and C on day 3 were associated with toxicity (see results). Furthermore the plasma levels in group A were always below that value throughout the experiment. It was found in a previous study that plasma levels of fluoride in Icelandic sheep show up to tenfold seasonal variation which is determined by the fluoride content of the fodder (Kristinsson *et al.*, 1991). The highest levels, 160–330 ng/ml (average 252 ng/ml) were found in the spring in sheep that had been fed on fishmeal supplementary to hay during the last 7–8 weeks. It is obvious that these sheep are badly situated to cope with a fluoride contamination from a volcanic eruption if that happens in the spring.

No information seems to be available on the pharmacokinetics of toxic doses of fluoride in the sheep. It has been demonstrated that it is dose independent (linear) at subtoxic doses ranging from 0.15 to 0.75 mg/kg (Joseph-Enriques *et al*., 1990). The linear relationship between dose and fluoride levels in plasma and mandibles, respectively, as shown in Figures 3 and 4 indicates that this is also valid for the doses given here.

Acute fluoride poisoning in the sheep has been associated with hypoglycemia (*Kessabi et al.*, 1985) and hypocalcemia (Georgsson and Pétursson, 1972; Kessabi *et al.*, 1985). In spite of the toxic symptoms in groups B and C no such changes were seen in this experiment. It has been suggested that the hypoglycemia is caused by blockade of the bacterial production of propionate and amino acids in the rumen by fluoride (Kessabi *et al.*, 1985). These substances are converted to glucose through the Krebs cycle and are the main source of glucose in ruminants. When fluoride is administered once a day as in the present study it is probably rapidly removed from the intestine by absorption and its effect is therefore of short duration. This together with the moderate doses given may therefore explain the absence of hypoglycemia in these lambs. Rapid removal of fluoride might also explain the absence of hypocalcemia which has been attributed to inhibition of calcium absorption from the intestine. Fluoride inhibits calcium absorption by reacting with calcium ions to form a poorly soluble calcium fluoride (Kessabi *et al.*, 1985).

Dental fluorosis (in Icelandic “gaddur” or “gostönn”) has been the most common signs of chronic fluoride poisoning in sheep fol-

lowing volcanic eruptions in Iceland (Sigurðsson and Pálsson, 1957; Georgsson and Pétursson, 1972). Another, but less common sign is periostal hyperosteosis. Dental fluorosis is a late emerging symptom. The first fluorotic teeth in sheep exposed to ashes from the eruption in Hekla in 1970 appeared 4–9 months after the exposure (Georgsson and Pétursson, 1972). It is noteworthy that even lower doses than given in the present study can produce fluorotic lesions in teeth. Milhaud *et al.* (1987) were able to produce dental fluorosis in sheep fed 3.5 mg/kg fluoride pr day for 4 months. However, these lesions were small and could not be observed in the live animals but were found at necropsy almost three years later. The duration of the present study (20 weeks) was probably too short for these symptoms to develop.

It can be concluded from this study that diarrhea, loss of appetite and general prostration are the most overt signs of toxicity after sublethal intake of fluoride by sheep. Plasma levels of fluoride markedly above 500 ng/ml after a short time exposure should be considered potientially toxic. Even though dental fluorosis is a reliable sign of chronic exposure it is occuring too late to be of preventive value.

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