Analytica Chimica Acta, 275 (1993) 335–340
Elsevier Science Publishers B.V., Amsterdam

Ion chromatographic determination of perchlorate in cattle urine

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(Received 20th May 1992; revised manuscript received 21st September 1992)

Abstract

Much research has been done over the years on the subject of thyreostatic drugs of the thiouracil type in cattle fattening. These substances increase body weight by enlarged filling of the gastro-intestinal tract and by augmented water retention. On the other hand, little work has been done on the elimination and the presence of residues of inorganic thyreostatics such as perchlorate. The aim of this study was to evaluate the elimination of ammonium perchlorate in cattle urine according to the concentration given and the duration of administration. Perchlorate concentrations were measured by mobile phase ion chromatography. The detection limit was 0.1 mg kg^{-1} for tenfold diluted urine.

Keywords: Ion chromatography; Cattle; Perchlorate; Thyreostatic drugs; Urine

Thyreostatic drugs (TS) are used to cause fraudulently increased weight gain in cattle by inhibition of the thyroid gland. The weight gain consists mainly of an increased filling of the gastro-intestinal tract and augmented extracellular water retention in slaughtered animals [1]. Meat from TS-treated animals may be exudative and thus of inferior quality. The presence of residues of these highly potent antithyroid drugs in meat may constitute a human health hazard. Therefore, the use of these drugs for cattle fattening is prohibited in the European Economic Community (EEC).

Thyreostatic drugs were commonly abused in cattle fattening in the 1970s. The most frequently used substances in those days were of the thiouracil type, such as methyl-, propyl- and phenylthiouracil and tapazole [2,3]. These drugs can be analysed for by high-performance thin-layer chromatography and gas chromatography–mass spectrometry [4]. A large number of inorganic anions also show thyreostatic activity, and perchlorate salts belong to this group of inorganic thyreostatic drugs [5]. For regulatory control of the abuse of these thyreostatics, a method of analysis had to be developed and more information was needed on the excretion and the residue levels.

Data on inorganic TS originate mainly from East European countries. Previous investigations have concerned mostly the zootechnical effects of these compounds, but little work has been done on the possible residues in meat and the thyroid gland and the excretion of these inorganic growth promoters in urine [6–13].

The aim of this study was to investigate whether perchlorate could be found in cattle urine after administration of a single dose of ammonium

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perchlorate to a cow and, if so, how the elimination occurred for different doses of a single oral ingestion. After these preliminary trials, the withdrawal period was determined after prolonged administration of a daily dose of 4 g of ClO_4^- for 10 days.

EXPERIMENTAL

Reagents and reference compounds

Ammonium perchlorate (NH_4ClO_4) and potassium perchlorate ($KClO_4$) were obtained from Janssen Chimica (Geel, Belgium) and Aldrich-Chemie (Steinheim, Germany), respectively. The eluent consisted of a mixture of sodium carbonate (Na_2CO_3) (Riedel-de Haën, Seelze, Germany), 0.1 M tetrabutylammonium hydroxide (TBAOH) (Dionex, Sunnyvale, CA) and acetonitrile (ACN) (Merck, Darmstadt, Germany). All other reagents were of analytical-reagent grade and used as received.

Solutions

Stock standard solutions of ammonium and potassium perchlorate were prepared by dissolving 1 g of these products in 1 l of LC-grade water (Merck). These stock standard solutions were diluted 100-fold to give working standard solutions of 10 mg kg^{-1}. From the latter solutions, dilutions equivalent to 1, 0.8, 0.6, 0.4, 0.2, 0.1 and 0.05 mg kg^{-1} were prepared.

Chromatographic conditions

The analyses were carried out with a Dionex DX-100 ion chromatograph. The method is based on mobile phase ion chromatography (MPIC).

MPIC is a patented technique that utilizes a hydrophobic column packing, a hydrophilic mobile phase (1 mM Na_2CO_3–2 mM TBAOH–27% ACN) containing an ion-pairing reagent (TBAOH) and a suppressor (12.5 mM H_2SO_4) to lower the mobile phase background conductivity before entering a conductivity detector. The eluent flow-rate was 1 ml min^{-1} and the regenerant flow-rate was ca. 4 ml min^{-1}. Separation by MPIC depends on the partitioning of neutral ion pairs between the hydrophilic mobile phase and the hydrophobic stationary phase.

The column was a neutral polystyrene resin that contained no fixed ion-exchanging sites. For this reason the column can be used for either anion or cation determinations by appropriate choice of the pairing reagent.

The retention time of perchlorate, whether the ammonium or the potassium salt was used, was ca. 10.00 min.

The data were recorded with a Shimadzu C-R5A Chromatopac integrator coupled to the ion chromatograph.

Procedure

One 3-year-old black-pied cow was used for these experiments. Several doses were given in ascending order of concentration (2, 4, 6 and ten times 4 g). Between each dosing a rest period of about 2 weeks, starting at the time when the analytical results for the previous experiment were below the detection limit, was inserted. This period allowed the cow to recover slightly and to ensure that it was free from residues from the previous dose before starting a new experiment. Urine samples were taken at several times after ingestion (1, 7, 12, 24, 36, 48, 72 h, etc.). Each sample consisted of ca. 80 ml of urine collected by catherization of the bladder.

For analysis, 1 ml of urine was diluted tenfold with water. For macroscopically visible contaminants, the urine was first filtered and subsequently diluted. This diluted filtrate was loaded directly into the ion chromatograph using a 1-ml tuberculin syringe. Only 25 μl of this volume was retained in the 25-μl loop of the injection pump. By switching the valve system the loop was connected with the eluent flow and the sample was taken up in the eluent flow.

RESULTS

Preliminary experiments

Before starting the experiments on the elimination of perchlorate after oral administration to a cow, a method of analysis had to be developed. Dionex proposed certain analytical conditions

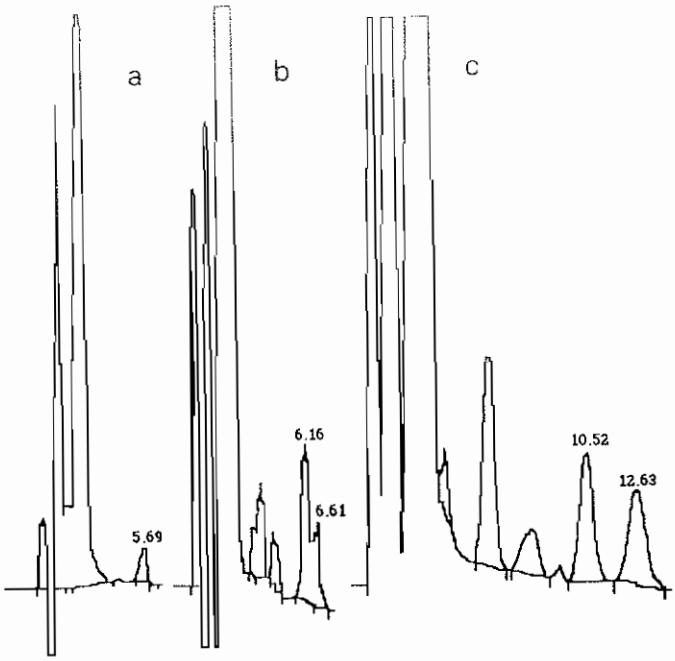

Fig. 1. (a) Chromatogram of porcine urine spiked with ClO_4^- ($t_R = 5.69$ min) (eluent containing 35% ACN); (b) chromatogram of bovine urine spiked with ClO_4^- ($t_R = 6.61$ min), interfering peak with $t_R = 6.16$ min (eluent containing 35% ACN); (c) chromatogram of bovine urine spiked with ClO_4^- ($t_R = 10.52$ min), interfering peak with $t_R = 12.63$ min (modified eluent containing 27% ACN).

based on studies with human urine spiked with perchlorate (eluent, 1 mM Na_2CO_3–2 mM TBAOH–35% ACN at a flow-rate of 1 ml min^{-1}; regenerant, 12.5 mM H_2SO_4 at a flow-rate of 3–4 ml min^{-1}). Under these conditions perchlorate could be determined within 6 min (Fig. 1a). However, using these conditions on cattle urine, perchlorate could not be separated from an endogeneous substance inherent in the urine (Fig. 1b). It has not been possible to identify this compound.

The next step was to search for an appropriate composition of the eluent that would enable the interference from this unknown peak to be eliminated. The best resolution and separation were obtained with an eluent of almost the same composition but with a slightly different acetonitrile content, i.e., 27% instead of 35%. Under these conditions perchlorate was virtually baseline separated from the interfering peak (Fig. 1c).

The results are expressed as mg ClO_4^- per kg urine. The detection limit (3σ) was ca. 0.1 mg kg^{-1} for a tenfold dilution of urine.

Elimination of ammonium perchlorate after oral administration of three different single doses

This first study used one 3-year-old black-pied cow to explore the possibility of analysing cattle urine for perchlorate residues after oral administration of a single dose of ClO_4^-. From a practical point of view the ammonium salt was used because of its greater solubility in water than magnesium or potassium perchlorate, so that the whole dose could be given at one time in a maximum volume of 50 ml.

The elimination of ClO_4^- residues was followed after oral administration of three different single doses of ClO_4^-, viz., 2, 4 and 6 g administered at intervals of ca. 40 days to the same cow. The results are shown in Fig. 2.

Excretion starts rapidly after oral ingestion, independent to the dose given. Maximum ClO_4^- levels in the urine were found 5–12 h after ingestion depending on the concentration administered. The interval in which the maximum ClO_4^- level was reached varied with the dose (Table 1). The maximum residue level after oral ingestion of 2 g of ClO_4^- was observed 5 h after administration. For a 4-g dose it was found after 9 h, and for 6 g after 12 h.

The rate of elimination was equivalent to the concentration, so that the concentration of ClO_4^- in the urine decreased to ca. one third of the maximum amount for all three doses 24 h after oral ingestion (33/104 for 2 g; 80/220 for 4 g; 130/414 for 6 g). The higher the dose given, the

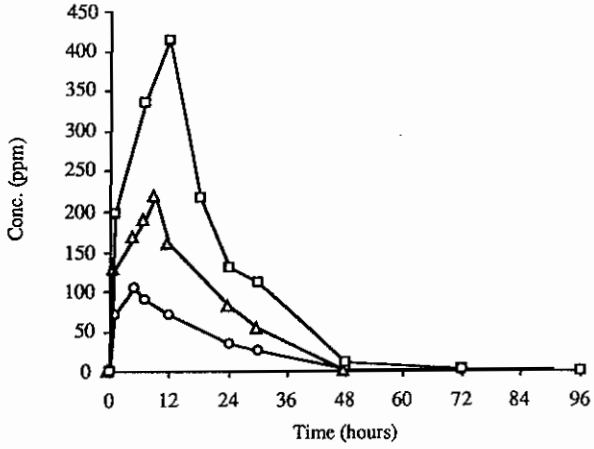

Fig. 2. Elimination curves for three different doses of ClO_4^- (○ = 2 g; △ = 4 g; □ = 6 g).

TABLE 1

Comparison of some elimination characteristics of the three different single doses and the multiple dose treatment

Parameter	Dose of ClO_4^-			
	2 g	4 g	6 g	10×4 g
Maximum concentration of ClO_4^- residues (mg kg^{-1})	104.5	219	414	151.4
Time of maximum excretion (h)	5	9	12	12
Concentration of ClO_4^- at 24 h (mg kg^{-1})	33.4	81	129	43.6
Rate of elimination until 24 h (mg kg^{-1} h^{-1})	3.7	9.2	23.8	9.0
Time of elimination (h)	48	48	72	72

faster was the rate of elimination. The rates of elimination for the three different doses are summarized in Table 1.

The lowest dose of 2 g of ClO_4^- reached its maximum value in 5 h and decreased to about one third of this maximum in 19 h (= 3.7 mg kg^{-1} h^{-1}). When 4 g of ClO_4^- were ingested, maximum excretion was obtained after 9 h, and 24 h later this amount was reduced to ca. one third at a rate of 9.2 mg kg^{-1} h^{-1}. For the highest concentration of 6 g of ClO_4^- the rate of elimination, calculated after reaching its maximum excretion level, was about 23.8 mg kg^{-1} h^{-1}. This concentration-linked elimination rate occurred until 48 h after administration, at which time the residue level of the 4-g dose was undetectable. For the 2-g dose small traces of ClO_4^- residues could be detected. After administration of 6 g of ClO_4^-, traces of ClO_4^- were still present 72 h after ingestion; a further 24 h later no ClO_4^- residues were found.

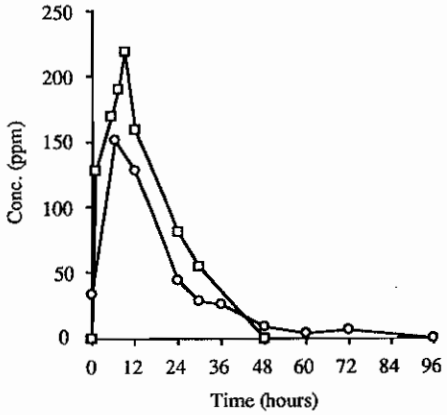

Fig. 3. Elimination curve for 4 g of ClO_4^- in (○) a single dose and (□) ten doses.

Hence there is evidence for a concentration-linked elimination rate in the first phase of the elimination (until 48 h) and in a second phase the rate of elimination decreased, so that the higher the concentration given, the longer small traces of residues could be found in the urine.

Elimination of ammonium perchlorate after prolonged administration

In a second experiment, a single cow was treated with a daily dose of 4 g of ClO_4^- for ten successive days. The results obtained are shown in Fig. 3.

After this treatment period, the ClO_4^- level observed in urine was in the same range as after the ingestion of a single dose. After withdrawal of the drug, an almost identical elimination rate as for the single dose was observed. In the first 2 days after stopping the treatment, the ClO_4^- levels in urine decreased very rapidly. The amounts and the rate of excretion were comparable to those after ingestion of a single dose. The difference between application of a single dose and repeated doses during 10 days was that residues were detected until 72 h after stopping the treatment in the case of the long-term administration whereas for the single dose the residue levels were below the detection limit of 0.1 mg kg^{-1} after 48 h.

This evolution of excretion probably results from the fact that small amounts of ClO_4^- were stored for some time in the thyroid gland and were released after stopping the treatment. On the other hand, results for urine samples taken during the period of administration indicated that a concentration of 4 g of ClO_4^- per day was not sufficient to obtain a steady-state. This lack of accumulation was probably also the consequence of the very rapid decline of ClO_4^- in the urine.

DISCUSSION

Data on the excretion and distribution of inorganic thyreostatics are scarce. Most of them origi-

nate from East European countries and were concerned with the zootechnical effects of these drugs in cattle fattening. The results differ from West European opinion on the quality of the meat after treatment with TS. According to some East European researchers, the use of inorganic TS, i.e., ammonium or magnesium perchlorate or a combination, increased the average daily weight gain of cattle in a short time and reduced the feed costs without decreasing the meat quality [10,11]. In contrast, in pigs they even decreased the moisture content and increased the intramuscular fat and protein concentration. They improved the biological value of pig meat [6,9]. Also in cattle, meat quality regarding tenderness and moisture content was ameliorated using ClO_4^- [7,8]. On the other hand, in some investigations no difference among treatments was found; it was concluded that the use of ClO_4^- in cattle fattening did not give a significant gain in daily liveweight [13,14].

When using ammonium or magnesium perchlorate as a growth promoter, an optimum dose of 1.5–5.0 mg kg^{-1} liveweight is recommended, the feeding period should not exceed 3 months and the animals should be slaughtered at least 5 days after the withdrawal of the salts from the diet [11]. Apart from these biological and zootechnical data, little has been published concerning the distribution and the elimination of perchloric acid salts in farm animals. In calves given ClO_4^- at a concentration of 200, 20 or 2 mg kg^{-1}, peak concentrations of ClO_4^- in blood occurred 5 h after application. Excretion in urine was up to 8.5% of that given. The amount of the anion retained in the body increased as the amount given was reduced [12]. This was not confirmed in this work, where a higher concentration gave residues for a longer time.

During the period of treatment, no significant changes in the behaviour or reactions of the animal were observed. Repulsive reactions against oral administration occurred after a few days. At this high concentration of 10 mg kg^{-1} in the present experiments (4 g), the excretion was characterized by a rapid start with a maximum concentration after 12 h. The results confirmed the fact that only slight accumulation occurred during prolonged administration of even this high concentration of 10 mg kg^{-1} liveweight [11,12]. During the whole period of administration a steady state was never reached because of the rapid excretion of ClO_4^-. At the time of the following daily dose, the residue level had returned to a concentration of approximately one third of the maximum concentration. At this elimination rate, storage of ClO_4^- residues seemed almost impossible.

Conclusions

Perchlorate was excreted in the urine after oral administration of a single dose of ClO_4^- and excretion started very rapidly. The three different doses (2, 4 and 6 g) showed an elimination curve equivalent to the dose given. The rate of elimination was dose dependent, so that the highest amount gave small residue amounts for a longer time. The higher the dose, the longer was the time during which residues could be found in the urine. ClO_4^- levels in urine showed a biphasic elimination: after an initial rapid decline, observed in the first 2 days after treatment, the ClO_4^- excretion slowed by a factor 16 depending on the treatment. Prolonged ClO_4^- administration at a dose of 4 g per day for 10 days resulted in a longer excretion period in the urine. Long-term treatment influenced the second phase of elimination. Small traces of ClO_4^- could be found for a longer time. This suggests that ClO_4^- administered over a longer period, as practised in cattle fattening, accumulates in the body and give residues in urine for longer periods than found with single doses. ClO_4^- as a growth promoter has the unfortunate characteristic (for analysts) of a rapid decline of ClO_4^- residues in cattle urine, which makes it difficult to detect and control its abuse in cattle fattening. Lowering the detection limit may result in a longer period during which residues can be found. Therefore, adjusted clean-up procedures are needed in order to lower the detection limit in this preliminary technique.

This work was supported by the Institute of Veterinary Inspection (IVK–IEV), Belgian Ministry of Public Health. The authors are indebted

to Professor Dr. A. De Kruif and Dr. P. De Winter for help with the animal experiments.

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