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Excretion profile of corticosteroids in bovine urine compared with tissue residues after therapeutic and growth-promoting administration of dexamethasone

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ABSTRACT

The illicit use of dexamethasone as growth-promoting agent in animal breeding is still practiced within the EU constituting a health risk for meat consumers. An experimental study was developed to assess dexamethasone urinary excretion and tissue distribution (liver, kidney, and muscle) in male calves after therapeutic and growth-promoting administration. Urine and tissue samples collected from treated and untreated bovines were also investigated for the presence of other natural and synthetic corticosteroids (prednisolone, prednisone, hydrocortisone, and cortisone), in order to study a possible correlation with dexamethasone administration and to clarify prednisolone origin.

Analyses were performed by a multi-residue LC–MS/MS method developed and validated according to the Commission Decision 2002/657/EC.

The results confirm the rapid rate of dexamethasone urinary excretion, irrespective of the dosage, the duration and the route of administration, and the disappearance of cortisone and hydrocortisone during the treatment. Dexamethasone was distributed to the tissues where the elimination rate proceeded relatively slower as suggested by the presence of residues one month after the withdrawal of the therapeutic treatment.

An increase in the number of positive findings for prednisolone, in association with higher levels of cortisone and hydrocortisone, was observed in urine samples collected from slaughterhouse rather than those collected at the farm. Prednisone residues were found only in one urine sample that showed the highest levels of prednisolone, hydrocortisone, and cortisone.

The occurrence of prednisolone residues in urine and even in tissue samples confirms the endogenous nature of this molecule.

1. Introduction

Among the numerous synthetic corticosteroids available in veterinary medicine, dexamethasone remains the most widely used for the treatment of metabolic and inflammatory diseases in ruminants [1–3]. Although their use is intended for therapeutic purpose, dexamethasone and other synthetic glucocorticosteroids are also illegally used in cattle fattening, alone or in association with other drugs, because of their well known capacity to increase weight gain and to reduce the feed conversion rate [4,5]. Due to the potential toxic effects that glucocorticoid residues can exert on meat consumers, the Council Directive 2003/74/EC [6] has been issued for banning their illicit application. Their use in livestock is also strictly regulated with withdrawal periods between treatment and slaughtering, and maximum residue limits (*MRLs*) established in edible biological matrices and milk for some compounds [7]: the *MRLs* established for dexamethasone are 2 $\mu g\,kg^{-1}$ for liver, 0.75 $\mu g\,kg^{-1}$ for muscle, 0.75 $\mu g\,kg^{-1}$ for kidney and 0.3 $\mu g\,kg^{-1}$ for milk, and for prednisolone are 4 $\mu g\,kg^{-1}$ for muscle and fat, 6 $\mu g\,kg^{-1}$ for milk and 10 $\mu g\,kg^{-1}$ for liver and kidney.

Despite the ban, the administration of these substances for growth-promoting purposes is still practiced within the European Union [8]. In the attempt to fight these illegal practices, a National Surveillance Plan for steroid abuse was adopted by each individual Member State for the detection of the parent compound in target biological matrices such as liver, kidney, and muscle at slaughterhouse, hair and urine at farms. Because of the complexity of the matrices and the very low-dose corticosteroid regimes commonly used in growth-promoting practices, the analytical detection needs highly sensitive, specific and selective methods which must be in compliance with the criteria established in the Commission Decision 2002/657/EC [9].

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The knowledge on the pharmacokinetics, in particular the metabolic fate and elimination profile of administered drugs plays a crucial role in residue analysis. Dexamethasone at low dosages is rapidly metabolized and excreted, thus its urinary concentration is very low during the treatment and completely absent some days after the end of the treatment preventing the long term survey [10,12]. The knowledge on drug distribution in tissues is also matter of interest in residue control because it supplies information to identify the more relevant matrices that have to be collected and analysed. Although there are many studies concerning dexamethasone excretion kinetics in cattle urine [11–13], only few papers assess its urinary excretion and distribution profile in cattle tissues depending on the type of treatment. The aim of this study is to evaluate the urinary elimination of dexamethasone after therapeutic and growth-promoting treatments and to compare the obtained results with those found in liver, kidney, and muscle samples. Further, we investigated the correlation between endogenous corticosteroids and administered dexamethasone levels and we also measured the urinary and tissue concentration of prednisolone and prednisone (Fig. 1) to obtain additional information with respect to their source [14–17].

2. Experimental

2.1. Animals and experimental protocol

The experimental plan was designed according to the guidelines of Italian law for care and use of experimental animals [18] and the study was approved by the Ministry of Health and the local Committee for animal welfare. Thirty Friesian male calves (aged two weeks) were farmed for 6 months under controlled experimentally conditions. They were fed a diet available on the market, usually employed in zootechnical practice with *ad libitum* access to water. Feed ingredients were dairy-products, oils and fats, oilseed products and by-products, cereal products and by-products and minerals (21.5% proteins, 20% fats, 0.3% fiber and 7.5% ash). During the sixth month of breeding, 10 animals underwent growth-promoting dexamethasone treatment (0.4 mg/day of dexamethasone 21-disodium phosphate "*Desashock*" orally administrated per capita/day for 20 consecutive days) other 10 animals underwent therapeutic dexamethasone treatment (2 mg of dexamethasone 21-disodium phosphate "*Desashock*" per kg of b.w. intramuscularly administrated for 3 consecutive days); the remaining 10 animals were used as controls of both groups of treated bovines. Appropriate measures were taken to avoid any kind of cross-contamination between the three different groups of animals.

2.2. Samples collection

Samples from growth-promoting treated bovines: urine samples were collected before the first administration, then at the 2nd, 4th, 8th, 15th, 20th, 21st, 22nd, 23rd, and 30th day; liver, muscle and kidney samples were collected after slaughtering.

Samples from therapeutic treated bovines: urine samples were collected before the first administration then at the 3rd, 4th, 5th, 6th, 7th, and 31st day. The last samples were collected directly from the bladder after slaughtering (32nd day) together with liver, muscle, and kidney samples.

Samples from control bovines: urine samples were collected in the same sampling days of both treated bovines; after slaughtering the same tissue samples were collected.

Urine samples were collected (taking care to prevent faecal contamination) after milk administration and waiting for spontaneous micturition and were immediately stored in the dark at −20 °C until analysis. Tissue samples were stored at −20 °C until analysis.

2.3. Chemicals and reagents

All solvents were HPLC or analytical grade and purchased from Riedel-de Haën (Seelze, Germany). Water was purified by Milli-Q System (Millipore, Bedford, MA, USA). Sodium acetate anhydrous and β-glucuronidase-arylsulphatase (*Helix pomatia*) were obtained from Merck (Darmstadt, Germany), this latter was used as supplied. Sodium hydroxide was purchased from J.T. Baker (Deventer, Netherlands), protease (from *Bacillus licheniformis*) and acetic acid were obtained from Sigma–Aldrich (St. Louis, MO), Tris was purchased from Carlo Erba Reagents (Milan, Italy). OASIS HLB SPE cartridges (3 mL, 60 mg) were supplied by Waters (Milford, MA, USA).

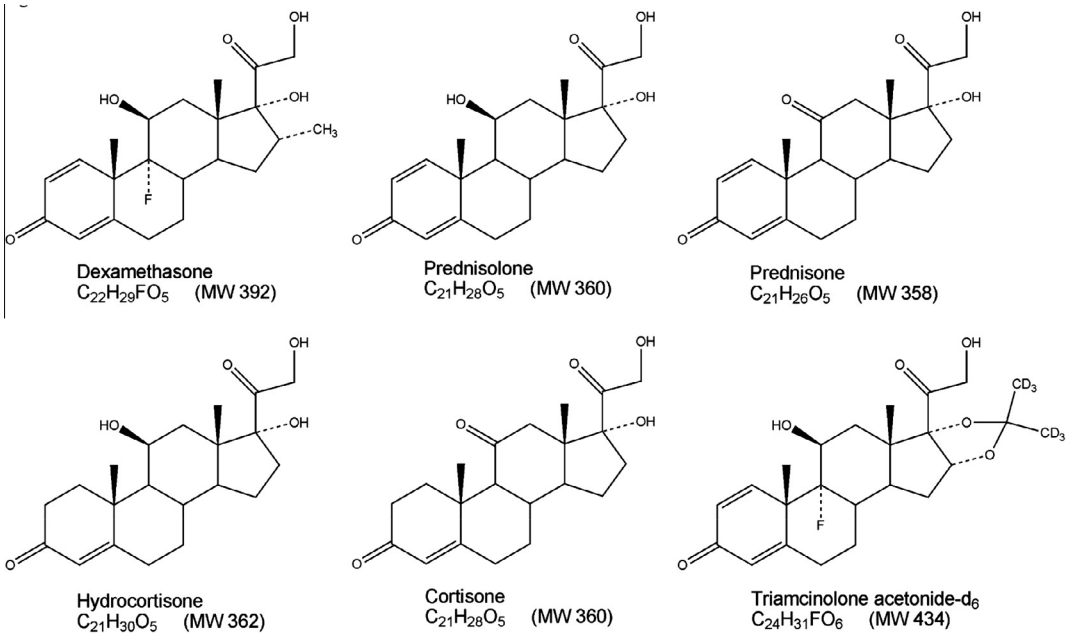

Fig. 1. Structures of the corticosteroids.