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Oral bioavailability, tissue distribution and depletion of flumequine in the food producing animal, chicken for fattening

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Abstract

Chickens were used to investigate kinetic properties including metabolism of flumequine after single IV and oral dose, and to study tissue depletion of flumequine after multiple oral doses. Plasma and tissue (muscle, kidney, liver and skin plus fat) concentrations of flumequine and its metabolite 7-hydroxyflumequine were determined using a HPLC method. After IV and oral administration (single-dose of 12 mg flumequine/kg bw), plasma concentration–time curves were best described by a two-compartment open model. Elimination half-life and mean residence time of flumequine in plasma were 6.91 and 5.90 h, respectively, after IV administration and 10.32 and 8.95 h after oral administration. Maximum plasma concentration was 3.62 μg/ml and interval from oral administration until maximum concentration was 1.43 h. Oral bioavailability was found to be 57%. Flumequine was converted to 7-hydroxyflumequine. After oral administration (24 mg/kg bw every 24 h for 5 days), renal and hepatic concentrations of flumequine (18–25 μg/kg) persisted for 4 days; however, at that time, flumequine residues were not detected in skin plus fat and muscle tissues. Flumequine administered at a dosage of 24 mg/kg bw every 24 h for 5 days, with a withdrawal time of 2 days, resulted in flumequine concentrations in target tissues that were less than the European Union maximal residue limits.

Keywords: Flumequine; Kinetics; Tissue depletion; Withdrawal time; Chickens for fattening

1. Introduction

Quinolones are often used in livestock and fish farm industries in cases of pulmonary, urinary and digestive infections as they act by inhibiting bacterial DNA-gyrase. Flumequine (9-fluoruro-6,7-dihydro-5-methyl-1-oxo-1H,5H-benzo-quinolizine-2-carboxylic acid) is a member of the halogenated quinoline carboxylic acid group of antibacterial agents with antimicrobial activity against a wide range of Gram-negative bacteria (Neuman, 1978). Flumequine is particularly active *in vitro* against *Escherichia coli*, *Salmonella* spp. and *Pasteurella* spp. (Greenwood, 1978; Steer et al., 1981) and is commonly used in food producing species (ruminants, pigs, birds, fish) (Dorrestein et al., 1983; Ziv et al., 1986; Pijpers et al., 1989; Mevius et al., 1990a,b; Delmas et al., 1997; Samuelsen, 1997; Hansen and Horsberg, 1999). The minimal inhibitory concentrations (MIC) of flumequine for these bacteria are reported in the range 0.09–1 μg/ml (Dorrestein et al., 1983; Ziv et al., 1986; Pijpers et al., 1989; Mevius et al., 1990b; Goren et al., 1982).

Studies on the kinetic behaviour of flumequine in rats, dogs, calves, sheep, goats, pigs and fish are available (Ziv et al., 1986; Mevius et al., 1990a; Delmas et al., 1997; Ruiz-Garcia et al., 1999; Harrison et al., 1986; Meijer et al., 1994; Villa et al., 2005a,b; Rogstad et al., 1993). However, limited information is available on disposition, metabolism and safety of flumequine in birds (Dorrestein et al., 1983; Goren et al., 1982; Samaha et al., 1991; Atef et al., 1987). While several studies have described the

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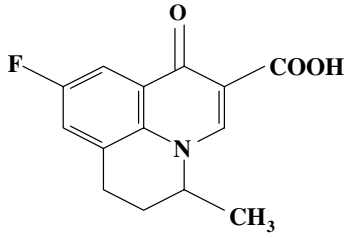
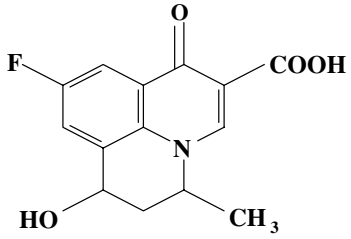
pharmacokinetics and metabolism of flumequine in rat, dog, calves and sheep, there is no such information available for chickens. In calves (Mevius, 1990a), sheep (Delmas et al., 1997), rat and dog (Harrison et al., 1986) and man (Vree et al., 1992), flumequine is glucuronidated and to a lesser extent hydroxylated to 7-hydroxyflumequine. This metabolite exhibits approximately one-eight the antimicrobial activity of flumequine (Schuppan et al., 1985) (Fig. 1).

The major pharmacodynamic effect of flumequine is its antimicrobial activity. From a clinical point of view, flumequine and microbiologically active metabolites should be assayed in the plasma to compare these plasma concentrations with the MIC values of potential pathogens. On the other hand, there is a strict legislative framework controlling the use of quinolone substances, with the aim of minimizing the risk to human health associated with consumption of their residues. Therefore, to ensure human food safety, the European Union (EU) has set tolerance levels for these compounds as maximum residue limits (LMR). The LMR in chicken and turkey was fixed for flumequine at 400 μg/kg in muscle, 250 μg/kg in skin + fat, 800 μg/kg in liver and 1000 μg/kg in kidney (EMEA, 2002a). Depletion of drugs from food producing animals must be assessed in order to determine the time needed before the antibiotic disappears from animal tissue and to assess in a definitive way when the treated animal can be safely consumed. In this framework, there is a demand for suitable and sensitive analytical methods that can be used to monitor the levels of quinolone residues in foods and to establish drug withdrawal times in chickens after pharmacological treatments.

The objectives of the present study were: (i) to describe the kinetic behavior of flumequine and its 7-hydroxy derivative following single-dose intravenous (IV) and oral administration of flumequine in chickens and (ii) to evaluate the depletion of flumequine in edible tissues (muscle, liver, kidney and skin + fat) of healthy chickens after multiple-dose oral administration.



Flumequine



7-hydroxy -flumequine

Fig. 1. Chemical structures of flumequine and its metabolite 7-hydroxyflumequine.

2. Materials and methods

2.1. Chemicals

Flumequine was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA) and 7-hydroxyflumequine was provided by CEVA Sante Animale (Libourne Cedex, France). All chromatographic solvents used in this study were HPLC grade. The other chemicals were of analytical grade.

2.2. Animals

The study was undertaken in accordance with the ethics requirements and authorized by the official ethical committee of our university. Forty six healthy Ross male broiler chickens that were 40-days old and that each weighed 2 kg were included in the study. All chickens were obtained from a poultry breeding farm (Nutreco, SA Sada Division, Cazalegas, Toledo, Spain). Chickens were placed individually in cages in the animal house. Chickens were allowed a 7-day acclimation period prior to the study. Animal house was maintained at room temperature (25 ± 2 °C) and at 45–65% relative humidity. Antibiotic free commercial feed and water were available ad libitum.

2.3. Experimental design

Chickens were randomly allotted to three groups. Group A and B animals (8 chickens/group) were used to investigate the kinetic characteristics of flumequine after a single IV and oral administration of an aqueous flumequine solution at a dose level of 12 mg/kg bw, respectively. Chickens of group C ($n = 30$) were used to study tissue depletion of flumequine and its metabolite 7-hydroxyflumequine. Chickens in group C were given serial daily doses of flumequine (24 mg/kg bw orally, every 24 h, for five consecutive days). All dosages were administered between 8 and 9 AM. The solution for IV administration was daily prepared by dissolving 480 mg flumequine in 10 ml NaOH 0.01 M (using sterilized bidistilled water) with the subsequent regulation of the pH to 10.3 with HCl 6 M and adjusted to a concentration of 48 mg/ml. Further lowering of the pH resulted in precipitation of flumequine. Flumequine for oral administration was also daily prepared from the flumequine solution of concentration 48 mg/ml adjusted with sterilized 0.9% saline solution to a concentration of 12 mg/ml and 24 mg/ml. A total volume of 0.5 ml (solution of concentration 48 mg/ml) or 2 ml (solution of concentration 12 mg/ml) was used for IV and oral administration (groups A and B), respectively. A total volume of 2 ml (solution of concentration 24 mg/ml) was used for oral administration of group C. Flumequine was administered IV into the right brachial vein of chickens in group A or was administered directly into the crop of chickens of groups B and C by use of a thin plastic tube attached to a syringe. Food but not water was withheld from 12 h before until 6 h after drug administration.

Blood samples (0.5–1 ml/sample) were collected from the left brachial vein of each chicken of groups A and B. Samples were collected into heparinized syringes through a cannula immediately before (time 0) and 10, 20, and 30 min and 1, 2, 4, 6, 8, 12 and 24 h after drug administration. Blood samples were centrifuged (1800×*g* for 10 min), and plasma was harvested and stored frozen at −45 °C until analyzed. Flumequine and 7-hydroxyflumequine concentrations were measured in plasma samples of chickens in group A and B.

Chickens of group C were euthanized by use of carbon dioxide and immediate 12, 24, 48, 72 and 96 h after the last dose of flumequine was administered. Six chickens were euthanatized at each time point. Birds were immediately exsanguinated and tissue specimens (2 g) of kidneys,