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In vivo and in vitro metabolism of dexamethasone in the camel

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Abstract

The metabolism of dexamethasone (DXM) in the camel was assessed by in vivo and in vitro techniques. Liver samples were collected at the abattoir from camels of either sex, and microsomes were isolated and characterized as to their protein and haemoprotein content as well as for their ability to metabolise several cytochrome P450 model substrates. The expression of different P450 enzymes was evaluated by means of immunoblotting, and the glucuronidating capacity was assessed with 1-naphthol as the substrate. The activity of 11β-hydroxysteroid dehydrogenase type 1 was assayed using metyrapone as a model substrate. To examine the in vitro metabolism of DXM, microsomes were incubated with the corticoid in the presence of either a NADPH-generating system or of uridindiphosphoglucuronic acid. In vivo metabolism of DXM was studied in two male camels, injected with a bolus intravenous dose of DXM (0.2 mg/kg body weight) and DXM metabolites were evaluated in urine samples collected at different times after the administration. DXM and metabolites were extracted using solid phase and liquid–liquid extraction, and analysed by liquid chromatography mass spectrometry (LC/MS) and by LC/MS/MS. Comparative results were obtained by in vitro and in vivo studies. Two phase I metabolites were detected: the major one resulted from reduction of the 3-carbonyl group in ring A and the minor metabolite from ring hydroxylation of ring A. Glucuronidation involved both phase I metabolites as well as the parent compound.

Keywords: Dexamethasone; Metabolism; Camel; In vivo; In vitro

1. Introduction

Dexamethasone (9α-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione, DXM) is a long-acting synthetic analogue of hydrocortisone (cortisol) and is widely used in veterinary practice (Ferguson and Hoenig, 1995; Pugh, 1991). DXM is the most potent among synthetic corticosteroids and has been in use for many years in various species to treat lameness attributable to joint injury or swollen bursae and tendon sheaths as well as immune-based inflammation (Friederich et al., 1992; Grundy and Barton, 2001). The pharmacokinetics of DXM have been reported in horses, dogs and cattle. Recently, the pharmacokinetics and pharmacodynamics of DXM have been studied in the camel (Al Katheeri et al., 2004a,b) but few reports on DXM metabolism in large animals are available in the literature.

As far as ruminants are concerned, the major metabolic route for DXM is thought to involve hydroxylation at the 6-position of the steroid ring and glucuronidation or sulphation of both the parent molecule and the –OH derivative (Antignac et al., 2002) leading to the formation of metabolites mainly devoid of corticosteroid activity. In vitro studies have revealed that in other mammalian species hydroxylation at positions other than C-6 may occur and it is generally recognized that cytochrome P450 (CYP), particularly that belonging to the 3A subfamily (CYP3A), is mainly involved in the hydroxylation of the steroid ring (Tomlinson et al., 1997a). The DXM side chain cleavage, probably mediated by CYP17A, followed by 6-hydroxylation has been

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also reported in human liver microsomes, but this pathway is of minor significance in vivo (Tomlinson et al., 1997b). Finally, 11β-hydroxysteroid dehydrogenases (HSDs) 1 and 2 have been shown to be involved in DXM metabolism. Unlike 11β-HSD2, which is mainly expressed in extrahepatic tissues, 11β-HSD1 is an NADP(H)-dependent microsomal enzyme expressed in a wide range of tissues including liver, catalysing the interconversion of the 11β-hydroxyl group into 11β-keto group and vice versa (Diederich et al., 2002).

Camel racing is a popular sport in the United Arab Emirates (UAE). Winners are subjected to a dope testing programme and a zero medication rule is adopted by the Racing Commissioner who imposes severe penalties if a camel fails the anti-dope testing. The aim of the present study was to identify DXM metabolite(s) by in vivo and in vitro techniques and verify its fragmentation pattern which clearly increases the reliability of anti-doping control analysis.

2. Materials and methods

2.1. Chemicals and antibodies

Bovine serum albumin, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, lactic dehydrogenase, $NADP^+$, and 4-aminophenol were purchased from Roche Diagnostics; Primary antibodies raised against rat CYP1A, CYP2C and CYP2B from Biotec and all other reagents came from Sigma–Aldrich.

2.2. In vitro studies

In order to examine the in vitro metabolism of DXM by liver microsomes, a number of biochemical and immunochemical assays were performed to characterise the expression and the activity of microsomal monooxygenases and transferases and HSDs that could be reasonably involved in the oxidative and conjugative biotransformation of the drug.

2.3. Liver specimens

Liver specimens were collected from a slaughterhouse from six camels (3 females and 3 males), ranging in age from 7 months to 10 years. For comparison, liver samples from one goat (1-year-old) and one calf (6 months) were also collected. None had received any drug for at least six months. Good quality hay and fresh lucerne (alfalfa) was fed once daily and water was allowed ad libitum.

2.4. Preparation of liver subcellular fractions

Microsomal fractions were isolated by differential centrifugation and stored at −80 °C as detailed elsewhere (Nebbia et al., 2003). The protein concentration in individual samples was estimated with the method by Lowry et al. (1951). The cytochrome P450 (CYP) content was determined as the difference in spectrum (450–490 nm) between carbon monoxide complexed versus uncomplexed microsomal suspensions after reduction with sodium dithionite (Rutten et al., 1987).

2.5. Enzyme assays

Microsomal suspensions were assayed for their ability to metabolise a number of substrates that in humans and other laboratory or livestock species are believed to be markers for the expression of different CYPs (Lewis, 1996; Sivapathasundaram et al., 2001). With the limitation that CYPs may have different substrate specificities (Fink-Gremmels and Van Miert, 1996; Nebbia, 2001), ethoxyresorufin, was selected as CYP1A-dependent substrate, benzphetamine as CYP2B-dependent substrate, aminopyrine as CYP2C-dependent substrate, erythromycin and ethylmorphine as CYP3A-dependent substrates, and aniline as CYP2E-dependent substrate.

All enzyme activities were assayed in aerobic incubations at 37 °C under conditions of maximal velocity with appropriate blanks. The rate of *N*-demethylation of ethylmorphine (6 mM) and erythromycin (1 mM) was measured in 1 mL assay mixtures containing 0.8–1.2 mg protein, 800 μL/mL of 0.1 M Tris–HCl and an NADPH generating system (0.32 mM βNADP+, 6.4 mM glucose 6-phosphate, 1.25 U glucose 6-phosphate dehydrogenase, 5 mM magnesium chloride and 1 mM EDTA). The formation of formaldehyde was estimated by Nash's reagent as described by Werringloer (1978). Ethoxyresorfin (2 μM) *O*-deethylase activities was assayed fluorometrically using 0.2–0.3 mg protein and measuring the amount of the released resorufin as detailed by Re et al. (1993). The rate of aniline (5 mM) hydroxylation was assayed with about 1 mg protein by measuring the amount of 4-aminophenol formed (Nebbia et al., 1993).

The activity of 11β-HSD1 was assayed by measuring the rate of NADPH oxidation at 340 nm using metyrapone 1 mM as a substrate (Maser and Bannenberg, 1994). Uridindiphosphoglucuronyl-transferase (UGT) activity toward 1-naphthol (0.3 mM) was determined on 0.25% Triton X100-activated microsomes using the method reported by Mulder and Van Doorn (1975) as modified by Antoine et al. (1988).

2.6. Western immunoblotting

Solubilised microsomes (10 μg) were electrophoresed in 10% sodium dodecyl sulphate polyacrylamide gels (SDS–PAGE) and transferred to nitrocellulose as previously reported by LaemmLi (1970) and Towbin et al.