Clenbuterol plasma pharmacokinetics in cattle†

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The pharmacokinetics of clenbuterol (Cb) were investigated to determine the extent to which analysis of plasma concentration can be used to discriminate between therapeutic and illicit growth promoting treatment of cattle. Analysis of plasma concentration enabled assessment of the extent of differences in pharmacokinetics between such dosing regimens. Cattle were treated with Cb using either a therapeutic (20 calves, 0.8 μg Cb kg^{-1}, twice daily in feed for 10 days), or growth promoting (30 calves, 10 μg Cb kg^{-1}, twice daily by drench for 20 days) dosing regimens. Blood samples were collected by jugular venepuncture, and plasma Cb concentrations determined by direct enzyme immunoassay. To determine plasma pharmacokinetics, use of a two compartment model was applied to the data and revealed that steady state kinetics were reached after 3 and 5 days following initiation of therapeutic and growth promoting dosing regimens, respectively. Tolerance limit analysis of concentrations during the therapeutic regimen indicated that a plasma Cb concentration greater than 1.63 ng ml^{-1} would be indicative ($p < 0.01$) of a growth promoting dose.

Introduction

Clenbuterol is used as a licensed respiratory and tocolytic veterinary medicine for bovine and equine species. Illegal use at higher doses for growth promoting purposes in food producing animals has been widely reported[1] and gives rise to concern regarding consumer safety. Despite such uses there is limited information regarding the plasma pharmacokinetics of clenbuterol at therapeutic and growth promoting doses to enable threshold concentrations indicative of illegal use to be determined. The value of immunoassays for clenbuterol residue analysis in a variety of matrices has been widely reported.[2, 3] Development of rapid and sensitive enzyme immunoassays has enabled the accurate analysis of low concentrations for clenbuterol in plasma and permitted the depletion kinetics in plasma to be readily determined.[4] Previous studies [5,6] in which growth promoting doses were administered to cattle have shown plasma half lives in cattle of approximately 18 and 56 h for the α and β phases respectively, reflecting the relatively prolonged retention of clenbuterol compared to other β agonists which has been associated with partitioning into fatty compartments and resistance to metabolism.[7,8] The concentrations reported in edible tissues (liver and kidney) may have appreciable implication for the consumer given the pharmacological potency of clenbuterol. The present study evaluates the pharmacokinetics of clenbuterol in plasma following administration of doses consistent with legal (therapeutic) and illegal (growth promoting) use, the possibility and practicality of discriminating between such treatments by plasma clenbuterol analysis.

Experimental design

Therapeutic dose

Friesian male calves (~18 weeks old, $n = 20$, weight 163 ± 6.17 kg) were dosed with Ventipulmin® granules (Boehringer Ingelheim, Germany) (0.8 μg clenbuterol hydrochloride kg^{-1} body weight) twice daily, in feed for 10 days. A further five untreated calves served as controls (body weight 159 ± 7.96 kg). Blood (20 ml) was collected by jugular venepuncture into heparinised tubes; the sampling interval ranged from 4 h, immediately after the final dose to 24 h 8 days later. Plasma was separated by centrifugation and stored at −20 °C.

Growth promoting dose

Male Friesian calves (~16 weeks old, $n = 30$, weight 116–188 kg) were dosed with clenbuterol hydrochloride solution (10 μg clenbuterol hydrochloride kg^{-1} body weight) twice daily by drench for 21 days at twice daily as described.[5] Animals were sampled in groups of 4 or 8 to minimise trauma to individuals. Blood (10 ml) was collected by jugular venepuncture into heparinised tubes and plasma separated and stored as above. Samples were collected intensively (1–16 h interval) for 198 h after the final dose (see Fig. 1). Plasma clenbuterol concentrations derived from this part of the study have been reported elsewhere.[5] Results are re-evaluated in the

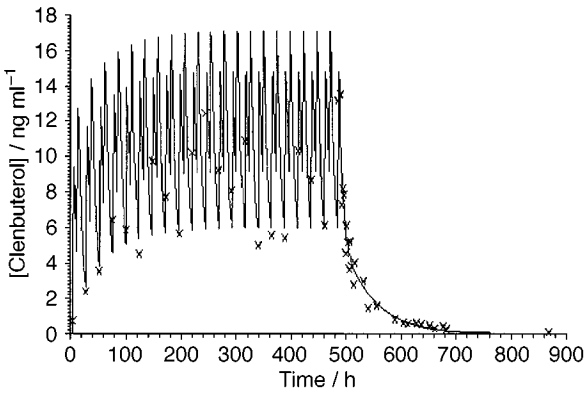

Fig. 1 Mean plasma concentration after initiation of growth promoting dosing regimen. Data were analysed using a 2 compartment model.

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current study for the purpose of comparison with data derived from the therapeutic dose regime.

Analysis of plasma samples

Plasma clenbuterol was quantitated directly by microwell enzyme immuno assay (EIA) as described previously.[9,10] Validation data generated using the growth promoting regimen have been reported elsewhere.[5] Plasma concentrations for the data derived following the therapeutic dosing regimen were calculated by interpolation from a clenbuterol calibration curve using a 4 parameter logistic fit (Argus 300 plate reader and EIAsmart™ software, Canberra Packard, Pangbourne, Berks, UK). Use of a Students *t*-test enabled a limit of quantitation (LOQ) of 0.12 ng ml^{-1} to be calculated by determination of the 99.9% probability that control values would not exceed this concentration. This LOQ was 4 times higher than that reported previously,[5] and reflects a more rigorous statistical analysis made possible by the availability of a large number of control plasma samples in the therapeutic dose study.

Analysis of blank plasma samples fortified to concentrations for 0.03, 0.1 and 0.5 ng ml^{-1} gave mean ± *s* (n = 4) recovery of 100 ± 22, 110 ± 20 and 112 ± 10% analytical recovery.

Linearity and bias was calculated by determination of concentration in incurred samples diluted in plasma. Least squares regression analysis for each sample gave correlation coefficients ranging from 0.988 to 0.998. The intercept on the *x*-axis indicated minimal bias ranging from 0.01 to −0.004 ng clenbuterol ml^{-1}.

The within-assay coefficients of variation of triplicate determination (n = 7) were found to be 33 and 8% at mean concentration 0.02 and 0.41 ng ml^{-1}, respectively. Between-assay relative standard deviations for triplicate determination (n = 33) were 16 and 9% at mean concentrations of 0.04 and 0.45 ng ml^{-1}.

Results and discussion

Following oral administration, changes to plasma clenbuterol concentration with increasing time were described by fitting a two compartment model of drug distribution and elimination.[11] Steady state clenbuterol concentrations were achieved at 3 days for the therapeutic protocol and at 5 days for the growth promoting regime. A plasma C_{max} of 15.34 ± 5.38 ng ml^{-1} was achieved after the growth promoting dose; this was in keeping with Stoffel and Meyer,[6] demonstrating peak plasma concentrations of approximately 5.5 ng ml^{-1} following a growth promoting dosing regimen of 5 μg clenbuterol kg^{-1} twice daily. Estimation of mean residence time indicated relatively slow elimination of clenbuterol during both therapeutic (36.3 ± 7.5 h) and growth promoting (38.5 ± 4.2 h) treatments. This was supported by prolonged $t_{1/2}$ β for both groups of animals (Table 1). Comparison of plasma clenbuterol contents showed that the maximal concentration was significantly greater (36.5 fold) in animals treated with a growth promoting dose than those which received a therapeutic regime (Fig. 1 and 2).

Analysis of data obtained from plasma sampled on the sixth day of dosing during the therapeutic regimen dosing (at which point maximum plasma concentration was achieved) using a tolerance limit calculation[12] indicated that a sample above 1.63 ng ml^{-1} is indicative of a growth promoting dose (Table 2).

Using the threshold value it was possible to determine from Fig. 1 that plasma clenbuterol concentration would be maintained above this value following the growth promoting regimen for up to 80 h.

A number of cases of food poisonings associated with the consumption of liver containing clenbuterol residues have been reported since 1990,[13–15] and concerns for consumer safety have prompted EU restriction (amendment 3112/96 to Council Regulation 2377/90) of clenbuterol use to respiratory disease in horses and for tocolysis in parturient cows and horses. A method for clenbuterol analysis based on live animal sampling has a practical application for surveillance of clenbuterol misuse.

These data indicate that the use of plasma analysis in conjunction with an appropriate threshold limit can provide a practical means of discriminating between therapeutic and growth promoting treatment within a time frame encompassing the dosing period and extending (depending on dose) to 80 h following cessation of a growth promoting dose. Applied to targeted on-farm surveillance, a plasma sampling approach would be well suited to a targeted monitoring programme since animals can be readily sampled and clenbuterol concentration rapidly quantitated by simple methods which do not require sample extraction such as described here. There are significant advantages in using plasma analysis compared to urine, in that threshold concentrations for urine cannot so readily be set because of wide variations in concentration reflecting fluctu-

Table 1 Summary of pharmacokinetic parameters

	Therapeutic dose (n = 20)	Growth promoting dose (n = 8)
C_{max}[a]/ng ml^{-1}	0.42 ± 0.18	15.34 ± 5.38[g]
MRT_{dist}[b]/h	17.2 ± 9.5	33.6 ± 5.2[g]
MRT_{abs}[b]/h	19.4 ± 7.5	4.9 ± 3.2[g]
MRT_{tot}[b]/h	36.3 ± 7.5	38.5 ± 4.2
CL[c]/ml min^{-1}	465.7 ± 173.7	248.7 ± 101.6[h]
$t_{1/2}$ α[d]/h	0.6 ± 1.3	2.27 ± 2.17
$t_{1/2}$ β[e]/h	97.5 ± 5.5	39.77 ± 6.73[h]
AUC[f]/ng ml^{-1} h^{-1}	5.4 ± 2.7	109.39 ± 28.94[h]

[a] C_{max}: mean maximum concentration achieved during study. Since the number of samples taken during the dosing period were insufficient to allow accurate modelling during the treatment phase, to represent the maximum mean concentration actual concentrations are reported. [b] MRT: mean residence time. [c] CL: clearance. [d] $t_{1/2}$ α: α phase half life. [e] $t_{1/2}$ β: β phase half life. [f] AUC: area under the curve. [g] $p < 0.001$ (Student's *t*-test). [h] $p < 0.0001$ (Student's *t*-test).

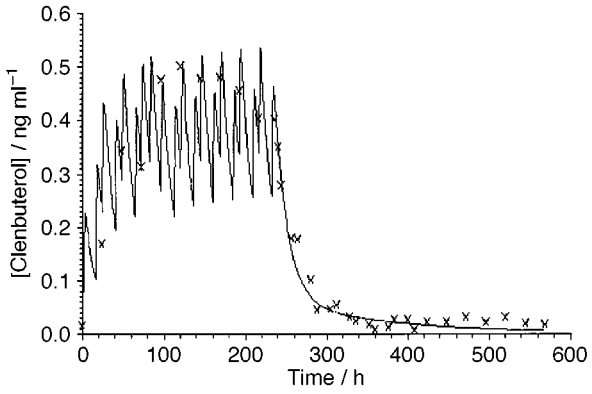

Fig. 2 Mean plasma concentration after initiation of therapeutic dosing regimen. Data were analysed using a 2 compartment model.

Table 2 Confidence levels for a plasma clenbuterol concentration permitting discrimination between therapeutic and growth promoting dose[a]

Confidence (probability)	Threshold (population lies below) (%)	Clenbuterol in plasma/ng ml^{-1}
0.95	95	0.96
0.99	95	1.10
0.95	99	1.36
0.99	99	1.63

[a] Values were calculated using the tolerance limit method.[12]

ation in urine output. Application of threshold values along with reference to treatment records (stipulation by EU directive 96/22/EC) may thus provide adequate provisional evidence of abuse pending confirmation analysis. Where no records of administration exist, measurement of any clenbuterol would be indicative of illegal use.

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