TERBUTALINE PHARMACOKINETICS IN COWS

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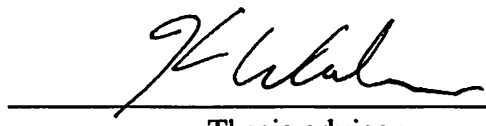

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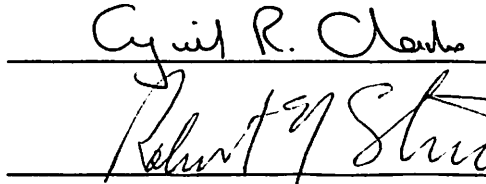

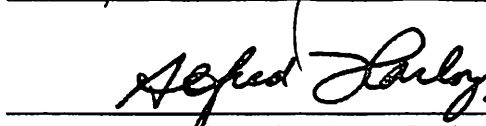

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Introduction

Effective management of dystocia in cattle is complicated by the limited availability of tocolytic drugs, which act to reduce myometrial tone and facilitate manipulation of the fetus and exteriorization of the uterus during cesarean section. Since the prohibition in 1997 in Europe and Canada of clenbuterol, another β_2 agonist used as a tocolytic in human medicine, terbutaline, has been employed only experimentally in veterinary medicine. Its utilization is problematic due to a paucity of pharmacokinetic information that can be used to estimate appropriate tissue residues. Such information is particularly relevant to terbutaline because of the history of abuse associated with its close relative, clenbuterol. While accurate withdrawal times can only be calculated by extensive sampling of a variety of relevant tissues, preliminary estimates can be obtained by investigating the serum pharmacokinetics after intravenous administration of a single dose. In addition to supporting estimation of withdrawal times, the availability of such pharmacokinetic data also will facilitate the design of future experiments investigating the distribution into milk, and the pharmacodynamic properties and safety of terbutaline.

Therefore, the goal of the study was to determine the serum pharmacokinetics of terbutaline in six healthy cows within 24 hours of parturition after parenteral administration.

Chapter I

Literature review

Dystocia

Definition

Dystocia can be defined as difficult parturition due to prolonged first or second stage labor, impedance of the fetal passage through the birth canal, or the necessity of assistance for delivery.[53,55,69] The first stage of parturition consists of progressive relaxation and dilatation of the cervix (average duration of 6 hours).[69] The calf is delivered during the second stage of parturition after rupture of chorioallantoid and amnionic membranes and application of intense abdominal pressure and myometrial contractions by the dam (average duration of 2 to 4 hours).[69]

Dystocia in cattle is considered a medical emergency and commands prompt resolution to afford the optimum prognosis for dam and fetus. The overall incidence of bovine dystocia ranges from 3-25 %, and first-calving heifers are most commonly affected because they have often not yet reached skeletal maturity.[50] Since the most common cause of calf mortality during the first 96 hours of life is dystocia, or injuries resulting from prolonged delivery,[7,68] a high incidence within a herd could have a strong negative economic impact. Also, cattle experiencing dystocia have been reported to have a subsequently reduced conception rate.[69]

Etiology

The etiology of dystocia in cattle is usually multifactorial and includes maternal factors, fetal factors, or a combination of both. In large animals, fetal causes of dystocia such as malpresentation, malposition, malposture or fetopelvic disproportion occur more commonly than those involving the dam.[55] Some examples of maternal causes include primary or secondary uterine inertia, abnormalities of the pelvic canal (inadequate size, pelvic deformities), incomplete dilatation of the cervix, stenosis of the vulva and uterine torsion.[69]

Management

History, length of gestation and duration of labor combined with a good physical, rectal and vaginal examination are necessary before attempting to manage cattle dystocia. Assessment of calf viability is also required prior to selection of the appropriate method of delivery.[69] Obstetrical procedures available for management and correction of dystocia include manipulation (repulsion, rotation, version, rectification of postural defects), extraction (mechanical), fetotomy, and as a last resort, cesarean section. However, the use of tocolytic drugs to relax the contracting pregnant uterus or undilated cervix could potentially represent another alternative for the management of bovine dystocia. Indeed, in cases of high uterine tone or intense myometrial activity, obstetrical manipulations can be very difficult to accomplish and, in these circumstances, the use of a myometrial relaxant could allow better access and easier manipulation of the fetus, or facilitate exteriorization or detorsion of the uterus during cesarean section.

History & Status of Clenbuterol

Clenbuterol is a β_2 adrenergic agonist that was used in Europe and Canada as a bronchodilator in horses or as a myometrial relaxant in cattle and sheep.[29,43] In a study conducted by Jonker, it was administered intravenously in 219 cows affected with fetal malpresentations (n=37), malposture (n=112), or uterine torsion (n=70).[29] The successful rate of vaginal delivery was 84 % (76/91) for malpresentations and malpostures and 77 % (70/91) for uterine torsion.[29] Good myometrial relaxation was obtained which made obstetrical manipulations easier and shorter for the veterinarian, and less traumatic for the dam.[29] Other recorded benefits included a lower need for epidural anesthesia and significantly lower incidence of retained fetal membranes.[29] A significant decrease of bovine myometrial activity was observed for at least 30 minutes after administration of clenbuterol in another study.[43]

Despite its aid for manual correction of bovine dystocia, clenbuterol has also been used illegally in food animals as a growth promoter (repartitioning agent) to reduce fat deposition, increase lean meat, and improve feed conversion efficiency.[33,44] To avoid the potential for human intoxication after consumption of liver or skeletal muscles of cattle treated with clenbuterol, its use was prohibited in 1997 in Europe and Canada. Common clinical signs associated with clenbuterol intoxication in humans include muscle tremors, tachycardia, and heart palpitations.[11,62] In the United States, this uterine relaxant has never been approved. There is no other myometrial relaxant approved for bovine obstetrical use.

Tocolytic therapy

Human medicine

In human medicine, many drugs have been employed to treat premature labor and reduce perinatal morbidity and mortality.[42] In an attempt to prevent the after-effects of premature delivery, attention logically has centered on efforts to find safe and effective tocolytic drugs. Beta 2 adrenergic agonists (ritodrine, terbutaline, isoxsuprine, epinephrine), calcium antagonists (magnesium sulfate), calcium-channel blockers (nifedipine), prostaglandin synthetase inhibitors (indomethacin), and oxytocin antagonists (atosiban) represent the classes of myometrial relaxants investigated. These studies involve the administration of different drugs at variable dosages, routes of administration, and variable efficacies while noting adverse effects associated with their use. The majority of clinical trials performed in the United States involve the utilization of β_2 adrenergic agonists such as terbutaline or ritodrine,[10,15,54] the latter being the only drug approved by the Food and Drug Administration for human tocolysis.[15,42]

Veterinary medicine

The effects of different tocolytic drugs has been investigated only experimentally in domestic animals. A significant proportion of published studies used a sheep model,[1,3,4,46,48,56] possibly due to their small size, docile nature, short gestation (5 months compared to 9 months in the bovine) and low cost.

Based on the literature, many tocolytic drugs have been used in domestic animals. Ritodrine, terbutaline, magnesium sulfate, and nifedipine are reported most commonly.[1,4,19,60] Experimental trials using isoxsuprine, epinephrine, indomethacin and atosiban have been reported less frequently.[20,46,48,56] Each drug has advantages and

disadvantages of various degrees, hence there is no ideal tocolytic drug. However, choosing the best myorelaxant for management of dystocia in large animals should be based on defined criteria such as efficacy, safety, availability, existing parenteral formulation, and cost (**Table 1**).

Calcium-channel blockers appear to be effective as myometrial relaxants in the ewe.[19] However, the product is only approved for humans and does not come in an injectable formulation. Oral administration in ruminants is undesirable due to the effects the ruminal flora may have of the drug metabolism. Magnesium sulfate has been used in domestic animals, however it appears to be less efficacious than β_2 agonists.[1] Commercially available sources of magnesium in veterinary medicine include an oral paste (Magnagel),[a] a powder (Carmilax),[b] or a salt (Epsom® salts).[c] Intravenous preparation of magnesium[d] approved in veterinary medicine are combined with calcium because hypomagnesemia is frequently accompanied by hypocalcemia in large animal species. Magnesium sulfate has been administrated IV in 15 ewes, however, no information or reference concerning the drug used in the study was provided.[1] To the author's knowledge, Epsom® salts has not been reported to be administered parenterally in domestic animals and needs further investigation. The efficacies of isoxsuprine and epinephrine to relax the myometrium have not been well demonstrated in domestic animals. Due to their non-specific action on β agonist receptors (β_1 and β_2), they also induce significant adverse effects.[5,48,56] Indomethacin and atosiban are approved only for humans and may be difficult to administer intravenously because of drug formulation and dosage requirements.[37] However, both induce myometrial relaxation with fewer side effects than β_2 agonists, and may represent potential tocolytic drugs to investigate further

in domestic animals.[20,37,46] Beta 2 agonists (ritodrine, terbutaline) are reported to be very efficacious for uterine relaxation in domestic animals.[12,52,60] Ritodrine is expensive, not available in the United States, and causes significant maternal and fetal side effects.[17,60] Terbutaline is available in the United States and is formulated for parenteral administration. Variable but overall moderate adverse effects have been associated with its administration in veterinary medicine.[4,14] Despite the higher expense, terbutaline was selected among the other tocolytic drugs and investigated for tocolytic use in bovine obstetrics based on its efficacy to relax the myometrium in domestic animals, moderate adverse effects associated with its administration, and the availability of a parenteral formulation of this drug (**Table 1**).

Table 1. Characteristics of tocolytic drugs

Tocolytic	Efficacy	Adverse effects	Drug availability	Parenteral formulation	Cost (adult cow)
Nifedipine	+++	++	+	No	$ 2.00
Mg Sulfate	++	++	+	Yes	< $ 3.00 (Epsom salt)
Isoxsuprine	+	+++	+	No	<$ 1.00
Epinephrine	+	+++	+++	Yes	$ 5.00
Indomethacin	++	+	+	No	$ 40.00
Atosiban	++	+	+	Yes	$ 8.00
Ritodrine	+++	+++	-	Yes	$ 32.00
Terbutaline	+++	++	+++	Yes	$ 50.00*

Legend: -: Not available in the United States
+: Low
++: Medium
+++: High
*: Terbutaline sulfate, Brethine ®

Considerations:

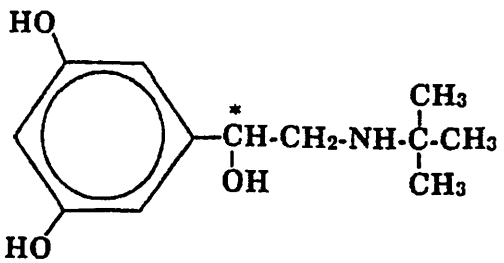
The ability of a drug to relax the myometrium was based on the quality and duration of relaxation, and the number of studies reporting significant myometrial relaxation in domestic animals. Adverse effects were evaluated by total number and severity. Drug availability was based on whether the product was available in the United States. The cost of a particular drug was determined for an adult bovine with an average body weight of 500 kg (1100 lbs).

Terbutaline

Physicochemical characteristics

Terbutaline [1-(3,5-dihydroxyphenyl)-2-t-butylamino ethanol] is a synthetic sympathomimetic amine that selectively stimulates β adrenergic receptors, particularly β_2 receptors located in bronchial, vascular and uterine smooth muscles, rather than β_1 receptors located in cardiac muscle.[16,51] Stimulation of β_2 receptors results in relaxation of smooth muscle. When administered either orally or intravenously, the molecule exists as a mixture of 2 stereoisomers (enantiomers), however only the (-)enantiomer exerts the desired pharmacological effects, while the (+)enantiomer is devoid of effects in the pharmacodynamic test models used.[9] Beta 2 receptors can bind β agonist drugs at 3 points on the molecule: the β hydroxyl group, aliphatic nitrogen, or the aromatic ring (**Figure 1**).[61]

Figure 1. Molecular structure of Terbutaline



The biological activity of terbutaline is due to the aromatic ring that is substituted with a hydroxyl group which binds the β adrenergic agonist to its receptor.[61] The biological activity is also dependent on the ionization status of the molecule. The 2 phenolic groups of the molecule have a pKa value of 8.8 and 11.2, and the aliphatic amine pKa is also alkaline at 10.1.[61] Thus, the drug is in an ionized form in blood (pH=7.4) after intravenous administration, according to the Henderson-Hasselbalch

equation.[16,54,61] Plasma or serum protein binding for terbutaline is reported to be low at 14 to 25%.[51]

The tocolytic effect of β_2 agonists is mediated by activation of adenyl cyclase that converts adenosine triphosphate (ATP) to intracellular cyclic adenosine monophosphate (cAMP).[63] The rise in cAMP activates the enzyme cAMP dependant protein kinase and stimulates the removal of myometrial intracellular calcium. Activity of myosin light-chain kinase (MLCK) is also reduced and myometrial relaxation occurs.[63]

Terbutaline undergoes hepatic metabolism. In humans and dogs, it is conjugated to sulfate and in rats, primarily to glucuronic acid.[47,64] Terbutaline is excreted predominately by the kidneys. The drug can cross the placental barrier in women. Fetal plasma levels up to 55 % of the maternal plasma level were reported in one study involving 10 women following the administration of the β_2 agonist.[28] To the author's knowledge, there is no study published on fetal plasma concentration of terbutaline in domestic animals. Therefore, the transfer of terbutaline across the placenta in domestic animals is unknown. Approximately 1 % of the dose administered to pregnant women is excreted in breast milk.[45] Excretion of terbutaline in the mammary gland of domestic animals has not been reported and needs further investigation.

Indications in human & veterinary medicine

Terbutaline is widely used in human medicine for treatment of bronchopulmonary disorders involving bronchospasms such as asthma, chronic bronchitis or emphysema. Many studies involving women in preterm and term labor investigated terbutaline as a tocolytic agent.[6,15,26,57] In women, terbutaline administered intravenously in emergency situations, rapidly stopped uterine contractions.[26,36]

In veterinary medicine, terbutaline is used as a bronchodilating agent in the treatment of cardiopulmonary diseases such as tracheobronchitis, allergic bronchitis, pulmonary edema, and collapsing trachea in small animals.[51] This drug is also used for treatment of bronchoconstriction in horses suffering from chronic obstructive pulmonary disease (COPD) also named recurrent airway obstruction (RAO).[51] The use of terbutaline in domestic animals as a tocolytic agent is limited and only experimental. Very few studies describe its effect on uterine blood flow and myometrial activity in rats and ewes.[12,14]

Adverse effects in human & domestic animals

Adverse effects (primarily cardiovascular and respiratory) remain a major consideration in the use of β_2 agonists to arrest premature labor in human patients.[42] Adverse effects reported in women following the administration of terbutaline include tremors, vomiting, tachycardia, premature ventricular contractions, hypotension, hyperglycemia, hyperinsulinemia, metabolic acidosis, hypokalemia, hypocalcemia, decreased glomerular filtration rate and pulmonary edema.[10,30,42] The mechanism by which pulmonary edema develops is still unknown but is most likely multifactorial. The main contributing factors include an increased blood volume (35-40 %) in pregnancy, the stimulation of the renin-angiotensin-aldosterone system and antidiuretic hormone production by β agonists (terbutaline), increasing fluid retention.[42] Other factors have also been suggested.[34] Fetal tachycardia and hyperglycemia have also been reported following maternal administration of terbutaline.[25,28] However, this β_2 agonist was found to be beneficial in a fetus with prolonged bradycardia.[27]

In veterinary medicine, maternal and fetal side effects appear to be similar but overall lower in severity compared to those reported in human medicine. Continuous infusion of 1500 micrograms (~ 2 μg/kg) of terbutaline over 30 minutes in pregnant ewes caused a transient maternal tachycardia and hypotension in 2 studies.[3,4] Other maternal and fetal adverse effects included metabolic acidosis, hyperglycemia, and lactic acidemia.[3] Administration of a bolus of 250 ug of terbutaline intravenously in 5 near-term pregnant ewes induced significant but transient maternal tachycardia and systemic hypotension, and decreased pulse pressure and blood flow in the main uterine artery.[14]

Pharmacokinetic disposition in humans & domestic animals

Many studies have investigated the pharmacokinetics of terbutaline in humans.[9,36,49] Different patients (pregnant women in premature labor, healthy men, asthmatic children), dosages, routes of administration (oral, IV, subcutaneous (SC), nebulization), and body fluids (plasma, serum, urine) have been investigated.[2,9,24,36,45,49] Relatively few trials have been conducted in domestic animals and these have been restricted to the investigation of intravenous pharmacokinetics in dogs, rats[47] and horses[66] and oral pharmacokinetics in broiler chickens[39] and dairy cattle.[67] The latter study was very limited in scope, published only as a doctoral thesis, and failed to provide sufficient data to allow estimation of withdrawal times in either meat or milk. The pharmacokinetic determinants described in these studies included therapeutic plasma concentration, bioavailability, volume of distribution, clearance (renal, total), mean residence time, and terminal half-life of terbutaline.

Therapeutic plasma concentration is based on the observed relationship between the measured plasma concentration of the drug, its pharmacologic and toxic effects.[54] A

common tocolytic dose for terbutaline in women is reported to be ranging between 200 and 250 micrograms.[9,26,35,36,42,49,57] Cessation of uterine contractions in women in preterm labor occurred when the plasma concentration of terbutaline ranged between 5-15 ng/ml in one study[8] and between 12.8 and 31.5 ng/ml in another study.[36] After administration of oral terbutaline once a day for 6 days to dairy cows at 50 μg/kg and for 14 days to broiler chicken at 10 μg/kg, plasma concentrations of terbutaline were 4 ng/ml and 42.8 ng/ml, respectively.[38,67] In a study involving 6 horses, median maximal serum terbutaline concentration was 9.3 ng/ml after administration of 10 μg/kg intravenously.[66]

Bioavailability can be defined by the degree to which a drug becomes available to the target tissue and is dependent on the route of administration.[54] In men, most oral β agonists are well absorbed (up to 75 %), except for terbutaline (14.8 %).[9] Peak plasma concentration is generally reached within 1 to 4 hours.[45] Due to extensive first-pass sulphation occurring in the liver, the bioavailability is low (8-15%).[2,45,49] The intestinal wall (mucosal epithelium) metabolism and the presence of food may affect the absorption of terbutaline after oral administration, contributing to a low bioavailability.[2,48] In the equine species, bioavailability of oral terbutaline was close to zero in one study.[65] To avoid the first-pass sulfation, tocolytic drugs have to be administered parenterally (IV, SC), thereby increasing their bioavailability to approximately 100 %.[35,45] In general, pulmonary administration of β_2 agonists with aerosol or nebulization shows 10 to 20 % absorption, and a maximum plasma peak concentration at 2 to 4 hours following administration.[45]

Mean residence time (MRT) can be defined as the mean time required for an intact drug molecule to transit through the body.[36] A MRT value of 9 hours was found in

healthy men,[49] and a value of 3.4 hours was reported in pregnant women.[36] In horses, the MRT was 30 minutes on average in one study.[66]

The volume of distribution represents the calculated body space available for distribution of a drug and varies from 0.8 to 1.9 L/kg in human and domestic animals[9,24,36,49,66] Because of the drug's low protein-binding property, the volume of distribution is not likely to be changed, even in the presence of a low serum albumin concentration.[51,58]

Total body clearance corresponds to the volume of distribution of a drug in the body cleared of a substance per unit of time and includes mainly renal and hepatic clearances (and others). Total body clearance has been reported to be 0.16 to 0.19 L/h/kg[9,24] in humans and approximately 1.9 L/h/kg in horses.[66] Renal clearance represents the volume of blood cleared of a substance by the kidney per unit of time.[53] Terbutaline is filtered and actively secreted by the kidney.[8] The renal clearance of the (+)enantiomer of terbutaline is greater than the (-)enantiomer, showing the stereoselectivity of its excretion.[9] In men and dogs, more than 90 % of a parenteral dose of terbutaline is excreted in the urine, 66 % of which is unchanged drug. Only 1 % is excreted via bile.[64]

Terminal half-life can be defined as the time required for a given plasma concentration of a drug to decline by 50 %. Terminal half-life for terbutaline reported in the literature in horses was 1.2 hour[66] and varied from 3.7, to 12.1, 14 or 17 hours in humans.[9,24,36,49]

Methods for assessment of pharmacokinetics of terbutaline

In the majority of the pharmacokinetic studies conducted on terbutaline, serum or plasma was obtained after centrifugation of whole blood collected via an intravenous catheter placed in a peripheral vein (jugular, femoral). The concentration of terbutaline present was measured mainly via 2 different techniques: gas chromatography with mass spectrometry (GC-MS)[8,9,24,66] or high pressure liquid chromatography (HPLC) using Ultra-Violet (UV) or fluorescence detection.[22,32,59] Either method appears to be adequate to determine the concentration of terbutaline in serum, plasma or urine, although GS-MS is more sensitive (0.15 ng/ml)[66] than HPLC fluorescence detection (0.3 ng/ml)[32] or HPLC-UV (0.1 μg/ml).[22] To the author's knowledge, there is no reported pharmacokinetic study of terbutaline using milk either from humans or domestic animals. However, HPLC and ELISA were used in a study detecting the presence of β_2 agonists (clenbuterol, salbutamol and terbutaline) in milk replacer for calves.[13]

Basic principles of High Liquid Performance Chromatography (HPLC)

Chemical separation

Certain compounds have different migration rates that are determined by the choice of a particular stationary phase and mobile phase. The stationary phase in HPLC refers to the immobile packing material contained within the chromatographic column over which the mobile phase continuously flows.[31] The mobile phase in HPLC refers to the solvent and carrier of the compounds present in the sample solution that passes continuously and under high pressure through the chromatographic column.[23,31] The chemical interactions of the stationary phase and the sample solution contained within the

mobile phase will determine the degree of separation of the components present in the sample solution. For example, samples that have stronger interactions with the stationary phase than the mobile phase will elute from the column less quickly, thus their retention time will be longer. The inverse is also true. Various types of stationary phases are commercially available and include liquid-solid (adsorption), liquid-liquid, and size exclusion. Liquid-solid stationary phase functions on the basis of polarity. Polar compounds that possess functional groups capable of strong hydrogen bonding will adhere more tightly to the stationary phase than less polar compounds.[23] Therefore, less polar compounds will elute from the column faster than highly polar compounds. However, when reverse phase HPLC is used (C_{18} column), less polar compounds will elute from the column slower than higher polar compounds.

Identification

Identification of a compound via HPLC involves the selection of a detector and the development of a separation assay. The detector is the component of an HPLC that emits a response to the compounds as they elute from the stationary phase and subsequently signals a peak on the chromatogram.[23] Some examples of the more common detectors include Ultra-Violet, Refractive Index, and Fluorescent.

The separation assay should be developed to obtain a clean peak of the target compound on the chromatogram with a reasonable retention time (usually 10 to 20 minutes). A known compound must be utilized in order to assure identification of the unknown target compound. Consequently, the selection of the parameters for the separation assay is accomplished by researching the literature for previously published methods and trial and error.

Quantification

Quantification is the process of determining the unknown concentration of a compound using a known solution of pre-determined concentration.[23] A series of known concentrations of the target compound is injected onto the HPLC for detection in order to obtain a standard curve. The chromatograph of these known concentrations will give a series of peaks that correlate to the concentration of the compound injected. The area under each peak (AUC) is then calculated, using the trapezoidal method $[0.5 \cdot (t_{i+1} - t_i) \cdot (C_{i+1} - C_i)]$ where t_{i+1} and t_i are times associated with sequential blood samples and C_{i+1} and C_i are the corresponding sample concentration.[39] The AUC (or the ratio of the AUC of the target compound and an internal standard) can be plotted with the known concentrations (target compound) on a graph. A best-fit line can be derived and the equation generated, also called polynomial response function (format: y=mx + b), will represent the calibration curve equation.[23] When a sample of unknown concentration "y" of the target sample is injected onto the HPLC, the chromatograph gives a peak output of area "x". The area under the curve "x" is then placed in the calibration curve equation, and the concentration of the unknown target compound is found by solving the equation for "y".

Chapter II

Materials and Methods

Animals

Six near-term pregnant beef cows purchased from a local livestock market were used in the study. Prior to parturition, animals were housed together in an outdoor large animal resource facility for 3 days. Normal health and pregnancy status were determined by physical examination and rectal palpation. Accurate weights were obtained and ranged from 437 to 664 kg. Parturition was induced on day 2 in order to synchronize delivery time. Induction of parturition was performed by administration of dexamethasone sodium phosphate at 20 mg/cow intramuscularly (IM), and dinoprost tromethamine ($PGF_{2\alpha}$) at 25 mg/cow IM. On day 3, the cows were transported to the Oklahoma State University (OSU) Boren Veterinary medical teaching hospital food animal barn where they were maintained in individual stalls (under the supervision of the principal investigator) until parturition. Each cow involved in the present experiment was cared for according to the guidelines of the Institutional Animal Care and Use Committee of Oklahoma State University. They were fed ad libitum grass hay and a quantity of commercial sweet feed sufficient to meet NRC requirements for late gestation cows.

Experimental design

Experiment I

Pharmacokinetic experiments were initiated within 24 hours post-partum. A 16 ga, 3 inch and a 12 ga, 5 inch intravenous catheter were placed in the right caudal auricular (ear) and jugular veins of each animal, respectively. Terbutaline sulfate (Brethine®)[e] was then administered as an IV bolus via the ear vein catheter at a dose of 5 μg/kg of body weight over a 30 second time period.

Blood samples were collected from the jugular catheter before drug administration and 5, 10, 20, 30, 45 minutes, and 1, 2, 3, 4, 6, 8, 12, 16, 18, 24, 48, 72 hours thereafter. After collection of each blood sample, the catheter was flushed with 5 ml of heparinized saline solution (10 U heparin/ ml of saline 0.9 %). All samples were collected into evacuated glass tubes without additive. Clotted blood samples were centrifuged (600 g for 15 minutes) and serum was harvested in plastic tubes and stored at -70°C until analysis.

Experiment II

A second experiment was performed using the same 6 cows, approximately 8 months after the initial experiment, using a higher dosage of 0.5 mg/kg of terbutaline sulfate (Sigma)[f] administered IV once. Prior to beginning the experiment II, this dosage was administered to a 2-year-old intact male alpine goat to screen for potential adverse effects. The goat's heart rate, respiratory rate and general health were monitored closely for 4 hours after the administration of the β_2 agonist (**Figure 2-3**).

For the second experiment, the cows were neither pregnant nor lactating. Each dose of terbutaline sulfate crystalline powder was measured with precision by using an

electronic scale.[f] The powder was then reconstituted using sterile water to obtain a final concentration of 12.5 mg/ml of terbutaline. The solution was passed through a 0.2 μm filter during the intravenous administration via the ear vein catheter. Blood collection timing was the same as in experiment I, except that the 72 hours blood sample was not collected. Heart rate and general health of each cow were monitored closely in the hour following the administration of terbutaline.

Terbutaline assay

The concentration of terbutaline in serum and milk samples was measured by high performance liquid chromatography (HPLC) with fluorescence detection, using a combination of 2 previously published methods with slight modifications in order to optimize the assay.[22,32]

Materials

Terbutaline sulfate, betaxolol, acetonitrile, and methanol were obtained from Sigma.[g] Terbutaline sulfate for injection (Brethine®) was manufactured by Novartis Pharmaceuticals (Summit, NJ, USA). Solid-phase extraction columns were 6 ml polypropylene columns packed with 1 g of C_{18} bonded phase manufactured by Varian (Palo Alto, CA, USA).

Serum analysis

Extraction protocol

One hundred microliters of an internal standard solution (betaxolol, 1 μg/ml) and 1 ml of acetonitrile were added to 1 ml of each serum sample and vortexed for 30 seconds. Each sample was centrifuged at 3000g for 15 minutes at 4 degree C°. The serum samples were injected into C_{18} solid-phase extraction columns that had been preconditioned twice with 3 ml of ethanol and 3 ml of distilled water. After loading of each sample, the columns were rinsed twice with 3 ml of distilled water. The serum samples were eluted with 1 ml of ethanol containing 50 mM of ammonium chloride buffer (pH 8.5, 95:5 vol/vol) and placed in receiver tubes in a vaccum manifold. The solvent was removed by drying the samples by speed-vac at 45 degrees C°. The dried residue was reconstituted with 100 μl of mobile phase [25 mM sodium phosphate buffer (pH 7.4): methanol (77:23 vol/vol)], vortexed, and centrifuged at 13,400 g for 2 minutes. The supernatant was transferred into new tubes and stored at –20 degrees C. The samples were passed through the C_{18} solid-phase extraction columns within the mobile phase and terbutaline was detected via fluorescence at excitation and emission wavelengths of 224 and 310 nm, respectively (**Appendix A**).

Standard curve

Standard curves (0, 25, 50, 100, 250, 500, 1000, 2000 ng of terbutaline/ml) were prepared by adding known amounts of terbutaline sulfate to drug-free bovine serum and measuring terbutaline concentration as described in the extraction protocol. The detection limit was 25 ng/ml.

Pharmacokinetic analysis

Concentration-time data were subjected to pharmacokinetic analysis by use of noncompartmental methods, based on statistical moment theory. Terbutaline pharmacokinetic parameters in the bovine were calculated with standard formulae. The area under the curve (AUC) was measured by trapezoidal method described elsewhere.[39,54] The volume of distribution at steady state (Vd_{SS}) was calculated via the formula $Vd_{SS} = Cl_B \bullet MRT$.[54] The formula Cl_B = Dose / AUC was used to calulate total body clearance (Cl_B).[54] The mean residence time (MRT) was calculated via the formula MRT = AUMC / AUC where AUMC represent the area under the moment curve.[54] The formula $t\ ½ = 0.693 \bullet Cl_B / Vd_{SS}$ was used to calculate the half-life (t ½) of terbutaline.[54]

Chapter III

Results

The concentration of terbutaline in the serum samples of experiment I was too low to be determined, despite a sensitive assay (detection limit: 25 ng/ml). Therefore, no pharmacokinetic data could be calculated.

The results of the trial administration of terbutaline to a healthy goat, prior to experiment II are depicted in **Figures 2-3.**

In experiment II (bovine species), the serum concentration-time curves (individual, mean) obtained for all 6 cows after IV bolus administration of 0.5 mg/kg of terbutaline are shown in **Figures 5-6.** After preliminary analysis, the concentration of terbutaline in the first 2 cows was below the detection limit of the assay after approximately 30 minutes following administration of the β_2 agonist. Therefore, the assay was performed only on the serum samples collected up to 4 hours (240 minutes) in the 4 remaining cows.

Significant tachycardia (mean heart rate: 174 +/- 10.4 beats/min) was found at approximately 2 minutes following the administration of terbutaline **(Figure 4),** compared to baseline (58 +/- 7.5 beats/min). Cardiac arrhythmias and muscle fasciculations were also noted in cows 1 and 5 respectively, and lasted approximately 45 minutes. Their recovery was uneventful and did not necessitate therapy.

In all 6 cows, the mean serum concentration of terbutaline detected approximately 5 minutes after the IV bolus of terbutaline was 708.22 +/- 509.6 ng/mL (**Table 2**). The mean area under the curve to infinity (AUC_{inf}) was 13692.8 +/- 6869 min·ng/mL (**Table 2**). The mean half-life for terbutaline was found to be 6.93 +/- 2.4 minutes. The MRT was calculated to be 6.75 +/- 3.6 minutes. The total body clearance was 43.29 +/- 17.2 mL/min/kg. The volume of distribution at steady state ranged from 0.080 to 0.805 L/kg with a mean of 0.34 +/- 0.28 L/kg.

Results of noncompartmental analysis of the distribution and elimination of terbutaline following IV administration to 6 healthy cows are depicted graphically in **Figure 7**.

Table 2-Pharmacokinetic analysis of Terbutaline following IV administration (0.5 mg/kg) to six healthy cows (Experiment II)

Parameters	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Mean*
$C_{t=5}$ ($ng \cdot ml^{-1}$)	1263.47	418.346	671.43	579.38	522.98	1495.94	708.22 +/- 509.6
AUC_{inf} ($min \cdot ng \cdot mL^{-1}$)	17875.21	7362.25	10531.8	10470.1	10160.8	25756.7	13692.8 +/- 6869
$t_{½}$ (min)	4.53	10.21	6.62	8.46	7.92	3.81	6.93 +/- 2.4
MRT (min)	2.87	11.85	7.72	9.71	4.57	3.78	6.75 +/- 3.6
Cl ($mL \cdot min^{-1} \cdot kg^{-1}$)	27.97	67.90	47.48	47.75	49.21	19.41	43.29 +/- 17.2
Vd_{ss} ($L \cdot kg^{-1}$)	0.080	0.805	0.366	0.464	0.225	0.073	0.34 +/- 0.28

*Values are expressed as mean +/- s.d. $C_{t=5}$ = serum concentration detected 5 min after the administration of terbutaline. AUC_{inf} = Area under the concentration-versus-time curve to infinity. $t_{½}$ = half-life. MRT = Mean residence time. Cl = Clearance. Vd_{ss} = Volume of distribution at steady state.

Figure 2-Heart rate in one healthy goat following the administration of Terbutaline (0.5 mg/kg IV)

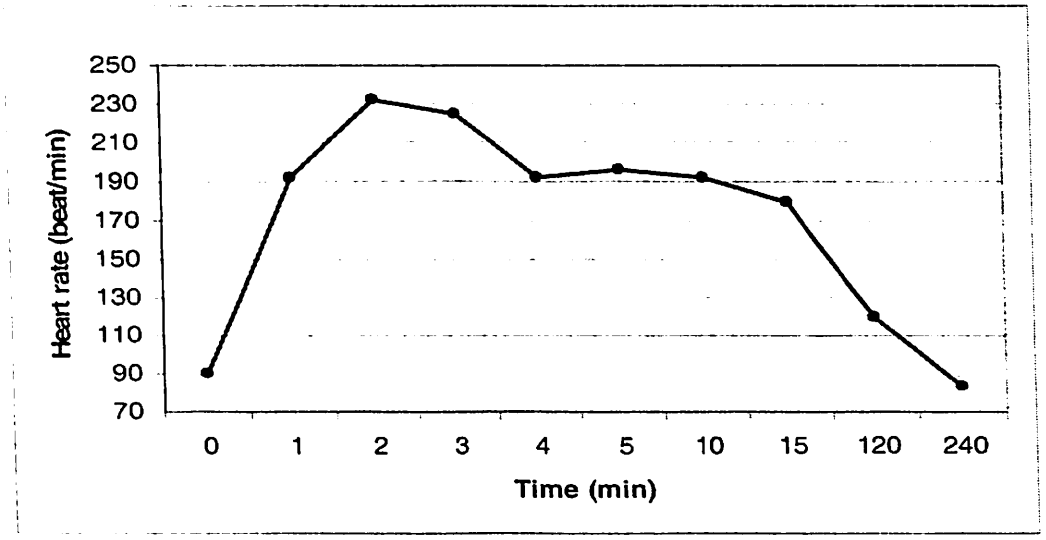


Figure 3. Respiratory rate in one healthy goat following the administration of Terbutaline (0.5 mg/kg)

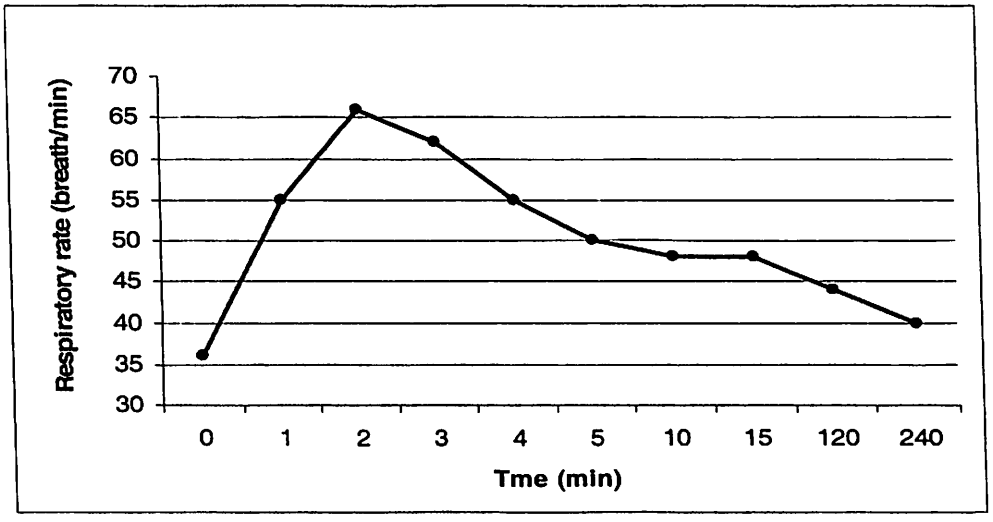


Figure 4. Mean heart rate of six cows following the administration of Terbutaline (0.5 mg/kg)

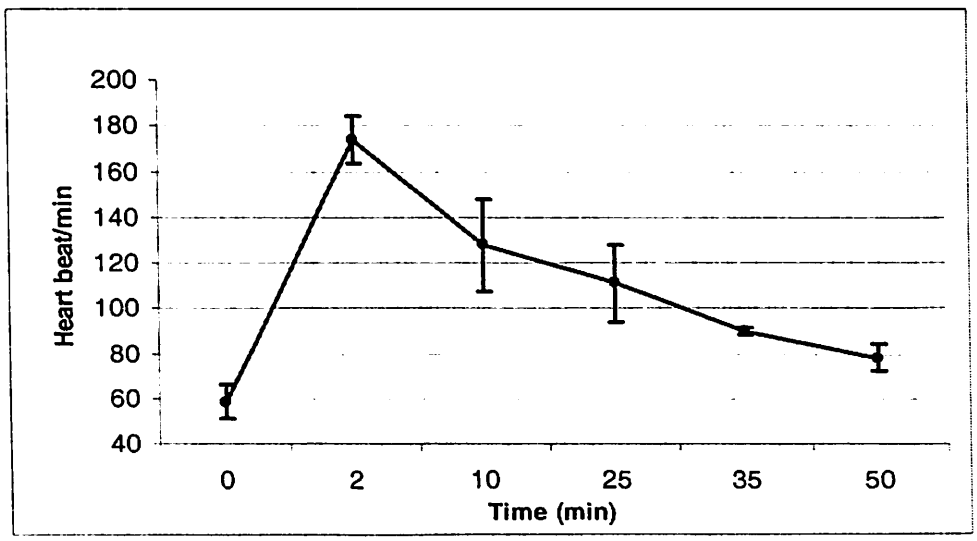


Figure 5. Terbutaline serum concentration after administration of 0.5mg/kg IV in six cows

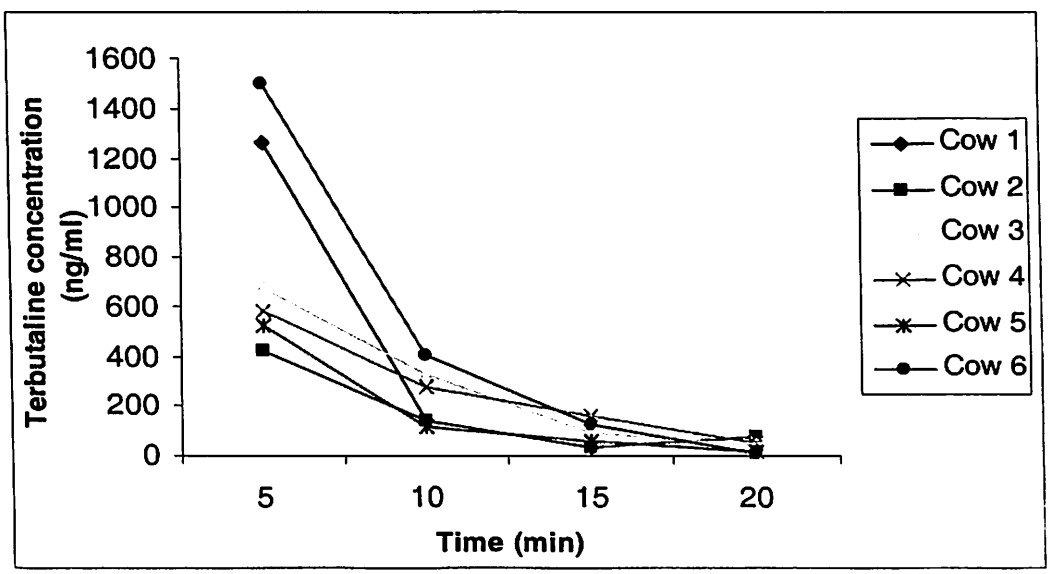


Figure 6. Mean serum concentration of Terbutaline administered to six cows at 0.5 mg/kg

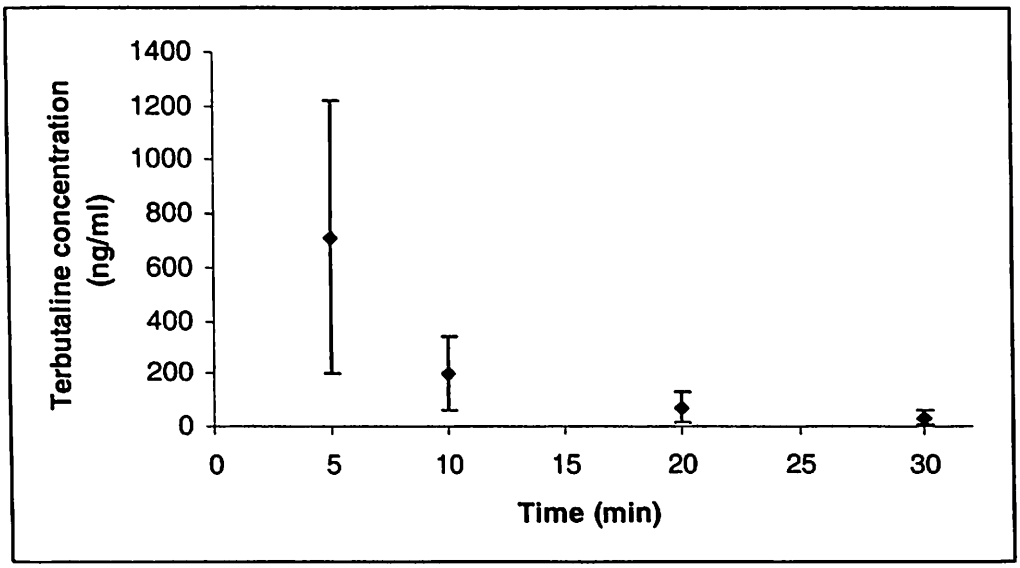
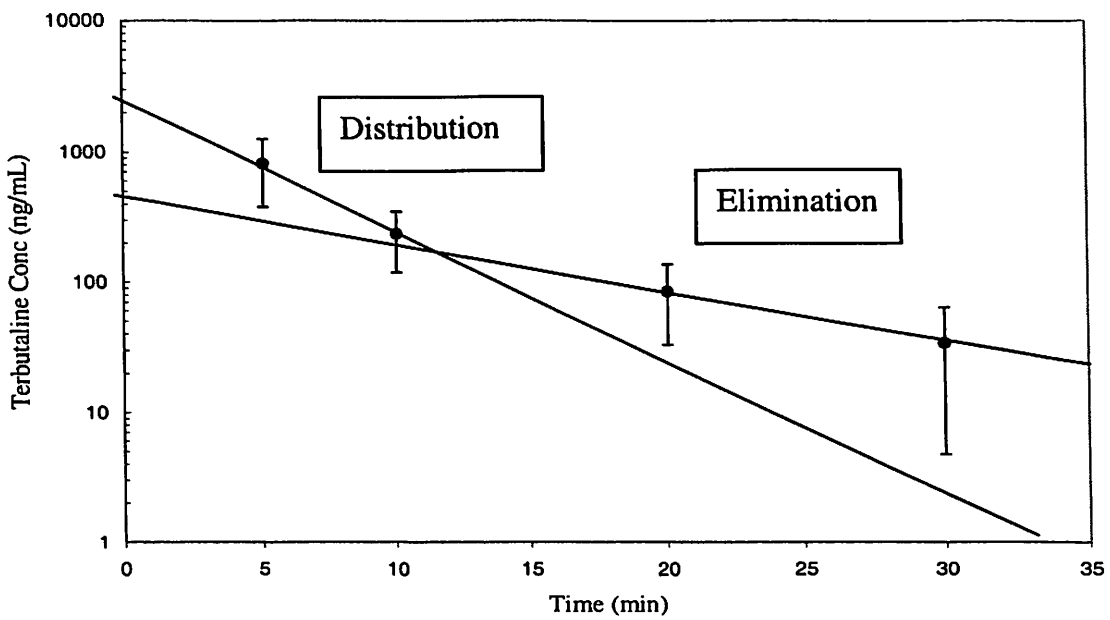


Figure 7. Serum Terbutaline concentrations (mean +/- s.d.) vs. time following a single 0.5 mg/kg dose administered IV to six cows using a noncompartmental model



Chapter IV

Discussion

The dosage of terbutaline selected for the first experiment was extrapolated from the most commonly reported dose used in women for tocolysis; 250-300 μg/min for an average 60 kg woman (0.5 μg/kg).[26,36,57] Comparisons between the pharmacokinetics of terbutaline in humans and domestic animals cannot be made. In a pilot study (non-published) using 4 late pregnant cows in which a cesarean was performed, a clinically detectable relaxation of the uterus was subjectively appreciated via sight and palpation, following a single administration of 5 μg/kg of terbutaline.

The assay used in the first experiment (HPLC with fluorescent detection) was developed based on Kim[32] and Herring[22] and the detection limit was 25 ng/ml. Despite this high sensitivity, the concentration of terbutaline in the initial experimental samples was too low to be determined, therefore, a second experiment using an increased dosage was performed.

To calculate a dosage of a drug, one required factor is volume of distribution. To the author's knowledge, a volume of distribution for terbutaline in the bovine has not been reported previously. However, it was found to be 0.9 L/kg in horses[66] and from 0.8 to 1.9 L/kg in humans.[9,24,36,49] Therefore, the volume of distribution was empirically selected at 0.5 L/kg (500 ml/kg) for cattle. Using a target maximal serum peak concentration of 1 μg/ml (empirical), and given that dosage of a drug equals its serum

concentration multiplied by its volume of distribution (Dose = Conc. x Vd), the new dosage of 500 μg/kg of terbutaline was calculated for the second experiment. This dosage was much higher than previously reported in humans and domestic animals for terbutaline.[3,26,49,57,66]

According to AMDUCA,[h] extralabel drug use (ELDU) in veterinary medicine is permitted only by or under the supervision of a veterinarian, allowed only for FDA approved animal drugs, and can only be used for therapeutic purposes (animal is suffering or its life is threatened), rather than production. The extralabel drug use should also meet the "label requirements" of AMDUCA: name and address of the prescribing veterinarian, established name of the drug, specified directions for use, identification of the animal, the dosage frequency, route of administration, duration of therapy, cautionary statements, and specified withdrawal time. However, in a research setting, a non-approved experimental drug such as terbutaline can be administered to food animals (bovine) as long as they do not enter the human food chain.

Induction of severe adverse effects (especially cardiovascular) using 100 times the initial dose of terbutaline was considered. However, the dose at which 50 % of animals die (LD_{50}) in rats for this β_2 agonist has been reported to be much higher (165 mg/kg).[i] To screen for possible adverse or fatal effects in a ruminant species, a pilot study using one 2-year-old healthy intact male alpine goat was conducted using 0.5 mg/kg administered via a jugular catheter over a 30 second time period. The goat showed significant tachycardia and tachypnea associated with terbutaline administration (**Figures 2-3**) but recovered uneventfully within 4 hours. Tachycardia associated with terbutaline administration in the goat and all 6 cows involved in the second experiment could be a

direct effect of terbutaline on the cardiac muscle via the stimulation of β_1 receptors, or secondary to systemic vasodilation (leading to hypotension) in response to β_2 adrenoreceptor stimulation of smooth muscle tissue in blood vessels. Cardiac arrhythmias found in cow 1 were most likely due to the stimulation of β_1 receptors located in cardiac muscle. The cause of muscle fasciculations exhibited by cow 5 after terbutaline administration may be due to hypokalemia, as reported in humans by Katz.[30] Suspected mechanisms leading to hypokalemia include a direct effect of the drug on the pancreatic islet cells inducing insulin release, stimulating cellular potassium uptake.[10] Other reported hypothesis include a direct stimulation of β_2 receptors in skeletal muscle, leading to direct cellular potassium uptake[10,66]

High pressure liquid chromatography data obtained in experiment II showed a variable serum concentration peak of terbutaline averaging 708.22 +/- 509.6 ng/mL, 5 minutes following its administration (**Figure 6, Table 2**). The exponentially decreasing curve following the peak was unexpectedly steep. Serum terbutaline concentrations were below the detection limit of our assay after 30 minutes (on average) following its administration (**Figures 5-6**). A similar decline in serum drug concentration was reported in equine species, although the drug was administered as a continuous infusion for an average of 33 minutes (10 μg/kg total) [66] instead of an IV bolus.

The pharmacokinetic (PK) analysis of concentration-time data was performed by use of a noncompartmental model. This particular model was selected based on the limited number of concentration-time data points obtained per cow. This model analyses using statistical moment theory.[54,58] Furthermore, the pharmacokinetic data do not need to be adjusted for the higher dosage (0.5 mg/kg) due to the fact that linear or first-order

kinetics is assumed with a noncompartmental model.[58] However, evidence of two different phases (distribution and elimination) was found when concentration-time data were plotted in a graph using a logarithmic scale (**Figure 7**). The two different phases suggested that a multicompartment pharmacokinetic model might be more appropriate for the PK analysis of terbutaline in bovine species than a noncompartmental model. Noncompartmental and one, two and three compartmental models have all been used for the pharmacokinetic analysis of terbutaline in other reports.[18,35,66]

In experiment II, the mean area under the curve (AUC) was 13692 ng·min/ml which corresponds to 228.2 μg·h/L. In order to make comparisons with the reported AUC for terbutaline in human and domestic animals,[26,66] a "dose normalized AUC" was used by dividing the AUC by the total dose of terbutaline administered. The dose normalized AUC obtained in the experiment II was 0.81×10^{-3} h/L (228.2 μg·h/L / 281000 μg), which was lower than the other AUC reported in humans, but similar to the one calculated in equine species. Indeed, in adult healthy men, the dose normalized AUC was calculated to be $69.71 \cdot 10^{-3}$ h/L (14.5 μg·h/L / 208 μg).[18] In horses, the dose normalized AUC was calculated at 1.18×10^{-3} h/L (5.37 μg·h/L/ 4525 μg).[66] Differences in AUC may be due to modeling error.[41] It may also be the result of failure to accurately define the terminal half life of a drug depending on the extent of which the blood concentration/time profile is described.[41]

Variable terminal half-lives for terbutaline (1.2, 3.7, 12.1 and 17 hours) have been reported in human and veterinary medicine.[24,36,49,66] A short half-life such as the one found in this experiment (t ½: 6.93 +/- 2.4 min) probably does not reflect the terminal elimination phase.[18] Based on **Figure 7**, it rather suggests a rapid half-life of distribution

throughout the body although this assumption remains uncertain. The variability in PK studies for this parameter appear to be due to the differences in duration of blood collection and serum concentration determination following the administration of the β_2 agonist.[49] It may also depend on the achievement of a steady-state of the drug.

The mean residence time calculated in this experiment was 6.75 +/- 3.6 minutes which was shorter than the MRT of 30 minutes reported in horses by Torneke.[66]

Total body clearance for terbutaline in experiment was 43.29 +/- 17.2 mL/min/kg (2.58 L/h/kg), which is comparable to that reported in horses (1.9 L/h/kg).[66]

The volume of distribution at steady state (Vd_{ss}) provides an estimate of drug distribution that is independent of the elimination process.[40] It also represents the sum total of all compartmental volumes and it is used to estimate the extent of extravascular drug distribution.[40] The mean Vd_{ss} for terbutaline in experiment II, 0.34 +/- 0.28 L/kg, was lower than the reported value for this parameter in humans and domestic animals.[9,24,36,49,66] This small Vd_{ss} (< 1 L/kg) suggests that terbutaline does not have a good extravascular distribution: it remains in the vasculature at a greater extent than in the tissue. The small Vd also suggests that the β_2 agonist should have an adequate watersolubility. Indeed, in the second experiment, terbutaline powder was diluted with sterile water at a concentration of 12.5 mg/mL without evidence of precipitation.

Compounding of drugs are prohibited for extra-label use in food-producing animals, because the drugs present a risk to the public health. To limit the cost of the drug due to the increased dosage, terbutaline powder (Sigma) was used in the second experiment to provide a parenteral formulation of the beta-2 agonist for administration to 6 cows that did not enter the human food chain.

Chapter V

Summary and Conclusions

Dystocia in cattle could be managed by the utilization of a β_2 agonist such as terbutaline. This drug has been efficacious for prevention and treatment of preterm labor in human medicine. However, it has only been used in an experimental manner in veterinary medicine. Since the prohibition of another β_2 agonist, clenbuterol, in Europe, Canada and the United States in food producing animals, the establishment of appropriate withdrawal times for terbutaline was indicated before pursuing its tocolytic potential in cattle.

The serum pharmacokinetics after intravenous administration of a single dose was therefore investigated in cattle, as a reliable preliminary estimate for establishment of tissue residues. High pressure liquid chromatography with fluorescence detection was used to detect the concentration of terbutaline in serum samples. The data obtained showed that this β_2 agonist disappeared abruptly from the serum after its administration. The limited number of significant concentration-time data points obtained per cow restricted the pharmacokinetic analysis to a noncompartmental model. All PK parameters were overall, dissimilar to those reported in the literature, with the exception of total clearance. The short half-life obtained was almost certainly not terminal, precluding the establishment of withdrawal times for meat (using 10 times the half-life of elimination).

Chapter VI

Future experiments

The pharmacokinetic data obtained in this experiment will be used as a basis for a future study in which a loading dose (30 μg/kg) and continuous IV infusion (258 μg/h/kg) of terbutaline in six cows will be maintained to achieve a steady state of serum drug concentration. This may allow the recognition of a distribution and elimination phase in serum and a distribution phase in milk, the selection of a more appropriate pharmacokinetic model for this β_2 agonist, calculation of its terminal half-life of elimination and subsequently, extrapolation of withdrawal times in meat and milk. To prevent inaccurate measurement of terbutaline concentrations, the method of detection and extraction of the drug will be based on the most sensitive detection method: gas chromatography with mass-spectrophotometry.

These pharmacokinetic data for terbutaline in bovine species will then serve as a basis for future experiments designed to evaluate the pharmacodynamics and safety of terbutaline and will correlate uterine electromyographic responses with concentrations of the drug in the systemic, uterine, and fetal circulation. A pharmacokinetic-pharmacodynamic (PK-PD) model will be constructed and then tested in a large population of bovine patients, with the ultimate goal of identifying demographic covariates that can be included in a population PK-PD model. The availability of such a model would allow estimation of dosage regimens appropriate for individual patients.

Footnotes

a. Magnagel: magnesium chloride, PRN Pharmacal, Pensacola

b. Carmilax®: magnesium hydroxyde: Pfizer, NY

c. Epsom® salt: magnesium sulfate·7 H_20, Cumberland Swan, TN

d. Caldex 2: calcium-combination therapy containing calcium, phosphorus, magnesium, dextrose, Fort Dodge animal health, N.W., IA

e. Brethine®: Terbutaline sulfate for injection (1 mg/ml), Novartis Pharmaceuticals, Summit, NJ, USA

f. Electronic scale: Ohaus Analytical Plus, Aldinger company, model AP250D-0, Switzerland

g. Sigma: pharmaceutical company, St-Louis, MO, USA

h. AMDUCA: Animal Medicinal Drug Use Clarification Act 2003. 21 CFR parts 530

i. PDR®: Physicians' Desk Reference, 56th edition. Medical Economics Company. 2002; 2314.

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Appendix A

Terbutaline extraction protocol using HPLC with fluorescent detection

Standard curve

Standard curve concentrations are 2000, 1000, 500, 250, 100, 50, 25, and 0 ng/ml of terbutaline (TBL).
Validation samples are six each of 25 and 1000ng/ml terbutaline.
Each standard curve, validation and experimental sample will contain 100ug/ml betaxolol (BXL).
All stock solutions are in methanol.

Validation samples

25ng/ml: 400ul of 500ng/ml TBL stock solution in 3600ul serum.
1000ng/ml: 800 ul of 5ug/ml TBL stock solution in 3200ul serum.
For each concentration pipet 7 500ul aliquots into glass screw-cap tubes and discard the rest. Use one aliquot of each concentration as a point in the standard curve.

Standard curve: in a glass screw-cap tube:
2000ng/ml: 200ul of 5ug/ml stock TBL in 300ul serum
1000ng/ml: 100ul of 5ug/ml stock TBL in 400ul serum
500ng/ml: 50ul of 5ug/ml TBL in 450ul serum
250ng/ml: 25ul of 5ug/ml TBL in 475ul serum
100ng/ml: 100ul of 500ng/ml TBL in 400ul serum
50ng/ml: 50ul of 500ng/ml TBL in 450ul serum
25ng/ml: 25ul of 500ng/ml TBL in 475ul serum
0ng/ml: 500ul serum

Extraction

- Add 100ul of 1mg/ml BXL stock solution to each tube. (5ml tip #1)
- Add 1ml of acetonitrile to each tube. (25ml tip #2)
- Vortex for 30 seconds
- Centrifuge at 3000g for 15 minutes at 4 degrees C.
- Precondition C_{18} solid-phase extraction columns with 2X3ml ethanol and 2X3 ml water.
- Pass serum through columns.
- Rinse columns with 2X3ml water.
- Place receiver tubes in vacuum manifold.
- Elute drugs from column with 1ml of ethanol:50mM ammonium chloride buffer, pH 8.5 (95:5, v/v).
- Dry in speed-vac at 45 degrees C.
- Reconstitute samples with 100ul mobile phase (5ml tip #1) and vortex.
- Centrifuge at 13,400g for 2 minutes.
- Transfer supernatant into a new tube.

- Store samples at –20 degrees C.
- Run through C_{18} column with the following mobile phase: 25mM sodium phosphate buffer (pH 7.4): methanol (77:23, v/v).
- Detect fluorescence at excitation and emmision wavelengths of 224 and 310nm.

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