Veterinary Journal of Kastamonu University Research Article
2022 Volume 1 (Issue 2) Pages 10-16

The effect of pentoxifylline administration at different doses on hematological and biochemical parameters in sheep

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Received 23.11.2022 - Accepted 30.12.2022 - Published 31.12.2022

Abstract: The aim of the present study was to determine the effect of administration of single increasing doses of pentoxifylline on biochemical and hematological parameters in sheep. The study was carried out on six healthy Merino sheep. Pentoxifylline was given to sheep intravenously at doses of 10, 20, and 40 mg/kg. Blood samples were taken from the jugular vein before (0 hour) and after (12 hour) pentoxifylline administration. Biochemical parameters such as creatine kinase, albumin, blood urea nitrogen, creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, cholesterol, triglyceride, total bilirubin and total protein were measured in autoanalyzer. Hematological parameters such as hemoglobin, hematocrit, platelet, red blood cells and white blood cells were determined in blood cell counter. Hematological parameters did not alter between the groups, although it was found that the hematocrit value in 20 mg/kg dosage group dropped. There were no significant changes in biochemical values following administration of the pentoxifylline at all dose level. These results show that pentoxifylline does not cause significant changes in biochemical and hematological parameters in sheep after intravenous administration at different doses. However, the safety study on pentoxifylline is also also necessary for multiple ascending doses in sheep.
Keywords: Biochemical, hematological, sheep, pentoxifylline.

Koyunlarda farklı dozlarda pentoksifilin uygulamasının hematolojik ve biyokimyasal parametreler üzerine etkisi

Özet: Bu araştırmanın amacı, koyunlarda artan tek doz pentoksifilin uygulamasının hematolojik ve biyokimyasal değerler üzerine etkisini belirlemektir. Araştırma 6 baş sağlıklı Merinos koyun üzerinde gerçekleştirildi. Pentoksifilin koyunlara 10, 20 ve 40 mg/kg dozlarında intravenöz olarak uygulandı. Kan örnekleri pentoksifilin uygulaması öncesi (0. saat) ve uygulama sonrası 12. saatte juguler venden alındı. Kreatin kinaz, albümin, kan üre nitrojen, kreatinin, alkalin fosfataz, aspartat aminotransferaz, alanin aminotransferaz, gama-glutamil transpeptidaz, kolesterol, trigliserit, total bilirubin ve total protein gibi biyokimyasal parametreler otoanalizör cihazında ölçüldü. Hemoglobin, hematokrit, trombosit, alyuvar ve akyuvar gibi hematolojik parametreler ise kan hücresi sayım cihazında belirlendi. Hematolojik parametrelerde gruplar arasında fark bulunmazken, 20 mg/kg doz grubunda hematokrit değerinin düştüğü belirlendi. Tüm doz gruplarında pentoksifilin uygulaması sonrası biyokimyasal parametrelerde önemli bir değişiklik görülmedi. Bu sonuçlar, farklı dozlarda intravenöz yolla uygulama sonrası pentoksifilinin koyunlarda hematolojik ve biyokimyasal parametrelerde önemli değişikliklere neden olmadığını göstermektedir. Bununla birlikte, koyunlarda çoklu artan dozlar içinde pentoksifilin güvenlik çalışmalarının yapılması gereklidir.
Anahtar kelimeler: Biyokimyasal, hematolojik, koyun, pentoksifilin.

Introduction

Pentoxifylline is a non-selective phosphodiesterase inhibitor used especially in the treatment of circulatory disorders (Uney et al., 2019). Pentoxifylline is preferred in circulatory disorders because it reduces the viscosity of the blood and provides oxygenation of peripheral tissues. In addition, it has anti-inflammatory and antioxidant effects (Corum et al., 2018; Sezik et al., 2020). Pentoxifylline is recommended for the treatment of lameness, vasculitis, collagen disorders, endotoxemia, septicemia, diabetic disorders and cancer in humans (Corum et al., 2019; Samlaska & Winfield, 1994). It has been reported that pentoxifylline can be used in the veterinary field in cases such as vasculitis, atopic dermatitis, dermatomyositis, contact allergy and systemic lupus erythematosus in dogs, cutaneous vasculitis in horses, laminitis, endometritis-placentitis, septicemia in foals, and vasculitis, laminitis, endometritis-placentitis in cattle (Sykes & Papich, 2014; Uney et al., 2019).

Although pentoxifylline is not approved in sheep, it has been used extra-label at different doses (10 to 60 mg/kg) in conditions such as septic shock, endotoxemia, preeclampsia, and bronchopulmonary injury (Chalmeh et al., 2016; Ogura et al., 1994; Sigurdsson & Youssef, 1993; Tálosi et al., 2001). In extra-label use, adverse effects should be assessed for effective and safe use of drugs. Biochemical and hematological parameters may be relevant in assessing adverse drug effects (Corum et al., 2015; Corum et al., 2016). However, no information was found about the effect of pentoxifylline on biochemical and hematological values in sheep. This study was conducted to assess the effect of pentoxifylline on biochemical and hematological parameters in sheep following a single intravenous injection of 10, 20, and 40 mg/kg.

Materials and Methods

Animals: The investigation was conducted on six female Merino sheep (57 ± 4 kg of body weight and 1.6 ± 0.3 years old) defined to be healthy by general clinical examination, biochemistry panel, and complete blood count. All animals were housed in individual pens throughout the study and were fed with commercial feed, and water was given *ad-libitum*. The experiment was approved by The Ethics Committee of the Faculty of Veterinary Medicine (University of Selcuk, Konya, Turkiye).

Experimental design: The study consisted of three periods with a 15-day washout period between treatments according to the crossover design. For drug administration to sheep, the analytical standard of pentoxifylline (Tokyo Chemical Industry, Europe) was dissolved with physiological saline (50 mg/mL). Pentoxifylline was administered to sheep via rapid intravenous (1 min) bolus injection at doses of 10, 20 and 40 mg/kg. Blood samples were collected into gel-containing tubes for biochemical analyses (3 mL) and into EDTA-containing

tubes for hematological analyses (2 mL) through jugular venipuncture (jugular vein) prior to pentoxifylline administration (0 h, control) and at 12 h after the pentoxifylline administration. Hematological parameters were analyzed immediately after taking blood samples. The blood samples collected for biochemical analyzes were centrifuged at 4.000 x g for 10 minutes, and the serum samples obtained were stored at −80 °C until the time of analysis. During the investigation, animals were also clinically observed.

Hematological and biochemical analyzes: The hemocell counter (Auto Hematology Analyzer, Shenzhen Mindray Bio-Medical Electronics, BC-2800, China) measured hematological characteristics such as hemoglobin, hematocrit, platelet, red blood cell (RBC) and white blood cell (WBC). Autoanalyzers (ILab-300 bioMerieux Diagnostics, Milan, Italy) were used to measure biochemical values such as creatine kinase (CK), albumin, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), cholesterol, triglyceride, total bilirubin (TBIL) and total protein (TP).

Statistical analysis: Hematological and biochemical parameters were presented as mean±SD. Shapiro-Wilk and Levene tests were used to determine the homogeneity and normality of the data distribution. The paired-t test was used to statistical analysis within the group. For the evaluation between groups, one-way analysis of variance (ANOVA) and post hoc Tukey tests were used. SPSS 19.0. (IBM Corp, Armonk, NY) statistics program was used for the statistical analysis. Statistical significance was considered as $P< 0.05$.

Results

No clinical adverse effects were observed in animals in the 10 and 20 mg/kg dose groups. In the 40 mg/kg dose group, tachycardia and hypersalivation were observed and the animals were agitated for approximately 4 hours. Hematological and biochemical parameters in sheep following intravenous administrations of pentoxifylline at different doses are presented in Table 1 and Table 2, respectively. There was no difference in biochemical parameters (BUN, creatinine, albumin, ALT, ALP, AST, cholesterol, CK, GGT, TBIL, TP, and triglyceride) in the in-group and between-group comparisons ($P> 0.05$). While there was no difference between the groups in hematological parameters ($P> 0.05$), it was determined that the hematocrit value decreased in the 20 mg/kg dose group ($P< 0.05$).

Table 1. Hematological parameters in sheep (n=6) following single intravenous administrations of pentoxifylline at doses of 10, 20 and 40 mg /kg (mean ± SD).

Parameters	**10 mg/kg**		**20 mg/kg**		**40 mg/kg**	
	0 h	**12 h**	**0 h**	**12 h**	**0 h**	**12 h**
WBC (*10^9/L)	7.15±0.76	7.68±1.09	7.29±1.13	7.40±1.06	7.48±1.05	7.28±1.33
RBC (*10^{12}/L)	11.29±1.65	11.62±1.45	10.99±0.89	11.07±0.84	10.69±0.98	11.63±1.73
HGB (g/dL)	10.12±1.73	10.23±1.34	11.17±1.25	10.40±1.00	9.52±1.17	10.02±1.79
HCT (%)	31.77±4.64	32.87±4.21	38.50±3.15	31.45±3.28*	30.53±4.37	31.80±3.75
Platelet (*10^9/L)	240.17±51.57	257.33±51.15	238.00±42.31	243.17±37.08	239.50±31.73	246.33±43.79

*; The value is statistically different from the 0 h in the same group.
WBC; white blood cells, RBC; red blood cells, HGB; hemoglobin, HCT; hematocrit.

Table 2. Biochemical parameters in sheep (n=6) following single intravenous administrations of pentoxifylline at doses of 10, 20 and 40 mg/kg (mean ± SD).

Parameters	**10 mg/kg**		**20 mg/kg**		**40 mg/kg**	
	0 h	**12 h**	**0 h**	**12 h**	**0 h**	**12 h**
ALB (g/dL)	3.44±0.30	3.40±0.29	3.54±0.27	3.51±0.41	3.57±0.41	3.40±0.36
ALP (U/L)	147.67±32.72	156.83±29.69	159.33±17.10	159.83±12.67	146.00±34.09	164.17±30.86
ALT (U/L)	16.50±3.56	16.67±2.66	16.17±3.31	16.00±2.53	17.50±2.59	15.33±3.88
AST (U/L)	151.50±38.38	151.50±35.19	139.17±46.76	145.83±39.46	146.67±46.07	154.83±33.44
BUN (mg/dL)	21.93±3.33	20.78±2.58	20.73±2.05	20.68±3.49	21.25±3.11	21.17±3.22
CHOL (mg/dL)	52.83±8.80	53.00±9.19	50.17±8.68	50.83±9.60	53.50±7.42	54.00±8.29
CK (U/L)	168.67±38.30	169.67±22.21	176.67±38.26	178.83±32.36	177.83±32.39	187.00±36.52
CRE (mg/dL)	0.70±0.10	0.70±0.12	0.70±0.12	0.69±0.11	0.75±0.10	0.73±0.12
GGT (U/L)	57.50±6.09	57.17±7.33	57.17±8.13	59.00±7.67	59.50±9.71	59.17±7.19
TBIL (mg/dL)	0.09±0.04	0.07±0.03	0.08±0.04	0.07±0.05	0.09±0.07	0.06±0.04
TP (g/dL)	6.57±0.25	6.58±0.26	6.46±0.34	6.55±0.33	6.73±0.35	6.71±0.22
TRIG (mg/dL)	15.17±4.62	18.00±2.37	16.67±4.80	18.00±3.29	19.17±4.07	18.17±3.37

ALB; albumin, ALP; alkaline phosphatase, ALT; alanine aminotransferase, AST; aspartate aminotransferase, BUN; blood urea nitrogen, CHOL; cholesterol, CK; creatine kinase, CRE; creatinine, GGT; gamma glutamyltransferase, TBIL; total bilirubin, TP; total protein, TRIG; triglyceride.

Discussion

Pentoxifylline is used in human and veterinary medicine to promote microcirculation and to provide peripheral oxygenation (Sezik et al., 2020). In experimental studies in sheep, pentoxifylline was used in the dose range of 10-60 mg/kg (Chalmeh et al., 2016; Ogura et al., 1994; Sigurdsson & Youssef, 1993; Tálosi et al., 2001). However, the use of drugs in increased doses may cause adverse effects. Biochemical and hematological values are used to judge the effects of medications on physiological and pathological states (Corum et al., 2022). The biochemical values (BUN, creatinine, albumin, ALT, ALP, AST, cholesterol, CK, GGT, TBIL, TP, and triglyceride) indicate the functionality of the kidney, liver, muscle, heart, and lipid metabolism. Hematological parameters (hemoglobin, WBC, RBC, hematocrit, and platelet) reflect fluid-electrolyte balance situation and bone-marrow functions (Kerr, 2002a; Kerr, 2002b; Turgut, 2000).

In this study, when different dose groups of pentoxifylline were compared, there was no difference in hematological parameters. In the in-group comparison, it was seen that the hematocrit value decreased in the 20 mg/kg dose group. However, this change was within the reference values specified in merino sheep (Lepherd et al., 2009). Similarly, oral (30 mg/kg) and intravenous (8 mg/kg) administration of pentoxifylline did not cause any change in hematological parameters in dogs (Rees et al., 2003). Intravenous administration of pentoxifylline (10, 20 and 40 mg/kg) in goats caused changes in WBC and hemoglobin (Coskun et al., 2022). It has been reported that pentoxifylline increases the level of hemoglobin in hemodialysis-induced anemia by suppressing the production of pro-inflammatory cytokines, which inhibit the efficacy of erythropoietin (Cooper et al., 2004; Shahbazian et al., 2017). It also increased the value of hematocrit in humans (Antunes et al., 2008; Golbasi et al., 2003).

Intravenous administration of pentoxifylline (10, 20 and 40 mg/kg doses) to sheep did not cause any difference in biochemical parameters. It has been reported that there was no difference in biochemical parameters following intravenous administration of pentoxifylline at a dose of 10 mg/kg to cattle and 8.5 mg/kg to horses (Liska et al., 2006; Uney et al. 2019). However, administration of pentoxifylline at a 10, 20 and 40 mg/kg doses to goats caused significant changes in biochemical (BUN, ALT, AST, TP, GGT, creatinine, albumin) values (Coskun et al., 2022). In addition, pentoxifylline decreased AST, ALT, and GGT levels in non-dyslipidemic and non-alcoholic human with fatty liver disease, and BUN and creatinine values in male rats (Cioboată et al., 2017; El-Haggar & Mostafa 2015; Jalili et al., 2019).

While no clinical side effects were observed at 10 and 20 mg/kg doses of pentoxifylline in sheep, tachycardia and hypersalivation and transient agitation were observed at 40 mg/kg doses. The rapid (1 min) intravenous bolus injection of pentoxifylline at 40 mg/kg dose may

cause these side effects. In goats, administration of pentoxifylline at the same dose and route did not cause any clinical changes (Coskun et al., 2022). Pentoxifylline caused transient hypersalivation and discomfort in cattle (10 mg/kg, intravenous), increased heart rate, sweating, and fasciculations in muscles in horses (8.5 mg/kg, intravenous), and deep respiration, eye closing, and lethargy in chickens (100 mg/kg, intravenous and oral) (De Boever et al. 2005, Liska et al., 2006, Uney et al., 2019).

Conclusion

This study showed that pentoxifylline did not cause significant changes in biochemical and hematological values in sheep after intravenous administration at 10, 20 and 40 mg/kg doses. However, pentoxifylline caused clinical adverse effects at 40 mg/kg doses. Therefore, the slow bolus injection (>1 min) or continuous infusion of pentoxifylline at 40 mg/kg dose would likely decrease these adverse effects. However, in sheep, the safety study on pentoxifylline is also necessary for multiple ascending doses as pentoxifylline is often used in repeated doses.

Financial Support

This research received no grant from any funding agency/sector.

Ethics Statement

This study was confirmed by the Ethics Committee of the Faculty of Veterinary Medicine of Selcuk University (Konya/Türkiye) (2015/07).

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgements

The study was presented in the form of abstract as poster presentation in the 1th International Congress on Advances in Veterinary Sciences & Technics, August 25-29, 2016.

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