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The effects of the method and dose of cyclophosphamide administration on cashmere shedding

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ABSTRACT - The objective of this study was to investigate the effects of the method and dose of cyclophosphamide (CPA) administration on cashmere shedding. Thirty-two castrated Liaoning cashmere goats were randomly allotted to four groups, with eight replicates in each group. Goats in the four groups were injected intravenously or intramuscularly with CPA at doses of 20 or 25 mg kg^{-1} body weight (BW), respectively. Feed intake and BW were recorded, and erythrocyte count, hemoglobin content, and cashmere weight were determined. It was found that the CPA administration method had no significant effect on feed intake or BW of cashmere goats. Cyclophosphamide injection can significantly decrease the erythrocyte count and hemoglobin content of cashmere goats, but the effects are dependent on injection method and CPA dose. The injection method and dose did not significantly influence cashmere weight, but the method had significant effects on time to initiate shedding and regrown hair length. Regrown hair was longest by intramuscular injection with 20 mg kg^{-1} BW, which also caused the least erythema on the epidermis during the days after shedding. The results indicate that the CPA administration method can significantly influence cashmere shedding. Intramuscular injection of CPA at a dose of 20 mg kg^{-1} BW was found to be relatively beneficial for hair removal and regrowth in cashmere goats.

Keywords: cashmere goats, dose, injection method, regrown hair length, shedding start time

Introduction

Cyclophosphamide (CPA), as a kind of anticancer drug, can increase nuclear aberrations in hair follicle cells of patients receiving it (Goldberg et al., 1990) and cause hair loss (Stork and Schreffler, 2014). Accordingly, some studies have been conducted on the use of CPA to stimulate wool shedding in fine-wool sheep. These studies have shown that CPA can be used as a chemical defleecing agent for sheep (Dolnick et al., 1969; Reis and Chapman, 1974; Fahmy and Moride, 1984; Lu and Cai, 2007). The appropriate doses can defleece sheep within a determined period of time and have no harmful side effects (Fahmy and Moride, 1984). In fine-wool sheep, studies have already been performed after treatment with CPA, but there is no related study on CPA in cashmere goats, except our previous study, which indicated that intravenous injection with CPA (about 20 mg kg^{-1} body weight [BW]) could induce cashmere shedding, increase cashmere weight, and achieve the purpose of concentrated defleecing (Chen et al., 2018). In addition, our research team also found that intravenous injection can significantly decrease the erythrocyte count and hemoglobin content in Liaoning cashmere goats in the early days of postinjection.

Generally speaking, intravenous injection can have a more rapid effect than intramuscular injection, but the latter is much easier and safer. However, the question of whether the two CPA administration

methods affect the process remains unanswered. The purpose of this study was to investigate the effects of CPA administration method and dose on cashmere shedding in cashmere goats to develop the concentrated defleecing technique for cashmere fiber further.

Material and Methods

The experiment was conducted in Shenyang city (41.8° N, 123.4° E) of China in April of 2017. Research on animals was conducted according to the institutional committee on animal use (201706001).

The experimental design was two injection methods × two dose levels with time blocks. Two injection methods were adopted in this study: intravenous and intramuscular. The CPA doses (supplied by Shanxi Powerdone Pharmaceutical Co., Ltd.) were 20 and 25 mg kg^{-1} BW, respectively. Thirty-two yearling castrated Liaoning cashmere goats of 32.8±2.3 kg BW, with similar cashmere production and general good health, were randomly allotted to four groups, with eight replicates in each group. Four pens of the same size (16 m^2) were used, and each group occupied a single pen. An individual feeding trough was provided for each goat, and the animals were supervised while eating so that individual intakes were obtained. All the goats were offered the same diet and water *ad libitum*. The diet was a total mixed ration (TMR) whose roughage was hay; its metabolizable energy content was 8.90 MJ kg^{-1} dry matter with crude protein at 123 g kg^{-1} dry matter.

The goats were offered their feed twice daily for 1 h, refusals were taken at the end of the feeding period, and feed intake for the day was summed. Goats were weighed individually before feeding in the first and last mornings of the experiment.

Blood samples (5 mL) were collected by venipuncture from the jugular vein and mixed with K_2EDTA to preserve the whole blood on days 0, 2, 6, and 10 postinjection. Erythrocyte count and hemoglobin content of each goat were determined using a semiautomatic biochemical analyzer (Model PUS-2018G; Nanjing Perlong Medical Equipment Co., Ltd.). Hemoglobin content was analyzed by the cyanmethemoglobin method and the hemoglobin colorimetric assay kit was supplied by Sigma-Aldrich Co. LLC.

When the cashmere fiber on the neck of the goats started to shed, the number of days from injection was recorded as shedding start time. Cashmere weight was measured according to the following steps: weighing of the hair shed by the goats individually; sampling of hair (5 g) from the shoulder, side, and thigh of each goat before the fleece was removed; picking of the coarse wool completely, measuring the cashmere content and calculating the rate of cashmere fiber; and calculation of the cashmere weight using the hair weight multiplied by the rate of cashmere fiber. Regrown hair length was measured using the following method: just when the goat coat was peeled off, the regrown hair sample was shaved very closely to skin with a razor blade in a 10×10 cm patch area from the middle of the left-side of each goat, and 200 fibers from each goat were measured using a ruler of 0.1 cm precision with the aid of a magnifying glass.

Results are presented as mean ± standard error. All analyses were executed with SPSS19.0 for Windows, and multivariate analysis of variance was performed to analyze the effects of injection method and dose on all the parameters according to the following mathematical model:

$$Yij = \mu + \alpha i + \beta j + \varepsilon ij$$

When data on blood parameters were analyzed, one-way analysis of variance was performed to indicate the change over time in the same treatment according to the following mathematical model:

$$Yij = \mu + \gamma i + \varepsilon ij$$

In the two models above, Yij = observation j of experimental unit subjected to treatments i, μ = general constant, αi = effects of injection method, βj = effects of dose, γi = effects of time, and εij = random error associated to each observation. Significant differences were determined via Duncan's multiple range test. The significant level was 0.05.

Results

There were no significant differences among the four treatments for feed intake, initial BW, or final BW of cashmere goats (P>0.05; Table 1). Therefore, CPA injection method and dose had no significant effect on feed intake or BW in the cashmere goats.

The erythrocyte count and hemoglobin content decreased significantly in the cashmere goats after CPA injection (P<0.05; Table 2). The erythrocyte counts were significantly lower on day 2 than those on days 0 and 6 (P<0.05), and there was no significant difference between days 0 and 6 (P>0.05). On day 2, they were significantly lower with the injection dose of 25 mg/kg BW than those with 20 mg kg^{-1} BW (P<0.05); they were also significantly lower by intravenous injection than intramuscular injection (P<0.05). The hemoglobin content significantly decreased in all the groups on days 2 and 6 than those on day 0 (P<0.05); there was no significant difference, however, between days 0 and 10 (P>0.05). On days 2 and 6, the injection dose of 25 mg kg^{-1} BW significantly reduced hemoglobin content than the dose of 20 mg kg^{-1} BW (P<0.05); intravenous injection significantly reduced hemoglobin content than intramuscular injection (P<0.05). Therefore, it can be said that the CPA injection significantly reduced the erythrocyte count and hemoglobin content of cashmere goats in the days immediately following injection, and that the decrease in numbers of erythrocytes was related to the injection method and dose. The adverse effect of CPA injection on erythrocyte count diminished within six days, and the hemoglobin content returned to the pre-injection level within 10 days.

The CPA administration caused both guard hair and cashmere fiber to shed. When the hair was completely shed, the goats in all four groups experienced a regrowth of hair fiber that covered their skins. The CPA injection method and dose did not influence cashmere weights significantly (P>0.05; Table 3), but both had a significant effect on shedding start time and regrown hair length when the goat coat had just been peeled off (P<0.05). Shedding occurred sooner, and regrown hair was shorter by intravenous injection than by intramuscular injection (P<0.05). The regrown hair was longest in the group of intramuscular injection with 20 mg kg^{-1} BW.

Table 1 - Daily feed intake and initial and final body weights of cashmere goats with different injection methods and doses

Item	IV		IM		SEM	P-value	
	20	25	20	25		Method	Dose
Daily feed intake (kg d^{-1})	0.87	0.85	0.86	0.85	0.04	0.662	0.533
Initial body weight (kg)	33.1	32.9	32.6	32.5	2.0	-	-
Final body weight (kg)	33.3	32.7	32.5	32.5	2.1	0.546	0.384

IV - intravenous injection; IM - intramuscular injection; SEM - standard error of the mean.

Table 2 - Erythrocyte count and hemoglobin content in the blood of the cashmere goats with different injection methods and doses

Item	IV		IM		SEM	P-value	
	20	25	20	25		Method	Dose
Erythrocyte count ($\times 10^{12}$ L^{-1})							
Day 0	9.05A	9.13A	9.08A	9.10A	0.70	-	-
Day 2	8.33abB	8.03cB	8.44aB	8.18bB	0.65	0.041	0.033
Day 6	8.98A	8.95A	9.05A	8.93A	0.68	0.265	0.238
Hemoglobin content (g L^{-1})							
Day 0	39.56A	40.05A	39.94A	39.81A	1.86	-	-
Day 2	36.83abB	36.66bB	37.30aB	36.84abB	1.80	0.035	0.030
Day 6	37.91bAB	37.72bAB	38.66aAB	37.85bAB	1.52	0.047	0.039
Day 10	39.62A	39.92A	39.95A	39.68A	1.78	0.144	0.216

IV - intravenous injection; IM - intramuscular injection; SEM - standard error of the mean.
Values in the same row followed by different lowercase letters are significantly different (P<0.05); values in the same column followed by different uppercase letters are significantly different (P<0.05).

Table 3 - Cashmere shedding of cashmere goats with different injection methods and doses

Item	IV		IM		SEM	P-value	
	20	25	20	25		Method	Dose
Shedding start time (d)	12.2ab	9.8b	13.2a	11.2ab	1.6	0.033	0.025
Regrown hair length (mm)	1.7ab	1.1b	2.0a	1.5ab	0.2	0.040	0.028
Cashmere weight (g)	712	719	733	724	65	0.587	0.636

IV - intravenous injection; IM - intramuscular injection; SEM - standard error of the mean.
Values in the same row followed by different letters are significantly different (P<0.05).

It was observed that all goats had different degrees of erythema on their skins after shedding. The injection dose of 25 mg kg^{-1} BW demonstrated the greatest erythema (especially by intravenous injection), whereas the intramuscular injection with 20 mg kg^{-1} BW caused the least. The erythema disappeared, however, on about day 7 after shedding.

Discussion

The toxic effects of CPA administration mainly exist in the gastrointestinal function and marrow inhibition (Lu and Cai, 2007); nevertheless, in this study, the CPA administration method had no significant effects on feed intake or BW of cashmere goats. Li et al. (1980) found that CPA injection (22 and 24 mg kg^{-1} BW) had no significant effect on erythrocyte count but did significantly decrease hemoglobin content of fine-wool sheep on days 2, 3, and 4 postinjection. Guo et al. (2011) reported that intramuscular injection with CPA (26 mg kg^{-1} BW) reduced leucocyte concentration in cashmere goats, and Chatzinasiou et al. (2017) also observed that significant drops in blood leukocytes were induced by intravenous administration of CPA (37.5 mg kg^{-1} BW) in sheep. However, we found in our previous study that intravenous injection with CPA (15 to 25 mg kg^{-1} BW) did not affect the leucocyte number in cashmere goats (Chen et al., 2018). Besides the previous study, it was also found in the present study that erythrocyte counts and hemoglobin content decreased following CPA injection, and CPA dose had a significant effect on erythrocyte number. These findings indicate that the CPA injection can cause the hematopoietic function to be weakened under the conditions in this study, but the effects are minor, dose-dependent, and temporary. Therefore, the CPA injection at doses of 20 to 25 mg kg^{-1} BW could damage only the hematopoietic function temporarily without effect on the appetite and growth of cashmere goats.

In sheep, the wool-loosening response caused by CPA has been shown to be dose-dependent (Reynolds et al., 1972). This was also the case with the cashmere goats in this study. It was found that the goat coats could be peeled off easily during days 10 to 13 post-CPA injection, and the regenerated fiber covered the goat skins after the cashmere fiber had completely been removed. Furthermore, the CPA administration method also had significant effects on time to initiate shedding and regrown hair length. Specifically, intramuscular injection with CPA at a dose of 20 mg kg^{-1} BW delayed shedding and led to longer regrown hair. Chen et al. (1998) found that Xinjiang fine-wool sheep began shedding between days 6 and 8 following intramuscular injection with CPA at a dose of 30 mg kg^{-1} BW. In addition, Wansley et al. (1998) reported that an oral dose of CPA at 30 mg kg^{-1} BW could be used as a hair-loosening agent in Angora goats. Therefore, it can be inferred that the appropriate CPA dose is related to the administration method and animal species.

Cashmere goats are sensitive to cold, heat, and sunshine after shedding, especially during the first five days. The results of this study indicated that the selection of a suitable administration method for CPA serves to form a new layer of guard hair on the goat body, which then protects against sunlight radiation and cold stress and reduces the erythema that might appear on the skin in the days after shedding. The appropriate method and dose of CPA administration on cashmere shedding can benefit the concentrated defleecing technique for cashmere fiber in goats.

It is probable that the CPA injection induces cashmere shedding, because CPA inhibits the normal metabolic processes of hair-follicle and hair-root cells of cashmere goats, so that nutrients are withheld from the cells for a short period (see the experimental results of Lu and Cai (2007) in relation to fine-wool sheep). In addition, CPA administration reduces the levels of serum melatonin in cashmere goats (Luan et al., 2012), which is likely to prompt the cashmere fiber to fall off, because melatonin is a major factor influencing follicle activity (Cong et al., 2011).

Conclusions

Cyclophosphamide administration causes a temporary decrease in the erythrocyte count and hemoglobin content of cashmere goats, and the decrease is dependent on the method and dose of cyclophosphamide injection. The cyclophosphamide administration method has no significant effect on feed intake, body weight, or cashmere weight, but it does have significant effects on time to initiate shedding and regrown hair length. Intramuscular injection with cyclophosphamide at a dose of 20 mg kg^{-1} body weight can appropriately delay shedding and lead to longer regrown hair, which has been shown to be relatively beneficial for hair removal and regrowth in the cashmere goats in this study.

Author Contributions

Project administration: Q. Chen and X. Li. Writing-original draft: Y. Cong. Writing-review & editing: Y. Cong.

Conflict of Interest

The authors declare no conflict of interest.

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