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Adrenal Response to Stress and Plasma Levels of Cholesterol, Urea and Indol in Acetylsalicylic Acid Treated Lambs

D. GUDEV, S. POPOVA-RALCHEVA, I. YANCHEV and P. MONEVA
Institute of Animal Science, BG - 2232 Kostinbrod, Bulgaria

Abstract

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In this study we investigated the effects of acetylsalicylic acid on adrenal response and plasma levels of cholesterol, urea and indol in 3 months old lambs exposed to various stress stimuli: separation of the lambs from their mothers, transport (24 km) and accommodation to a new environment.

The animals (n=10) were allocated into 2 groups – control and experimental. The experimental lambs were given orally one tablet aspirin (325 mg) immediately before the transport and another tablet 3 days after the transport.

Acetylsalicylic acid pretreatment decreased plasma cortisol level at 1st ($P>0.05$) and 3rd d ($P<0.05$) as compared to control lambs but had no aftereffect as judged by cortisol levels measured at 6 d and 9 d. Plasma cholesterol levels tended to be lower in experimental lambs.

Plasma urea and indol levels in experimental lambs were higher throughout the studied period but the urea levels of significance were achieved at 3 d and 9 d ($P<0.05$) and those of indol levels at 1 d and 6 d ($P<0.01$).

It is suggested that the observed effect of acetylsalicylic acid on the investigated blood constituents was mediated by hypothalamic- pituitary- adrenal axis and digestive tract microflora.

Key words: acetylsalicylic acid, stress, cortisol, cholesterol, urea, indol, lambs

Introduction

It has been shown that acetylsalicylic acid exerts both stimulatory (Halter and Metz, 1982) and inhibitory (Cavagnini et al, 1979; Nye et al., 1997) effects on hypothalamic-pituitary- adrenal axis in response to various stimuli used to elicit adrenocorticotropin secretion. The effect of aspirin is largely due to its ability to inhibit prostaglandins synthesis, which is done by irreversibly blocking cyclooxygenase (prost-

taglandine synthetase), which catalyzes the conversion of arahidonic acid to endoperoxide compounds (Metz, 1981).

We have previously reported significantly decreased adrenocorticotropin response to transport and accommodation in a new environment in acetylsalicylic acid pretreated upper mountainous lambs (Gudev et al., 2002).

More recently Coetzee et al. (2007) have reported that intravenous sodium salicilate administration prior to castration attenuates acute plasma cortisol response in calves. We did not find any data concerning the effect of acetylsalicylic acid on rumen microflora activity.

In this study we examined the effects of acetylsalicylic acid on both adrenal response and some blood constituents used as indirect indices of ruminal gastrointestinal function in lambs after transportation and accommodation in a new environment.

Material and Methods

Ten 3-months old lambs were used in this study. The average live weight of the lambs was 18 kg. The lambs were abruptly separated from their mothers and transported from the Institute‘s branch “Zlatusha” to the Institute‘s physiological unit situated around 24 km apart from each other. The animals were in good health as judged by their health records appearance and food behavior.

The animals were allocated into two groups – control and experimental. The experimental lambs were given one tablet acetylsalicylic acid (325 mg, Asperan Perrigo Company, USA) prior to commencement of the transportation and one tablet at 3rd day following the transportation to the physiological unit. Each tablet was dissolved in 20 ml distilled water, administered orally 3 hours prior to the blood sampling, while control lambs received 20 ml distilled water instead.

The effectiveness of the administered dose of acetylsalicylic acid was determined in our previous study (Gudev et al., 2002).

Blood samples were taken by jugular vein puncture at 1, 3, 6 and 9 d following the transportation.

Adrenal response to the applied stress stimuli was assessed by the level of plasma cortisol as determined by radioimmunoassay (Kanchev et al., 1976). All assays were performed in duplicate.

Plasma cholesterol and indol were determined by the methods of Watson (1960) and Balahovskii (Chilov, 1959), respectively. Plasma urea levels were assayed as described by Rerat et al. (1979). The results of the statistical analysis are expressed as means ± S.E.M. and were analyzed by Student t-test.

Results and Discussion

Adrenal response of the lambs to the applied stress-eliciting stimuli (separation from their mothers, transport, accommodation in a new environment) was lower at 1st and 3rd d as compared to that of control lambs but the difference was significant ($P<0.05$) at 3rd d only (Figure 1). There were no significant differences between the two groups at 6 and 9 d. These data suggest that acetylsalicylic acid does not exert continuos aftereffect on adrenal response to stress and therefore should be given throughout the stress period. The varying results obtained from studies using different stress-eliciting stimuli may reflect different effects of prostaglandins in the regulation of hypothalamus and anterior pituitary which is species specific (Nye et al., 1997).

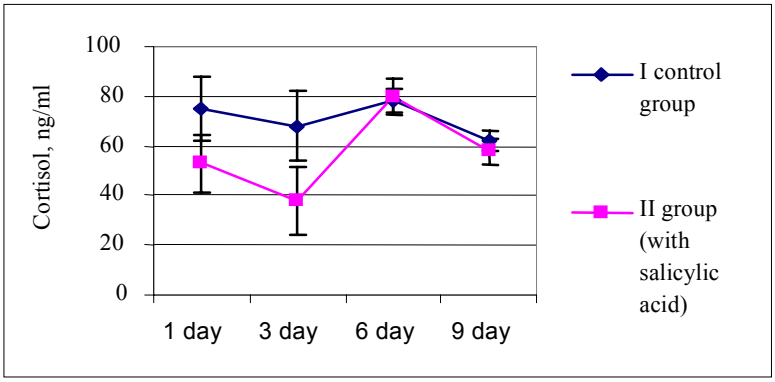

Fig. 1. Effect of acetylsalicylic acid on plasma cortisol response of lambs to weaning and transport

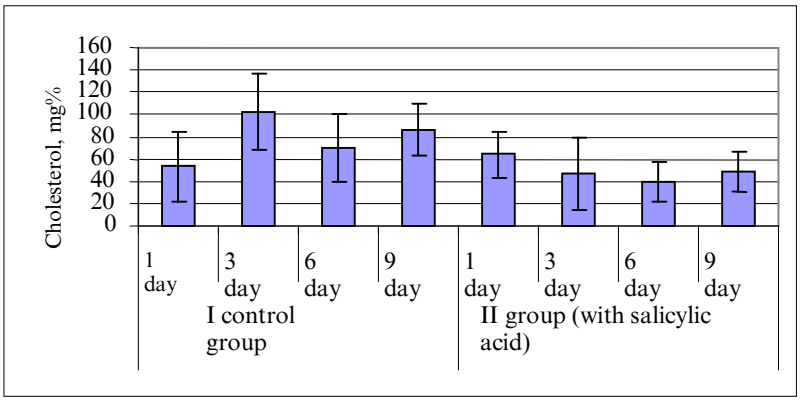

Fig. 2. Effect of acetylsalicylic acid on plasma cholesterol level after weaning and transport of lambs

In our previous experiment we have found that pretreatment of goats with acetylsalicylic acid does not influence adrenal response to exogenous adrenocorticotropin, but attenuates adrenal response to immobilization (Gudev et al., 2001). These results are consistent with those reported by Hockings et al. (1993) who found no direct effect of acetylsalicylic acid on human adrenal steroidogenesis.

Despite the controversial results concerning prostaglandins mediated effect of acetylsalicylic acid on hypothalamic pituitary adrenal axis (Vlaskovska et al., 1984; Brooks and Gibson, 1992; Zacharieva et al., 1992) our present and previous data demonstrate that acetylsalicylic acid given orally reduces lambs adrenal response to routine handling transport and novelty.

There were no significant differences in plasma cholesterol levels between control and experimental lambs (Figure 2). However plasma cholesterol level in the experimental lambs tended to be lower at

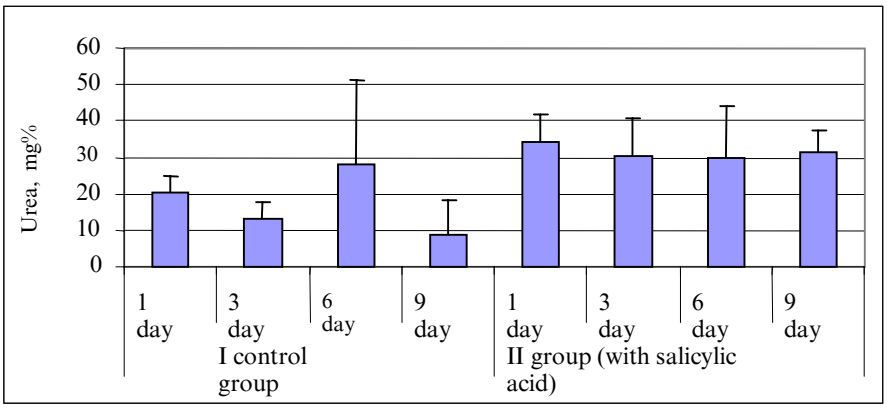

Fig. 3. Effect of acetylsalicylic acid on plasma urea level after weaning and transport of lambs

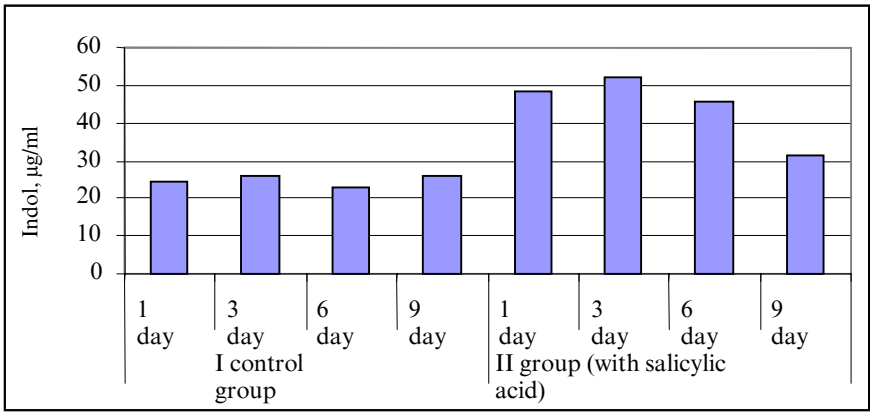

Fig. 4. Effect of acetylsalicylic acid on plasma indol level after weaning and transport of lambs

3rd d in comparison with that of control lambs. The observed trend corresponds to the lower level of cortisol at that time. It is known that prolonged adrenocorticotropin treatment suppresses hepatic scavenger receptor class B type 1 to provide precursor cholesterol for glucocorticoid syntesis resulting in elevated plasma low-density lipoprotein and high – density lipoprotein (Vieira-van Bruggen et al., 1998; Galman et al, 2002).

The lack of significance in plasma cholesterol level at 3rd day could be due to the relatively high individual differences. Alternatively, acetylsalicylic acid could exert stimulatory effect on plasma cholesterol by inhibition of gastrointestinal microorganisms or change the ratio between the friendly and unfriendly microorganisms. Some lactic acid bacteria are known to resist the detergent-like anti-microbial properties of bile salts that are continuosly

present in the gastrointestinal tract. They hydrolyse the conjugated bile salts which are synthesized mainly from cholesterol. This could lead to decline in plasma cholesterol level because of the enhanced cholesterol requirement for bile salts synthesis. Therefore, the proposed inhibitory effect of acetylsalicylic acid on gastrointestinal microorganisms may counteract the cholesterol-reducing effect exerted by gastrointestinal microorganisms. Consequently, the controversial results reported by numerous investigations may reflect prevalence of one of the two cholesterole monitoring mechanisms.The proposed inhibitory effect of acetylsalicylic acid on the friendly gastrointestinal microflora in our case may lead to elevation or no effect on plasma cholesterol (Bahar and Adrew, 1999).

We did not find any data concerning the effect of acetylsalicylic acid on ruminal or intestinal microflora. Indirect evidence implicating detrimental effect of acetylsalicylic acid on the friendly gastrointestinal microflora was obtained from the pronounced enhancement of plasma urea (Figure 3) and indol (Figure 4) levels in the experimental lambs.

Although plasma levels of urea were higher in acetylsalicylic acid treated lambs throughout the studied period the levels of significance were reached by 3rd and 9th d only. These data suggest that the observed higher urea levels at 6th and 9th d were due to the aftereffect exerted by acetylsalicylic acid since the lambs were treated twice – before the transport and by 3rd d following the accommodation to the new environment.

There are number of potential explanation for the acetylsalicylic acid augmented plasma urea level. It may reflect a direct or indirect effect acetylsalicylic acid on ruminal microflora. Possible indirect effect could be exerted by a change of ruminal ph, ruminal temperature and by influencing food consumption.

Recently it has been reported that therapeutic doses of aspirin lead to elevation in core body temperature during conditions of heat stress associated with induction of heat shock proteins (Fawcett et al., 1997).

Although ruminants eliminate salicylate rapidly the slow absorption of aspirin from the reticulo-rumen is rate limiting and therapeutically effective concentrations are maintained (Langston, 1993).

Plasma indol levels in acetylsalicylic acid treated lambs were higher throughout the studied period but levels of significance were reached by 1st and 6th day only (Figure 4).

These data are consistent with our view that acetylsalicylic acid may have a negative effect on the healthy composition of microflora. The possible reduction of the friendly bacteria could leave more food ingredients for the unfriendly bacteria. According to Claus and Raab (1999) tryptophan, which is indol predecessor is derived from mucosa cell debries. Thus reduction of friendly bacteria could ensure more tryptophan for growth of unfriendly bacteria which ultimately increases plasma indol levels. Further work is required to determine the exact effect of acetylsalicylic acid on growth rate of ruminal and intestinal microflora.

Conclusion

Acetylsalicylic acid attenuated adrenal response to stress stimuli elicited by transport and novelty. It had no significant effect on plasma cholesterol levels, but caused significant elevation of plasma urea and indol levels, suggesting a possible nega-

tive effect on ruminal and intestinal microflora.

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