

Sodium Salicylate Attenuates Acute Stress Responses to Castration in Calves

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Abstract: Acute stress following surgical castration of calves is a common animal welfare concern. Administration of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) can reduce post-castration stress but treatments vary in cost and efficacy. In this study, the effectiveness of sodium salicylate in relieving stress during invasive castration of calves was assessed on the basis of changes in serum cortisol levels and was compared with the effectiveness of ketoprofen. Twenty male Holstein calves were divided into 4 groups: a sham-operated untreated group, a castrated untreated group and 2 castrated groups receiving NSAIDs (sodium salicylate or ketoprofen). NSAIDs were injected intravenously immediately before administering local anesthetic. Blood samples were collected at regular time intervals for measurement of cortisol concentrations. Serum cortisol concentrations of the calves that were administered sodium salicylate were lower than those of the calves that were not administered any NSAID at 1, 1.5 and 2 h after castration and higher than those of the calves that were administered ketoprofen at 2 h after castration ($p < 0.05$). Although, the analgesic and anti-inflammatory effects of sodium salicylate are weaker than those of ketoprofen, the use of salicylic acid derivatives in surgical castration is useful from the viewpoint of animal welfare and entails a significantly lower cost.

Key words: Calf, cortisol, NSAID, stress, lower cost, salicylic acid

INTRODUCTION

Reduction of stress during castration of cattle is a common animal welfare concern which has prompted studies on appropriate timing for and methods of castration (Bretschneider, 2005; Stafford and Mellor, 2005). Intravenous administration of ketoprofen, a second-generation Non-Steroidal Anti-Inflammatory Drug (NSAID) that acts on both cyclooxygenase and lipoxigenase (Boothe, 2001) is known to reduce stress during castration. Stress is assessed by measuring blood cortisol levels and ketoprofen significantly suppresses castration-induced increases in cortisol levels (Earley and Crowe, 2002; Stafford *et al.*, 2002; Ting *et al.*, 2003a, b; Sedghi *et al.*, 2006; Zare *et al.*, 2007; Afaghi *et al.*, 2007; Mahjoor and Dehghan, 2008; Fatemeh *et al.*, 2008; Sattari *et al.*, 2009; Seyednour and Chekaniazar, 2011). However, calves that weigh >100 kg require fairly high doses of expensive second or third generation NSAIDs like ketoprofen. Therefore, NSAIDs are not widely used during castration with consequent compromise on animal welfare.

Salicylic acid derivatives which are a major type of first-generation NSAIDs are inexpensive and well understood therefore, it has been assumed that they would be effective even though they only inhibit cyclooxygenase-1. The *in vivo* kinetics of salicylic acid derivatives in cattle during castration have been recently studied in detail (Coetzee *et al.*, 2007), making the surgical application of these drugs an attractive option. In this study, the effectiveness of sodium salicylate, a salicylic acid derivative, in relieving stress during the invasive castration of male calves was assessed on the basis of changes in blood cortisol levels and was compared with the effectiveness of ketoprofen.

MATERIALS AND METHODS

Animals and treatments: Twenty, 5 months old male Holstein calves, weighing 169 ± 13 kg (mean \pm SD) were divided into 4 groups as follows: untreated, sham-operated; untreated, castrated; sodium salicylate treated, castrated and ketoprofen treated, castrated. Calf restraint, administration of local anesthesia and

administration of NSAIDs were performed according to previously reported methods (Earley and Crowe, 2002; Stafford *et al.*, 2002) with slight modification. In brief, 20 min before castration, the calves were restrained in a standing position and 4 mL of 2% procaine hydrochloride was injected as a local anesthetic into each testis parenchyma through the distal pole. The testis was then pushed dorsally off the needle and 1 mL of local anesthetic was injected into the subcutaneous area around the distal end of the scrotum. After both sides had been treated, the scrotum was massaged to facilitate the spread of the local anesthetic. In sham-operated calves, the scrotum was only massaged by hand after the administration of local anesthetic. In castrated calves, the scrotum was cleaned and the lower half of scrotum was cut off using a surgical scalpel. The testes and spermatic cord were exteriorized by gentle traction, the cord was knotted and the scrotum was cut off from the distal side of the knotted part. Procaine penicillin G was applied to the surgical field and injected intramuscularly ($100,000 \text{ IU kg}^{-1} \text{ BW}$) after castration. The NSAIDs, 25 mg kg^{-1} sodium salicylate (Salusobroka and dextrose injectable 5%; Mitaka, Japan) and 3 mg kg^{-1} ketoprofen (Ketofen 1% injectable; Merial Limited, France) were injected intravenously immediately before administering the local anesthetic. Two experienced veterinarians carried out all drug treatments and castrations. This study was approved by the Animal Care and Use Committee at Azabu University School of Veterinary Medicine (Approval number: 090120-2).

Blood collection and serum cortisol analysis: Blood samples were collected from the jugular vein at 0.5 h before castration, immediately after castration and at 0.5 or 1 h intervals for 7 h after castration. The collected blood was incubated at 37°C to allow clotting and centrifuged ($1500\times g$ for 20 min, 4°C) and the isolated serum was stored at -80°C until analysis. The serum cortisol concentration was measured by a radioimmunoassay (DPC cortisol kit; Siemens Healthcare Diagnostics, Tokyo, Japan).

Statistical analysis: Serum cortisol concentrations at each time interval were subjected to analysis of variance for repeated measurements by the general linear models procedure of the Statistical Analysis System (SAS) (Version 9.2, SAS Institute, Inc., Cary, NC). When significant changes were detected between groups ($p < 0.05$), the means in each group were compared using Duncan's multiple range test. The results were expressed as the Mean \pm Standard Error (SEM).

RESULTS AND DISCUSSION

Serum cortisol changes over time for the 4 groups of calves are shown in Fig. 1. Serum cortisol concentrations of the castrated calves that were administered local anesthetic without NSAID (untreated) peaked at 2 h after castration and then decreased gradually. The serum cortisol concentrations in the sham-operated calves remained unchanged during the study period. In the period starting from immediately after castration to 4 h after castration, the serum cortisol concentrations of the calves that were administered sodium salicylate were lower than those of the calves that were not administered any NSAID; the differences were statistically significant ($p < 0.05$) at 1, 1.5 and 2 h after castration. The serum cortisol concentrations of the sodium salicylate-administered calves were higher than those of the ketoprofen-administered calves from 1-3.5 h after castration and the differences were statistically significant ($p < 0.05$) at 2 h after castration.

NSAIDs are administered to small animals during surgery from the viewpoint of animal welfare through pain reduction. NSAID treatment also provides practical benefits including the hastening of postoperative recovery due to decreased stress reactions resulting from tissue injury and the accompanying inflammatory responses (Lemke *et al.*, 2002; Fowler *et al.*, 2003; Slingsby and Waterman-Pearson, 2002). Recent research

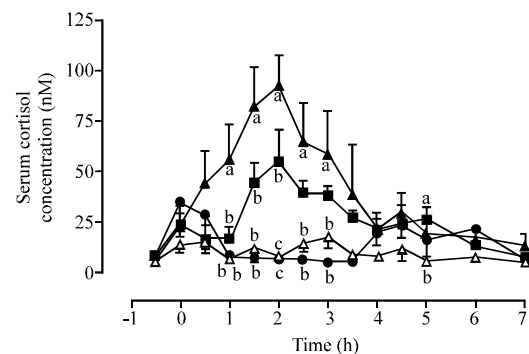


Fig. 1: Changes in the serum cortisol concentrations in calves treated as follows: castrated, untreated (▲, 5 calves), castrated with sodium salicylate administration (■, 5 calves); castrated with ketoprofen administration (●, 5 calves), sham-operated and untreated (△, 5 calves). Procaine hydrochloride was injected into the scrotum as a local anesthetic in all calves. Data are expressed as mean \pm SEM. Statistically significant differences ($p < 0.05$) were observed between the different treatments at the indicated times (marked a, b and c)

has shown that these drugs also have stress-relieving effects after clinical application to livestock animals. Notably, ketoprofen, a second-generation NSAID is very effective in reducing stress during both castration and dehorning (McMeekan *et al.*, 1998; Sutherland *et al.*, 2002). However, NSAIDs have not been widely used in these applications for several reasons. First, some producers express concerns about drug residue in edible meat. Second, some producers believe that the use of NSAIDs does not result in weight gain, shorten the fattening period or improve the quality of the meat. Finally, there remains a lack of awareness among veterinarians and livestock farmers about current improvements in animal welfare. Importantly, second and third-generation NSAIDs like ketoprofen are expensive. Salicylic acid derivatives are inexpensive NSAIDs that have traditionally been used in cattle and their effects and pharmacokinetics in cattle are well known. The *in vivo* kinetics of salicylic acid derivatives in cattle were first studied by Gingerich *et al.* (1975). If salicylic acid derivatives are found to be effective in stress reduction at low cost, they would be useful in veterinary medicine. The present study was conducted to address this question.

In this study, when castration was performed without NSAID administration, cortisol levels increased immediately after surgery and peaked after 2 h. With the administration of NSAIDs, cortisol levels increased at 0 h immediately after surgery but no increase was detected after 0.5 and 1 h. With sodium salicylate, the cortisol levels increased again at 1.5 h. With ketoprofen however, this second increase did not occur. Increased blood cortisol levels immediately after castration can be controlled by local anesthesia which should be used in all castrated cattle. The role of NSAIDs is to control the subsequent inflammatory reaction caused by tissue injury. In this regard, ketoprofen was found to be highly effective in the present study, consistent with previous reports.

In the study by Gingerich *et al.* (1975), animals were administered 50 and 100 mg kg⁻¹ sodium salicylate or aspirin however in the study, the calves were administered 25 mg kg⁻¹ sodium salicylate. Coetzee *et al.* (2007) reported that intravenous sodium salicylate administration at 50 mg kg⁻¹ immediately prior to surgical castration in cattle attenuates the acute plasma cortisol response however, the same dosage of aspirin by oral administration failed to mitigate the acute cortisol response. Moreover, the researchers showed that mean plasma cortisol concentration in the animals receiving sodium salicylate intravenously was lower than that in the

other experimental groups although, the difference was not statistically significant. Researchers therefore, chose the intravenous route due to uncertainty regarding absorption in the digestive tract; absorption is thought to vary based on physiological maturation, rumen development and feeding. We employed a lower concentration of sodium salicylate together with a local anesthetic in the present study because previous research also did not use local anesthesia (Coetzee *et al.*, 2007). The results show that intravenous sodium salicylate administration at 25 mg kg⁻¹ leads to significant attenuation of the acute serum cortisol response. Sodium salicylate was less effective than ketoprofen but when used in combination with an appropriate local anesthetic, 25 mg kg⁻¹ or a lower dose of sodium salicylate administered intravenously can provide safe and economical pain control. Although, Stafford *et al.* (2002) reported that local anesthetic had little effect on cortisol responses after surgical castration, the data show that the combination of sodium salicylate and local anesthetic might lead to a significant effect. The experiments did not examine the effect of NSAID treatment without local anesthetic, so future experiments are needed to address this question.

From a clinical point of view, second-generation NSAIDs do not currently contribute to animal welfare despite their efficacy in reducing stress responses in castrated calves because of their high cost. The cost of ketoprofen at the doses used in this study (sodium salicylate: 25 mg kg⁻¹, ketoprofen: 3 mg kg⁻¹), assuming an animal weight of 170 kg was 26 times that of sodium salicylate (Japan, 2011). In addition to the surgical fees for invasive castration, the cost of ketoprofen may make its use impractical in the field. Thus, the use of sodium salicylate in surgical procedures such as castration in livestock may have significant advantages.

CONCLUSION

The analgesic and anti-inflammatory effects of sodium salicylate are weaker than those of ketoprofen however, considering that the use of second-generation NSAIDs is impractical due to their high cost, salicylic acid derivatives have enormous potential when appropriate doses are used and proper administration methods are established. Although, some consideration must be given to potential drug residues in the final livestock product, the use of salicylic acid derivatives-classical NSAIDs in surgical castration is beneficial in terms of animal welfare and is economically advantageous.

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