Physiological and behavioural effects of intradermal injection of sodium lauryl sulphate as an alternative to mulesing in lambs

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**Objective** To assess the impact on physiology and behaviour of intradermal injection of sodium lauryl sulphate (SLS) as an alternative to mulesing.

**Procedures** Three groups of Merino lambs were studied: Control (n=10), SLS (n=11) and Mulesed (n=11). SLS group received SLS (7% w/v), benzyl alcohol (20mg/mL) in phosphate buffer, and Mulesed received 6 mL topical local anaesthetic as a wound dressing. Haematology, cortisol, beta-endorphin, haptoglobin, rectal temperatures, body weight and behaviours were monitored for up to 42 days.

**Results** SLS treatment induced mild swelling followed by thin scab formation. Fever (>40°C) was observed at 12 and 24 h, cortisol was elevated on d1 and d2, haptoglobin was highly elevated from d2 until d7, white cell count was elevated on d2 and d4 and average daily gain was not affected. Fever at 12 h was significantly higher in SLS than Mulesed group whereas maximum temperature, temperature area under the curve (AUC), occurrences of fever, cortisol profile, cortisol AUC, white cell counts and haptoglobin until d7 were comparable. The behaviours of normal standing, total standing and total lying where modified for 2 days by SLS treatment but changes were less marked and of shorter duration than Mulesed group. On d1, SLS group spent < 5% of time in total abnormal behaviours and Mulesed group 18%. SLS group tended to spend more time in abnormal behaviours on d1 than Controls.

**Conclusions** Behaviour of the SLS group was similar to unmulesed controls while physiological responses were intermediate between mulesed lambs receiving post-surgical analgesia and controls.

**Keywords** animal welfare, sodium lauryl sulphate, non-surgical mulesing
Susceptibility of sheep to fly strike on the breech is an important problem in many wool growing areas of the world, especially in Merinos. Modifications to breech conformation that reduce wrinkle and increase the perineal bare area decrease the risk of breech strike. A surgical method called mulesing achieves this goal by the removal of skin lateral to the perineum and on the sides of the tail. Non-surgical methods for improving breech conformation have been explored since the late 1930s and include topical or intradermal application of a wide range of caustic, sclerosing, escharotic and photoactive chemicals, digestive enzymes, freezing and irradiation. In addition, breech remodelling following avascular necrosis induced by application of occlusive clips to skin has been examined.

The potential for quaternary ammonium compounds to act as escharotic agents when applied topically or injected intradermally was examined by Chapman. Tissue remodelling and skin contraction during healing led to an increase in the bare area of the breech. A limitation of the most promising compound identified in these studies was its induction of cortisol responses and pain related behaviours of similar magnitude to mulesing. Modifications to the composition and delivery of escharotic formulations based on quaternary ammonium compounds were subsequently developed by Australian Wool Innovation with a view to reducing adverse effects. From this work, cetrimide was chosen for further research and development. Recent assessments of the response to intradermal injection of a modified formulation based on cetrimide in the breech and tail of lambs found physiological responses of similar magnitude to mulesing and behavioural responses generally of lesser magnitude than those seen in mulesed lambs. The results suggested
that further modifications to the delivery method or formulation may be needed to lessen the undesirable effects.

An alternative chemistry for modification of the breech based on intradermal injection of anionic surfactants has been investigated and a novel applicator was developed to control intradermal delivery of the chemical. The preferred active, sodium lauryl sulphate (SLS) is thought to rapidly denature proteins within minutes of injection in a concentration dependent manner leading to cell death. In a welfare assessment of an early prototype of the SLS method in weaned lambs aged 10 - 12 weeks, feed intake was reduced on day 2, cortisol concentration was elevated at 2 h, 12 h (day 1) and day 2, haptoglobin concentration was elevated on days 3 to 7, and neutrophil: lymphocyte ratio was elevated on day 2, in comparison with un-mulesed control lambs.

The current study compared treatment by intradermal injection of an improved SLS formulation into the breech and tail of lambs, with responses in un-mulesed control lambs and mulesed lambs that received a topical local anaesthetic formulation (Tri-Solfen, Bayer Australia Ltd) as a post-surgical dressing. The effect of treatments on a range of physiological and behavioural measures was assessed over 42 days following treatment.

Materials and Methods

Sheep

The study was approved by the institutional Animal Ethics Committee and conducted under APVMA permit number PER 7250. Thirty-two superfine wool Merino female and castrated male lambs, aged between 10 to 12 weeks were used. Three weeks before the commencement of the experiment, all lambs were tail docked with a gas-heated docking knife as per standard industry practice and male lambs castrated with elastrator rings.
Housing

Sheep were accommodated in an animal house in group pens with slatted timber floors. Lambs together with their dams were kept in large group pens to acclimatize them to the animal house for a 2-week period before commencement of the experiment, and were fed a ration of commercial sheep pellets (Ridley Agriproducts, Tamworth; 22% crude protein, 11.1 MJ/kg dry matter) at a rate of 2.0 kg/ewe and lamb unit/day plus 120g cereal chaff/ewe and lamb unit/day. During the study of the response of animals to the various treatments, pens with dimensions 4.5 × 3.0 m were used. Four ewes and their lambs were accommodated in each study pen. The sheep density was sufficiently low to allow freedom of movement and expression of behaviours.

Treatments

The study was performed sequentially on 2 cohorts providing replication in time, one week apart, commencing on 28 October 2008. Soon after being moved into the animal house, lambs were randomised on body weight and balanced for gender into three treatment groups of 10 - 11 animals per group. Lambs were randomly allocated to one of the two cohorts, and within each cohort they were randomly allocated into one of four pens. Each treatment was represented in every pen. Lambs were quietly handled for a 5-minute period on two occasions during the week before treatment with the aim of reducing sampling stress during the experiment.

On the day of treatment, Control lambs were placed in lamb marking cradles for approximately 2 minutes but were not mulesed and were then released back to their pens. Lambs to be mulesed were similarly placed into a marking cradle then mulesed in accord with standard industry practice and had a 6mL dose of topical anaesthetic (40.6 g/L lignocaine, 4.5 g/L bupivacaine, 24.8 mg/L adrenaline, 5.0 g/L cetrimide, Tri-Solfen, Bayer Australia Ltd) applied to the wound. The mulesing operator who performed the procedure has many years experience in mulesing lambs and is a
member of the Livestock Contractors Association. For the intradermal treatment, a formulation containing sodium lauryl sulphate (SLS) as a solution of sodium lauryl sulphate (7%), benzyl alcohol (20mg/mL), phosphate buffer and water for injection was supplied and administered by Mr Peter St Vincent Welch (Cobbett Technologies Pty Ltd) (Figure 1). The formulation (32.5mL) was administered to each lamb via a needle-less injector with compressed air at a pressure of 200 kPa. Lambs received 4 shots into each side of the breech and 5 shots into the tail in the pattern illustrated in Figure 2. Lambs in all treatments received an antibiotic injection (oxytetracycline at 10 mg/kg, i.m.) while in the marking cradle.

Physiological measurements

During the week before experimental treatments were applied, wool on the neck over the jugular vein was removed with clippers to facilitate blood sampling. Blood samples were taken from the jugular vein via venepuncture into 5 mL EDTA vacutainers (Becton Dickinson, Plymouth, UK). At each time point, blood samples were taken from animals in the same sequence. Blood samples were taken at 14 time points over the course of the experiment at -1.0 h, 0.5 h, 12 h (day 1), then at approximately 8:30 am on days 2 (24 h), 3, 4, 5, 6, 7, 14, 21, 28, 35 and 42 days after treatment. Blood was processed for total leukocyte counts on an automated haematology analyser (CellDyn 3500R, Abbott Diagnostics, North Ryde) calibrated for sheep blood. A fault in the instrument during the period of experimentation prevented the estimation of differential white cell counts. Thus neutrophil: lymphocyte ratios, a variable commonly measured in studies on the response to husbandry practices could not be calculated. Plasma was prepared and stored at -18°C for haptoglobin, cortisol and beta-endorphin determinations.

Haptoglobin was assessed as described previously. The inter-plate co-efficient of variation (CV) for quality control samples containing 0.12, 0.048 and 0.023 mg/mL were 4.1, 3.8 and 7.1 %
respectively for the lambs. Plasma cortisol concentrations were determined using a commercial radioimmunoassay (Spectria Cortisol RIA, Orion Diagnostica, Espoo, Finland), adapted and validated for ovine plasma as described previously. The calculated recovery of cortisol added to spiked ovine plasma was 102% and the sensitivity of the assay was 10 nmol/L. The stated cross-reactivities of the anti-cortisol antibody with corticosterone, cortisone, dexamethasone, prednisolone and prednisone were 0.2, <0.1, <0.1, 45.3 and 0.3 %, respectively. The intra-assay CV for samples containing 28.1, 61.5 and 135.7 nmol/l cortisol respectively, were 9.6, 10.2, and 7.6%. The inter-assay CVs for the same samples were 8.4, 8.0 and 11.6% respectively for the lambs.

Plasma beta-endorphin was determined using a commercial RIA kit (Phoenix Pharmaceuticals, California, USA) at sampling times -1, 12 hours, and days 2 and 3 following treatment. The mean recovery of added beta-endorphin to ovine plasma was 82% and the sensitivity of the assay was 10 pg/mL. The stated cross-reactivities of the anti-beta-endorphin antibody with met-enkephalin, leu-enkephalin, melanocyte stimulating hormone and adrenocorticotropic hormone were 0%. The intra-assay CV for samples containing 88.7, 118.2 and 169.9 pg/mL, were 8.7, 5.1, and 8.3% respectively. The inter-assay CVs for the same samples were 2.1, 5.9 and 4.4% respectively.

Rectal temperature was recorded at -1.0 h, 12 h (day 1), then between 8:00 and 9:00 am on days 2, 3, 4, 5, 6 and 7 with a digital thermometer to an accuracy of 0.1°C. A temperature of > 40 °C was interpreted as fever. Body weight was recorded approximately one week before treatment for allocation of sheep to treatment groups, then 1 day before and 7, 14, 21, 28, 35 and 42 days after treatment to an accuracy of 0.1 kg.

**Behavioural measurements**

Experimental animals were individually identified with coloured marks to assist with behavioural observations. Markings were randomized across experimental treatments. Behaviour was recorded...
by video on the day of treatment (day 1) and on days 2, 3, 4, 5, 7, and 13 following treatment for a 12-hour observation period (9:00am – 9:00pm) each day. Other details of data capture are as previously described.\(^7\)

Behaviours were observed at 15-minute intervals during each 12-hour observation period. A single operator, who was not informed of the treatment codes, classified behaviour into the following categories: normal standing, hunched standing, normal walking, stiff walking, ventral lying, lateral lying, lying intention, feeding, and pawing as previously described.\(^7\) From these classifications, the proportion of time spent in each behaviour on each observation day was calculated. Total time standing was calculated as standing + walking + feeding + pawing, and total abnormal behaviours were calculated as hunched standing + stiff walking + pawing + lateral lying + lying intention.

**Statistical analyses**

Where necessary, data were transformed to normalise the distribution of residuals. Transformations used were cortisol maximum (log\(_{10}\)), total white cell count (log\(_{10}\)), hunched standing, normal walking, drinking, pawing, total abnormal behaviours (log\(_e+1\)). For physiological variables measured repeatedly over time, repeated measures models fitting, when significant, the fixed effects treatment, cohort, sex, and pen and their interactions with time were used to analyse the results. For behavioural variables measured repeatedly over time, repeated measures models fitting, when significant, the fixed effects treatment, cohort, and sex and their interactions with time were used to analyse the results. The pre-treatment value was a significant covariate for total white cell count. Maximum value and area under the curve (AUC) for physiological variables were analysed by one way ANOVA. Behaviour data were analysed in ASReml\(^{15}\) and physiological data in Systat version 9.1 (SPSS Inc, Chicago, IL). When there was a significant time by treatment interaction, the significance of differences between treatments was estimated within each time point. The normality of haptoglobin data was not improved by transformation so the data were analysed by Kruskall
Wallis ANOVA on ranks at each time point. A probability of 0.05 was considered significant. For variables that were transformed before analysis, graphs depict least squares means back-transformed to the observed scale.

Results

Physiological data

There was a significant depression in average daily gain in Mulesed lambs in the week following treatment in comparison with Control and SLS groups. Average daily gain was comparable between Control group and SLS group at all time points (Figure 3).

Fever (> 40°C) was recorded in Mulesed group 12 and 24 h after surgery. Body temperature was significantly higher in Mulesed group than Control group until day 7. Fever was also observed in SLS group at 12 and 24 h after treatment. At 12 h body temperature was significantly higher in SLS group than in Mulesed group. Temperature of the SLS group was intermediate between Mulesed and Control groups on day 3 to day 5 and not significantly different from either group. Day 6 was the first day that body temperature in the SLS group was significantly lower than the body temperature of the Mulesed group (Figure 3). The maximum rectal temperate recorded at any time point during the study was identified for each lamb and group means were calculated. Maximum temperature did not differ between Mulesed (40.6 ± 0.09°C) and SLS groups (40.8 ± 0.09°C), which were both significantly higher than the Control group (40.2 ± 0.09°C). The AUC for body temperature was calculated from the time of treatment until day 7, after which time body temperatures in the Mulesed and SLS groups were both comparable to the Control group. AUC of both the SLS group (239.1 ± 0.38 degree days) and the Mulesed group (239.7 ± 0.38 degree days) was significantly greater than the AUC of Control lambs (237.6 ± 0.39 degree days). The mean number of days lambs in each group had a body temperature > 40.0°C was calculated. The number
of fever days in Mulesed (3.7 ± 0.49 days) and SLS groups (3.2 ± 0.49 days) was comparable and
was significantly higher than in the Control group (1.30 ± 0.51 days).

Cortisol concentrations in plasma were elevated in all groups 30 min after treatment (Figure 3). At
12 h and 24 h (d2) cortisol in Mulesed and SLS groups was significantly elevated, whereas the
Control group had returned to the pre treatment baseline. Cortisol concentrations in Mulesed and
SLS groups did not differ significantly. The maximum cortisol concentration recorded at any time
point during the study was identified for each lamb and group means were calculated. Maximum
cortisol did not differ between groups (Control 126.5, Mulesed 143.2, SLS 143.8 nmol/L, back
transformed means). The AUC for cortisol concentration was calculated from the time of treatment
until day 3, at which time cortisol concentrations in the SLS and Mulesed groups had returned to
normal. AUC of SLS (3699 ± 280 (nmol/L)h) and Mulesed (3690 ± 280 (nmol/L)h) groups was
significantly greater than the AUC of Control lambs (2394 ± 294 (nmol/L)h). There was no
significant difference between Mulesed and SLS groups in cortisol AUC.

Beta-endorphin did not differ between treatment groups at any time point (Figure 3).

There was a significant increase in haptoglobin concentrations in plasma by 24 h (d 2) in the
Mulesed and SLS groups (Figure 3). Haptoglobin was elevated in the Mulesed groups for at least 7
days after treatment whereas in the SLS group haptoglobin had returned to baseline concentrations
by day 7. Haptoglobin concentration was significantly higher in the Mulesed group than the SLS
group on day 7.

Total white cell count in blood was elevated on day 2 (24 hours after treatment) in Mulesed and
SLS groups (Figure 3). Following this peak, total white cell counts decreased towards normal over
the subsequent days but remained significantly elevated in SLS group and Mulesed groups on day 4. Total white cell count did not differ significantly between Mulesed and SLS groups on day 4.

**Behavioural responses**

The Mulesed group spent more time in normal standing postures than the Control group on the day of treatment (day 1) and on the subsequent 2 days (Figure 4). The SLS group spent more time standing normally on day 1 and day 2 than the Control group. On day 2 and day 3 the Mulesed group spent significantly more time standing normally than the SLS group.

The Mulesed group spent more time in a hunched standing posture on the day of treatment and on the subsequent day than the Control group (Figure 4). On the day of treatment, the time spent in hunched standing posture by the SLS group was intermediate between Control and Mulesed groups and not significantly different from either. On day 2, the Mulesed group spent significantly more time in hunched standing postures than the SLS group.

There were no significant differences between groups in the time spent walking normally (Figure 4). Stiff walking gait and pawing occurred too infrequently during the study for statistical analysis.

Across all observations time points, there was significantly less time spent feeding by Mulesed group than SLS or Control groups (Figure 4). The treatment group by time interaction on feeding was not significant.

The Mulesed group spent more time in combined standing postures (normal and hunched standing, walking, feeding and pawing) on days 1, 2 and 3 than the Control group and more time in combined standing postures on days 1 and 2 than the SLS group (Figure 4). The SLS group spent more time in combined standing postures on days 1 and 2 than the Control group.
The Mulesed group spent less time in combined lying postures than the Control group on days 1 and 2, and less time in combined lying postures on days 1, 2 and 3 than the SLS group (Figure 4). The SLS group spent less time lying on days 1 and 2 than the Control group.

Mulesed lambs spent more time in abnormal behaviours (hunched standing, stiff walking, pawing, lateral lying and lying intention) than Control and SLS groups on the day of treatment and on the following day (Figure 4). The percentage of time spent in abnormal behaviours by the SLS group was intermediate between Mulesed and Control groups on day 1 and day 2. There was a tendency for the SLS group to spend more time in total abnormal behaviours on day 1 (P = 0.068) than the Control group.

Response of the breech to SLS treatment

There was mild swelling of the injection sites by 12 hours after treatment. Some hardening of the injection sites was evident around day 4. Formation of scabs, generally less than 1 cm in diameter, became evident on the skin surface after one week. Some scabs had lifted from the skin at 42 days. No occurrence was seen of open wounds following treatment or during the healing process. A representative photograph of tissue swelling 24 hours after SLS administration is shown in Figure 5.

Discussion

The main findings of this study were that the intradermal injection of SLS caused an inflammatory reaction around the breech and tail, resulting in systemic sequelae of fever, increased cortisol and haptoglobin, and moderate changes in normal behavioural postures. Gross tissue reactions of swelling and scab formation were mild. Skin sites treated with SLS were mildly swollen for several days then became indurated. Small patches up to approximately 1 cm diameter of thin scab,
probably constituting an eschar or layer of necrotic tissue overlying a skin wound, developed at
some injection sites. These scabs subsequently lifted from the skin surface over several weeks
without creation of open wounds. This contrasts with the open wounds and subsequent heavily
crusted scabs formed from wound exudates that developed following mulesing.

As measured by body temperature, the inflammatory reaction to the intradermal treatment was well-
developed by 12 h. Fever resolved reasonably quickly and body temperature was within the normal
range by day 3. The tissue trauma induced by surgical mulesing produced an inflammatory response
of similar magnitude to SLS. In this study rectal temperature in Mulesed group remained elevated
until day 7. Maximum body temperature, area under the curve and the number of occurrences of
fever did not differ between SLS and Mulesed groups.

Tissue damage and inflammation also produce an increase in the acute phase protein haptoglobin,
which is measurable in the circulation until it is broken down by normal turn-over. SLS treatment
induced a large haptoglobin increase that was elevated until day 7. In comparison, the haptoglobin
peak in Mulesed animals was of similar magnitude but took longer to return to control levels
(sometime between day 7 and day 14). Control animals did not have increases in haptoglobin
concentrations above normal low levels. The changes in total leukocyte concentration were similar
in pattern to those seen for fever and cortisol. The increase in total leukocyte count suggests that the
systemic effects of treatment resulted in secondary consequences in immune cell populations, which
could affect immune function. No effects on beta-endorphin were observed in any treatment group.

Moderate changes were observed in normal behaviours in lambs receiving the SLS treatment. On
the first 2 days after treatment, lambs spent more time standing, and less time lying. Abnormal
behaviours of pawing and hunched standing were no more common in SLS lambs than in Control
lambs. Stiff walking occurred infrequently. In contrast, Mulesed lambs spent more time standing
normally and in a hunched posture than SLS lambs, and less time lying. Despite this the Mulesed lambs, which received topical anaesthetic formulation at the time of mulesing in this study, spent substantially less time in abnormal behaviours than mulesed lambs that received no analgesia in previous studies. The large proportion of time spent lying by Control lambs is in accord with previous observations on housed lambs. Across all observation time points, Mulesed lambs spent less time feeding than Control or SLS groups. Reduced feeding time was reflected in lower average daily gain in the first week following treatment in the Mulesed group. There was a tendency for time spent in total abnormal behaviours by SLS treated lambs to increase on day 1 in comparison with Control group.

Some comparisons can be made between the current study and recent assessments of the response of lambs to intradermal injection of the cetrimide alternative to mulesing. In contrast to the present study, mulesing wounds were not treated with topical anaesthetic in the previous studies in which mulesing treatment provided a positive control for assessment of physiological and behavioural impacts of SLS were less marked than those seen in lambs treated with cetrimide. In SLS treated lambs, fever was much less intense, body temperature and cortisol returned to normal more quickly, haptoglobin concentrations were lower and abnormal behaviours were less common than on cetrimide treated lambs.

The physiological responses to SLS treatment seen here were of similar magnitude and duration to those reported by Hemsworth et al. A greater impact on normal behaviours was observed in this study than was reported by Hemsworth et al., however they observed reduced feeding on day 2 which was not seen here. It is noteworthy that weaned lambs were studied by Hemsworth et al. whereas suckling lambs were studied here. Some differences also exist between the studies in the dose and method of application of SLS.
In conclusion, injection of SLS induced physiological changes indicative of local and systemic inflammation, moderate changes in normal behaviours and a tendency for time spent in abnormal behaviours to increase. These impacts, which occurred predominantly within the first 2 days following treatment, were less marked than the response of in mulesed lambs receiving post-surgical analgesia but greater than those seen in unmulesed controls.

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Figure 1. Injection of sodium lauryl sulphate.
Figure 2. Sequence and pattern for injection of SLS treatment
Figure 3. Physiological responses of lambs to treatments (Control, Mulesed, SLS). Within a time point, symbols with a different letter are significantly different.
**Figure 4.** The percent of time spent in defined behaviours during 12 hours period on each observation day in lambs (Controls, Mulesed, SLS). Within a time point, symbols with a different letter are significantly different. For time spent feeding, across all observation time points there was a significant treatment effect with Mulesed group significantly less than Control and SLS groups.
Figure 5. Representative illustration of breech and tail 24 hours (left) and 7 weeks (right) after intradermal injection of SLS.