Daily sodium cromoglycate treatment decreased mast cell and blood eosinophilic responses in _Haemonchus contortus_ resistant Black Belly sheep

G. TEREFE¹, K.C. NGUYEN², P. JACQUIET³, J.P. BERGEAUD³, C. GRIZEZ³, F. PREVOT³, J.C. BRUNEL⁴ and PH. DORCHIES³

¹ Addis Ababa University, Faculty of Veterinary Medicine, D. Zeit, P.O.Box 34, Ethiopia
² University d’Agriculture et de Foresterie, Ho Chi Minh Ville, Vietnam
³ INRA, Domaine expérimental de la Sapinière, 13 390 Osmoy, France
⁴ INRA, UMR INRA/DGER 1225, « Interactions Hôtes-Agents Pathogènes » Ecole Nationale Vétérinaire, 23, Chemin des Capelles, 31076 Toulouse, France

* Corresponding author : Tel.: +33-5-61193861; Fax: +33-5-61193944 - E-mail : p.dorchies@envt.fr

**SUMMARY**

Mast cells and eosinophils are known to participate in the processes of allergic inflammation and gastrointestinal parasitic diseases. Sodium cromoglycate (SCG), a non steroid anti-allergic drug is used in the prophylaxis and treatment of asthma and GIT allergic disorders in human. This experimental study has used SCG to reduce the population and degranulation of mast cells and the subsequent mobilisation of eosinophils during _Haemonchus contortus_ infection, in an attempt to rendering the relatively nematode resistant Black Belly breed of sheep more prone to the infection. Animals were divided in to three groups of 5 sheep (groups SH, H and C). SCG treated and infected, non-treated and infected and negative control respectively. Blood and bone marrow eosinophil counts, faecal egg count (FEC), tissue eosinophil, mast cell and globule leukocyte counts as well as measurement of other parasitological parameters were performed. Oral administration of SCG for 5 consecutive weeks successfully reduced the level of mast cell and bone marrow eosinophil number but not tissue eosinophil level. Furthermore, a significant reduction in mast cells, and a moderate reduction in globule leukocyte numbers were observed in the abomasal mucosa. Mean values for FEC and worm counts were higher in group SH. However, there was no significant difference between SCG-treated and non-treated group, which suggests the redaction in the inflammatory response alone, may not be sufficient enough to influence _H. contortus_ development in sheep. As the dose and mode of administration of this drug is not fully established for sheep, further studies might be required to make SCG a useful tool for experimental studies during gastrointestinal parasitism.

**Keywords :** _Haemonchus contortus_, Sodium cromoglycate, Eosinophils, Mast cells, resistant breed.

**RéSUMÉ**

Un traitement quotidien au cromoglycate de sodium diminue les réponses mastocytaires et éosinophiliques sanguines chez des moutons de race Black Belly résistants à _Haemonchus contortus_.

Mastocytes et éosinophiles sont des cellules impliquées dans les processus allergiques et les maladies parasitaires du tube digestif. Le cromoglycate de sodium (CGS), une drogue non stéroïdienne, est utilisée dans la prévention et le traitement de l’asthme bronchique et des manifestations allergiques du tube digestif. Cette expérimentation a utilisé le CGS afin de réduire la population et la dégranulation des mastocytes muqueux mais également la mobilisation des éosinophiles durant une infestation par _Haemonchus contortus_ chez des moutons de race Barbaros Black Belly réputés résistants à ce parasite. 15 agneaux de cette race ont été répartis en trois groupes de 5 animaux : i) traités au CGS et infestés par _H. contortus_ (SH), ii) non traités au CGS et infestés (H) et iii) témoins non traités et non infestés (C). Des comptages réguilier d’éosinophiles sanguins ont été réalisés de même que des comptages d’œufs dans les matières fécales. Après l’autopsie, 30 jours après l’infestation, une évaluation de la proportion d’éosinophiles dans la moelle osseuse, du nombre d’éosinophiles, de mastocytes et de leucocytes dans la muqueuse abomasale a été menée sur tous les animaux. L’administration quotidienne de CGS avant l’infestation puis durant toute la durée de l’infestation a réduit significativement l’éosinophilie sanguine et la proportion d’éosinophiles dans la moelle osseuse mais n’a pas eu d’effet sur le recrutement de ce type cellulaire dans la muqueuse abomasale. Une réduction significative du nombre de mastocytes et, dans une moindre mesure, de leucocytes est constatée chez les animaux du groupe SH. La diminution de réponse cellulaire dans ce dernier groupe ne s’est pourtant pas traduite par une installation significativement supérieure, un meilleur développement ou une plus grande fécondité des vers femelles même si des tendances apparaissent dans ce sens. Comme la posologie et le rythme d’administration du CGS ne sont pas complètement validés chez le mouton, d’autres expérimentations sont nécessaires pour faire du CGS un outil d’étude de la réponse cellulaire des ovis aux stronglyoses gastro-intestinales.

**Mots-clés :** _Haemonchus contortus_, cromoglycate de sodium, éosinophiles, mastocytes, race résistante.

**Introduction**

Mast cells and eosinophils are often incriminated for the pathogenesis of asthma and inflammatory gastrointestinal (GI) disorders in human (3, 20). Interestingly, helminth infections also are characterised by the induction of IgE synthesis and mast cell and eosinophilic accumulation suggesting some parallels in the pathogenesis of helminthosis and allergy (9). However, despite their in vitro activity (18), in vivo studies often produced controversial results on the role of these cells in GI parasitism (15). The invasion of GI mucosa with helminths causes the development of a Th2 type
of response, where mast cells exert their biological effects by releasing preformed and de novo synthesized mediators (histamines, prostaglandins and cytokines) up on stimulation. IL-5, one of the cytokines produced by the mast cells, is critical for the mobilisation of eosinophils from the bone marrow while IL-4 and leucotrienes facilitate their recruitment in tissues (17). Mast cells and eosinophils increase in number following gastro-intestinal nematode infections and are supposed to involve in the mounting of a certain degree of resistance which greatly varies between individuals and breeds of animals (1). In this regard, the Black Belly breed of sheep is known to resist *Haemonchus contortus* infection as compared to other breeds (7). One of the mechanisms envisaged to such resistance is the development of a high blood eosinophilia following experimental parasitic infections (1).

However, there is no detailed *in vitro* study to substantiate this role of eosinophils and/or mast cells in the resistance of the Black Belly breed to gastrointestinal parasites. Sodium cromoglycate (SCG), a non steroid anti-allergic drug is used in the prophylaxis and treatment of asthma and GI allergic disorders in human. It blocks the degranulation (14, 21) and reduces the population (8) of mast cells thereby reducing the severity of allergic diseases. This drug was also reported to act on eosinophilic gastroenteritis in humans (13). Different from the corticosteroid drugs (16), SCG is poorly absorbed through the digestive mucosa (11) and hence, its direct effect may be limited to the digestive tube after oral delivery. Assuming that similar mechanism of action of sodium cromoglycate could take place during parasitic infection in ruminants, this experiment attempted to reduce mast cell population and degranulation as well as mobilisation and recruitment of eosinophils that would influence the development of *H. contortus* in the abomasum of Black Belly breed of sheep. The association of this drug and the characteristics of parasite populations (establishment, egg count and female worm length) as well as local inflammatory responses in the abomasum have been described in this paper.

Materials and methods

Fifteen male lambs of Black-Belly breed (4 to 5 months old and 30-40 kg body weight) raised in worm free condition were generously provided by INRA (Bourges, Domaine de la Sapinière). Animals were divided in to three groups of 5 lambs (groups: H, SH and C). The *H. contortus* L3 (Humeau strain) were originally obtained from INRA (Tours-Nouzilly) and maintained by passages in donor sheep in our laboratory. A human preparation of oral Sodium Cromoglycate (Nalcron ND., Specia Laboratories, France) in 5ml (100mg) ampoules was purchased from a local pharmacy. The usual dose indicated for an adult person is 1-6 ampoules per day (corresponding to 2-10 mg/kg BW/day). Animals of group SH received three ampoules/day (10mg/kg) of Nalcron for 7 days before and for 30 consecutive days after *H. contortus* infection (D7 to D30) while groups H and C animals were left untreated. On D0, all animals in group SH and H were infected orally with 10000 L3 of *H. contortus* and group C served as negative control. Blood samples were collected in EDTA coated tubes once before the beginning of the treatment, and then daily until day 15 after infections followed by three times per week until the end of the experiment. 100μl of each blood sample was mixed in 900μl of eosin Y (Carpenter’s solution) and allowed to stain for 5 minutes. The solution was then diluted with similar volume of PBS (1 ml) to facilitate the visualisation of cells, and the mixture was filled into a FAST READ 102 cell counting chamber (ISL UK). Eosinophils were counted in the grids containing 1μl of the mixture (4). Egg counts were performed according to the modified McMaster technique (19) on faecal samples collected three times a week from D10 to D32. At the end of the experiment (D32), all sheep were killed by intravenous injection of 10 mg/kg pentobarbital sodium. Abomasal contents and washings were collected and sieved (40μm). The abomasum was digested in pepsin-hydrochloric acid solution (37˚C, 6h) to collect the tissue dwelling worms. The contents were then preserved in absolute alcohol. The volume of the material was adjusted to 1 litre and worms were counted in a 10% aliquot and classified according to their developmental stages. Furthermore, 20 adult female worms were randomly picked up from each sample for total parasite length measurement and counting of eggs *in utero*. For this purpose, individual female worms were allowed to disintegrate using 200 μl of Milton Sterilising fluid (contains: 2% w/v Sodium hypochlorite and 16.5% w/v Sodium chloride) diluted in a 1:4 ratio in distilled water (10) and all eggs liberated from the uterus were counted. Bone marrow smears were made from the iliac crest after autopsy and stained with May Grünwald-Giemsa. 400 leucocytes were counted and the percentage of eosinophils was determined. Tissue samples were collected from fundic and pyloric regions of the abomasum for histopathological examinations. They were preserved in 10% buffered formalin or Carnoy’s fixative. 5μm sections were stained with Hemalun and counterstained with Carbol-chromotrope for counting eosinophils and globule leucocytes while mast cells were stained with alcion blue-safranine (6). The cells were counted at X400 magnification in 10 microscopic fields. The evolution of faecal egg excretion and blood eosinophil counts was compared between the three groups of lambs using analysis of variance with repeated values (SYSTAT software). The number of worms recovered, female worm length, *in utero* egg counts, and abomasal eosinophils, mast cells and globule leucocytes were compared by the non-parametric, Kruskall-Wallis test (SYSTAT software) while the percentage of eosinophils in the bone marrow was compared using the Chi-square test between the three groups.

Results and discussion

In a separate trial before the start of this experiment, we have confirmed that SCG at various concentrations had no positive or negative effect on the mobility and viability of *H. contortus* infective larvae *in vitro*. In addition, no animal has shown overt symptoms of incompatibility or illness characteristic of the SCG treatment. The amount of SCG administered was based on the prescriptions designed for human beings (10mg/kg per day: maximum dose). Although this drug is
said to be poorly absorbed through the digestive mucosa (11), there is no data concerning the non-glandular compartments of the stomach in sheep. In spite of such ambiguity on the use of this drug in sheep, the results of our experiment have shown some important points deserving discussions. While control animals expressed a constant and low blood eosinophilia, a rise in the number of blood eosinophils was not observed in non-infected control animals. In sheep, eosinophils and mast cells are strongly involved in inflammatory reactions (12). In the present experiment, eosinophils and mast cells, were also observed in the abomasal mucosae of the two groups of sheep infected with Trichostrongylus colubriformis and Teladorsagia circumcincta between SCG-treated and non-treated sheep. It appears that the reduction in degree of blood eosinophilia and tissue mastocytosis was not to the level that could sufficiently influence H. contortus development in the abomasum. Possibly, sufficient numbers of cells were recruited into the abomasal tissue before a pronounced effect of SCG could be observed. This may either be attributed to the dose administered (under dosage) or the duration of administration (prolonged duration required) both before and after infection with H. contortus. Furthermore, though some IL-5 transgenic mice showed enhanced resistance in the control of worm development, the role of other immunological factors that could equally contribute in the control of worm development cannot be excluded. In conclusion, the changes in the number of mast cells and globule leucocytes in the abomasal mucosa of the two H. contortus infected groups have been recruited into the abomasal tissue before a pronounced effect of SCG could be observed. This may either be attributed to the dose administered (under dosage) or the duration of administration (prolonged duration required) both before and after infection with H. contortus. Furthermore, the role of other immunological factors that could equally contribute in the control of worm development cannot be excluded. Though some IL-5 transgenic mice showed enhanced resistance to Nippostrongylus brasiliensis (5), administration of anti-IL-5 monoclonal antibody to mice infected with Trichuris muris has greatly reduced the development of eosinophilia but had little effect on worm expulsion (2).

Figure 1. Evolution of blood eosinophilia in infected and control groups. Group SH has received a daily SCG treatment 7 days before and 30 days after infection (D7 to D30). Both Groups H and SH received 10000L3 of H. contortus on D0. Group C remained neither treated nor infected. Error bars indicate individual variations within a group.

Figure 2. Mean faecal egg count in the two groups of sheep infected with 10,000 Haemonchus contortus L3. Error bars indicate individual variations within a group.
pronounced enough to result in a significant difference between treated and non-treated groups. Although, inadequate administration of SCG could be taken as one cause for the absence of statistical differences between the two groups, it could also be the case that suppression of the inflammatory response alone may not be sufficient enough to influence parasite establishment and development.

**References**


