# EVALUATION OF CIMETIDINE AS A THERAPY FOR DERMAL MELANOMATOSIS IN GREY HORSE

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## ABSTRACT

This paper describes a clinically controlled study on the effectiveness of cimetidine an $H_2$ receptor antagonist for the treatment of melanoma in grey horses. Fifteen grey horses (age range 18 to 27 years) with dermal melanomatosis were divided in three groups. Group A served as the control group; groups B and C received cimetidine at two different protocols for 60 days (3.5 mg/kg PO, BID and 7.5 mg/kg PO, SID). The number and size of the masses was monitored before, during and after therapy. No differences were found between groups regarding number and size of melanomas before and after treatments ($p > 0.05$). Only in one horse it was possible to notice a slight size reduction of some of the masses however this may have been due to the anti-inflammatory effect of cimetidine as already demonstrated previously. It was concluded that cimetidine is not effective in treating dermal melanomatosis of grey horses. Differences of effectiveness in other studies could possibly be due to the variants of melanomas treated or to different treatment protocols. Further controlled studies are necessary to evaluate cimetidine effectiveness on different type of melanomas and other neoplasias arising from tissues other than skin. Also the mechanism of action of cimetidine on horse melanoma requires further investigation.

**Keywords:** Horse, melanoma, cimetidine, grey coat

## INTRODUCTION

Melanoma is one of the most common cutaneous tumors of the horse and represents 3.8% of the total equine neoplasias [1]. Such neoplasia affects mainly grey horses older than 6 years of age even though it can be found in horses of any coat color where it usually tends to be more aggressive [2, 3]. According to some authors, 80% of grey horses older than 15 years are likely to be affected by multiple melanomas (melanomatosis) [4, 5, 6]. The most common localizations of the tumor are the perineal and/or perianal region, the external genitalia, the ventral surface of the tail and parotid region, but it is possible to find it in many other sites like eyelids, coronary band, vertebral region, nasal cavity and theoretically in any other regions of the body [7, 8].

Although it represents a very common equine tumour, the exact nature of the grey horse melanoma is not completely understood. Some authors define it as a true malignancy [9] while other suggest that it may be a storage disorder associated with the depigmentation process that occurs in grey horses [6, 8, 10]. The graying of hair in horses represents a risk factor and it seems to be the result of a genetic mutation not detected in humans [11].

Valentine described four distinct syndromes [2]: melanocytic naevus (affecting mainly young horses), dermal melanoma (1 or 2 masses affecting mostly mature grey horses), dermal melanomatosis (affecting aged grey horse) and anaplastic malignant melanoma (reported in non-grey horse and with histological features of malignancy). Melanoma of grey horses presents usually as a benign tumor, probably due to the existence of unknown factors that prevent or delay the spread of the tumor [3, 12]. Metastatic spread of melanoma is possible to almost all organs of the body and is common in aged grey horses often without related symptoms [13, 14].

Benign melanoma of grey horses seem to share some features with human blue naevus which only occasionally undergoes malignant transformation [3, 12] and with animal-type melanoma, a recently reported rare variant of melanoma in humans, the name of which is derived from its histological appearance which is similar to that described in melanomas occurring in white or grey horses [15].

Cimetidine has been demonstrated to have anti-cancer effects on colorectal cancer, melanoma, renal cell carcinoma and salivary gland cancer in human [16, 17]. It seem to have an immunomodulatory effect [18] resulting in an increase

of natural killer cell activity [19]. Histamine $H_2$ receptors has been demonstrated on human melanoma cells [20, 21]. Cimetidine probably acts by three principal mechanisms: direct inhibition of tumour cell proliferation by antagonism of the $H_2$ receptor, activation of the local immune response characterized by interferon-gamma production by macrophages [22] and blocking of stimuli that histamine normally exerts on T-suppressor cells [23, 24]. Furthermore several other different mechanisms have been demonstrated or suggested [25, 26, 27]. A recent study seem to confirm the effectiveness of $H_2$ receptor antagonist famotidine against metastatic melanoma in man [28], but ranitidine, another $H_2$ receptor antagonist sometimes failed to obtain the inhibition of tumor proliferation both *in vivo* or *in vitro* [23, 27] confirming that different mechanisms, other than $H_2$ receptor antagonism, could be involved [22, 26]. Furthermore, there are different outcomes between its effectiveness in suppressing proliferation of melanoma cells *in vitro* or *in vivo* [29]. Strongly contrasting opinions about value of histamine antagonists for the treatment of melanomas or other tumors in man are reported in the scientific literature [21, 25, 30, 31, 32, 33, 34, 35, 36]. In most of cases cimetidine was used orally at a dosage of 300 mg four times daily. In a recent review it was shown that the association of cimetidine to other drugs used for melanoma treatment like interleukin-2 (IL-2) in humans is not deleterious however does not improve the response rate when compared to the treatment with the IL-2 alone [37].

In veterinary medicine, the importance of melanoma lies in its high prevalence, its repercussion on equine health (especially in regard to urogenital and gastrointestinal tract dysfunctions) and its negative effect on performance of the athletic horse.

At the present, therapeutic choices depend mainly on the localization, the number and the size of the masses. Surgery is a possibility in some cases but extensive tumor tissue plates (formed as a result of the union of small and individual nodules) which often develop on the ventral surface of the tail, the perineal and perianal region, are very invasive and difficult to remove completely. Consequently, the risk of relapse is very high. Local chemotherapy (cysplatin in sesame oil emulsion) or cryo-necrosis can be used individually or in association with surgery but are often ineffective [13].

Cimetidine has been reported to be effective for treating melanoma in horse [38, 23] but other investigation have failed to unambiguously obtain the same results [10, 14, 19, 39, 40, 41, 42, 43, 44, 45]. The schedule used for cimetidine administration in horses affected by melanomas in previous reports was very variable and is not yet well established, ranging from 1.6 to 4 mg/kg SID, BID or TID. Anyhow, clinical improvement and regression of the tumor has been obtained with dosage ranging from 1.6 mg/kg PO SID to 2.5 mg/kg PO BID or TID [38, 23]. Improvement of clinical appearance could be detected during the first 4-8 weeks but in some cases the therapy was continued for more than 13 months.

In this study we report the first clinical controlled study for effectiveness of two different protocols based on the administration of cimetidine, a $H_2$ receptor antagonist, as a single agent for melanoma of grey horses.

## MATERIALS AND METHODS

Fifteen grey horses with ages ranging between 18 and 27 years were used in this study. The group of horses belonged to the Veterinary Department - Military Athletic Horse Section in Rome, and included eight Italian Horse, five Lipizzaner, one Dutch Horse and one Lusitano. The horses are used for sports activities but at the time of the study they had all been retired from training. Cutaneous masses of varying locations had been detected and monitored for about seven months, between July 2008 and January 2009.

Horses were randomly divided into three groups of 5 animals each. A detailed history was collected, with emphasis on the development of neoplasias, clinical symptoms and treatments. At the first monitoring session, all the horses were subjected to a general clinical and integumetary examination. Inspection and palpation of skin surface was carried out with special attention to the sites predisposed to localizations of melanoma. Direct measurement of the lesions was made on each horse and in those horses with multiple lesions, measurement of a single mass was considered as a reference for the follow-up evaluations. Some lesions randomly selected on each subject were also measured by means of dedicated software (Adobe Photoshop CS3-Extended).

The number, location, stage of development and morphological characteristics of each melanoma was recorded. Image storing was made using a digital camera.

Blood was collected from all subjects from the jugular vein for blood smear and complete hematology and biochemical evaluations.

Cytological and biopsy sampling of the masses was performed for every horse. Cytological samples, obtained by needle aspiration with a 21 G needle, were stained with May-Gruenwald Giemsa and observed using a light microscope at different magnifications. Biopsies of the tumors were obtained after local anesthesia with 1% lidocaine. After shaving and surgical disinfection, an incision of 2-3 mm in length was made to allow for the insertion of a 14 G true-cut needle for biopsy sampling. Samples obtained were fixed in neutral 10% buffered formalin, processed and embedded in paraffin following standard histological procedures. Sections cut at of 4 μm thickness were stained with haematoxylin-eosin.

At the second monitoring session, held in August 2008, treatment was initiated with the following protocols:

**Group A**: control group, no administration.

**Group B**: administered 3.5 mg/kg of cimetidine PO, BID for 60 days.

**Group C**: administered 7.5 mg/kg of cimetidine PO, SID for 60 days.

Each horse were evaluated weekly during the sixty days of therapy and monthly after the end of therapy, for a total of 12 evaluations. On each session clinical evaluation, melanoma

number assessment, direct measurement of the masses and collection of digital photographs for computer measurements were carried out.

ANOVA has been used to test significant differences between sizes of melanomas at the beginning and at the end of therapy for each group. A p value of $<0.05$ was considered significant.

## RESULTS

The anamnestic data reported in all horses the presence of multiple cutaneous masses resembling melanomas for at least 3 years. The masses had been growing slowly and at the beginning of the study reached a stationary situation. No treatment had been attempted and no drugs had been administered in the last year. No horse had clinical symptoms relating to the presence of the masses with the exception of one horse which showed mild signs of difficulty extending his penis during urination. The number of the masses ranged from 8 to 22 with diameters ranging from 0.2 to 11 cm. The localizations of the masses for each horse are presented in table 1.

In all cytological samples there was a homogeneous population of strongly pigmented epithelioid cells with the presence of melanin granules. In some cases the nucleus was barely recognizable. Histopathological examination revealed atypical melanocytes organized in packets, sheets or cords. The melanocytes were predominantly epithelioid and/or spindle shaped. Cellular pleomorphism and mitoses were absent. The needle aspirate and biopsies confirmed the clinical diagnosis of "old grey horse melanoma" or dermal melanomatosis.

No significant hematologic abnormalities were found in any of the horses.

On examination and measurement of the masses during and at the end of the experiment none of the horse showed a reduction in size or in number of the tumors, except for horse No. 14 belonging to "Group C" where a slight size reduction of the masses localized on perineal region was observed.

## DISCUSSION

Analyzing the results of our study there is no evidence that the protocols applied led to any positive effects in terms of reduction of numbers and/or size of the tumors. Comparing the progression of melanomas in horses belonging to the control group with those subjected to either treatments, is clear that there was not a significant difference in size between the beginning and the end of the period of observation ($p > 0.05$) between groups. Only in one horse treated with 7.5 mg/kg PO SID was there evidence of a size reduction of the mass in the perianal region. The mass area decreased after the first week of treatment with a subsequent increase, before stabilizing at values still lower than the initial measurements. Interestingly, despite the fact that the number of masses remained unchanged, the size of the melanomas in the region ventral tail in the same horse showed a slight decrease in volume. Thus, it is possible that the protocol applied resulted in a positive effect in terms of volume reduction in one horse.

The observation that tumors growing slowly produce less histamine than those growing faster [24, 46] could account for the failure of effectiveness of the anti-histaminic property of cimetidine in our study, where all melanomas were in a clinically apparent "quiescent" state. This may be important, considering the suggestion of some authors that cimetidine could be more effective on actively growing tumors [14, 39]. If the $H_2$ antagonist mechanism of cimetidine was negligible, a different process, possibly an anti-inflammatory effect of cimetidine may have caused the reduction in size of the tumors and not the anti-neoplastic property [27]. These results support the fact that the exact mechanisms of action of cimetidine on tumors is not completely understood.

Some authors have reported successful effects of cimetidine in the treatment of grey horse melanoma, to a greater extent than reported in this study [38, 23]. Other studies have reported a 50 to 90% decrease in the number and size of masses using a dosage of 2.5 mg/kg PO, TID. It should be emphasized that some of the horses treated in the latter studies were affected by a quite different kind of melanoma with respect to malignancy and localization, and that some horses received a concomitant therapy such as surgery or cryonecrosis. Frederick (1990) observed that when surgical excision is performed early, the duration of remission was longer than expected and the results were far better than those obtained with cimetidine alone [47]. In contrast, melanomas presented in our study were fairly homogeneous for localization and biological behaviour and none of the horses received any kind of therapy other than cimetidine, making our population a good measure for evaluation of this drug om dermal melanomatosis.

Since an established protocol has not yet been accurately identified, we considered that it may be useful to test two doses of the drug on the assumption that horses would receive a roughly equivalent amount of cimetidine (7-7.5 mg/kg/day) but using different dosing interval, to avoid the inconvenience of a too frequent administration. As already pointed out, dosages as low as 1.6 mg/kg SID have been shown to improve the clinical condition of horses [23], and it seemed feasible to test if a one daily administration, which would be more suitable for horse owners, could be efficacious. It should also be borne in mind that pharmacokinetic studies of cimetidine have only been conducted in the horse concerning therapy of gastric ulceration and no studies relating to the distribution of cimetidine in neoplastic tissues have been performed [48]. Duration of the therapy (60 days) was chosen on the basis of the scientific literature, where a reduction in size and number of the tumors has always been noticed about three weeks after beginning of the treatment.

Regarding the positive results obtained by Goetz et al. [38, 23], other investigator have failed to unambiguously prove the effectiveness of cimetidine for the treatment of equine melanoma [10, 14, 19, 39, 40, 41, 42, 43, 44, 45]. Throughout the literature there is a lack of homogeneity of the methods, clinical presentation and treatment of the horses between investigations, making it impossible to carry out

a reasonable and logical comparison between studies. The variability in effectiveness of cimetidine, could be related to functional differentiation, cellular metabolism and embryonic derivation of tumours rather than their rate of growth. In our opinion, investigations regarding the presence of histamine $H_2$ receptors on horse melanoma cells would help to explain the inconsistency of results on grey horse melanoma.

In conclusion, even thought we can confirm that the use of cimetidine in dermal melanomatosis of grey horse was not effective in our trial, we believe that studies about cimetidine in horse should be continued, at least to evaluate its effectiveness on different type of melanomas or on neoplasms arising from tissues other than skin.

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**Table 1:** Localization of masses in horses on study by breed and study group.

| Horse | Breed | Tumor localization |
|---|---|---|
| **GROUP A** | | |
| 1 | Italian horse | Perineal region + ventral tail |
| 2 | Italian horse | Perineal region + ventral tail |
| 3 | Italian horse | Parotid region + intermandibular lymph nodes + ventral tail |
| 4 | Lipizzaner | Ventral tail |
| 5 | Lipizzaner | Perineal region + ventral tail |
| **GROUP B** | | |
| 6 | Italian horse | Perineal region + ventral tail |
| 7 | Italian horse | Perineal region + ventral tail |
| 8 | Italian horse | Periocular + parotid region+ perianal region |
| 9 | Lipizzaner | Perineal region+ perianal region + ventral tail |
| 10 | Dutch horse | Perineal region + ventral tail |
| **GROUP C** | | |
| 11 | Italian horse | External genitalia + perineal region + medial surface hindlimb |
| 12 | Italian horse | Ventral tail + medial surface hind limb |
| 13 | Lusitano | Perineal region + ventral tail |
| 14 | Lipizzaner | Perineal region + ventral tail + right shoulder |
| 15 | Lipizzaner | Perineal region + ventral tail |