Histometric Studies of the Equine Hoof Wall in Normal and Laminitic Horse

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Summary

The study was carried on 10 clinically normal horses and 10 horses subjected to experimental laminitis. Histometric examination of hoof specimens was done, after staining, using micrometer eyepiece. The results showed a significant ($p<0.05$) decrease in the number of horny tubules / mm^2, the maximum transverse diameter of the tubule, the thickness of the cortex, the quotient value of the thickness of the cortex / maximum transverse diameter of the tubule in horses affected by laminitis. Whereas, the maximum transverse diameter of the medulla, the quotient value of maximum transverse diameter of the medulla / thickness of the cortex, the quotient value of maximum transverse diameter of the medulla / maximum transverse diameter of the tubule, total surface area of the medulla, the quotient value of medullary surface area / tubular surface area were significantly ($p< 0.05$) increased in laminitis. It was concluded that the histometric changes in the microstructure of the tubular and intertubular horn play an important role in the production of poor quality hoof horn during laminitis.

Introduction

The hoof wall is composed of three layers: stratum externum, stratum medium and stratum lamellatum (Dellman, 1981). The stratum medium forming the bulk of the wall and consists of tubular and intertubular horn. The horny tubule consists of cortex and medulla. The cortex has three zones; the inner, middle and outer zones containing keratinized cells oriented around the medulla in a fairly tight coils.

The medulla contains loose elements (Wilkens, 1963; Stump, 1967; Steven, 1981 Dirks, 1985 and Christopher, 1995)

Equine laminitis is an inflammation of the dermo-epidermal junction especially in the dorsal hoof wall (Wintzer, 1982 and Stashak, 1987). Histopathological studies in horses and cattle affected by laminitis revealed disturbances in the epidermal keratinization process (Obel, 1948; Roberts et al, 1980 and Ekfalck et al, 1988).

Histopathological changes in the stratum medium during experimental laminitis in the horse showed cylindrical, oval or atrophied horny tubules associated with multiple cyst formation. The intertubular layers and the onychogenic fibers appeared swollen and fragmented (Mostfa, 1986). The developing horny cells are remarkably small and their intercellular space is widened (Marks and Budras, 1987).

Meyer (1985) concluded that the horn quality of the hoof is influenced by its microstructure which in turn can be determined by variable histometric parameters. The quality of the hoof horn depend on the number of tubules, the boundaries of tubules with the intertubular horn and the percentage of tubules to the intertubular horn (Tscherne, 1910; Dietz et al, 1971; Fuchs, 1976 and Kastner, 1976).

There is little literature concerning the histometric changes in tubular and intertubular horn during laminitis in horse. The aim of the present work was to describe the changes in the histometric parameters of the microstructure of the hoof wall in normal and in horses affected by laminitis to gain a basis for diagnosing the frequent pathological changes of the equine hoof.

Materials and Methods

Histometric examination of the normal hoof was done on specimens collected from euthanasized 10 horses. The hoof was obtained and splitted longitudinally at the middle of the toe by means of a saw. A specimen 1 cm x 1 cm including the structures of the whole thickness of the hoof was obtained from the upper, middle and lower parts of the hoof wall.

Laminitis was experimentally induced in horses by overfeeding of concentrated carbohydrate diet which consisted of 17 gm/kg b.w. of corn starch and 1.2 gm/kg of very fine saw dust (Mostafa, 1987). Hoof specimens were taken, at different times (24 and 48 hr., 6, 10, 20, 30, 40 and 75 days). The samples were preserved in 10% formal-saline solution and sectioned using freezing microtome at - 30 C into thin sections about 5 µm. Staining was done with haematoxyline and Eosin stain, periodic acid schiff reaction and Van Giesson stain (Carleton, et al, 1967).

The hoof sections were examined for the following histometric parameters: counting the number of horny tubules / mm^2 according to Geyer (1980), Hartel (1985) and El-Ghoul (1991); measurement of the maximum transverse diameter of the horny tubules and its medulla (Hofstetter,1985); calculation of the thickness of the cortex (Empel et al, 1982); calculation of the maximum transverse diameter of the medulla / thickness of the cortex (Dietz, 1976); calculation of the maximum transverse diameter of the medulla / maximum transverse diameter of the tubule (Hofstetter, 1985); calculation of the thickness of the cortex / maximum transverse diameter of the tubule (Empel et al, 1981); measurement of the total surface area of the medullary and horny tubules; calculation of the medullary surface area / tubular surface area (Hofstetter, 1985) and calculation of the surface density (Hartel, 1985).

Statistical analysis of the data were done by *t*- test and ANOVA using SPSS (Statistical Product & Service Solutions) (Kuehl, R., 1994). All data were presented as mean ± standard error, and $p < 0.05$ was considered significant.

Results

The results of the histometric examination revealed that, the average number of horny tubules, the maximum transverse diameter of the tubule, the thickness of the cortex and the ratio of the cortex thickness / tubular diameter in laminitis were significantly lower ($p < 0.05$) than that of the clinically healthy horses (table 1 and figures 1, 2 and 3).

The maximum transverse diameter of the medulla, the ratio of the medullary diameter / cortex thickness, medullary diameter / tubular diameter, medullary surface area / tubular surface area and the total surface area of the medulla were significantly higher ($p<0.05$) in laminitis than that of the clinically healthy horse (table 1 and figures 4, 5 and 6).

Insignificant differences were found in the total surface area of the tubule and the surface density between normal and laminitic horses (table 1).

Table 1: Histometric parameters in normal and laminitic horse.

Histometric parameters	**Normal hoofs**	**Laminitic hoofs**
	mean ± SE	**mean ± SE**
Number of horny tubules /mm^2	11.634 ± 0.196	10.477± 0.091**
Maximum transverse diameter of the medulla (µm)	15.241 ± 0.819	18.581 ± 0.989**
Maximum transverse diameter of the tubule (µm)	67.517 ± 4.351	56.706 ± 1.796**
Thickness of the cortex (µm)	25.732 ± 2.226	18.642 ± 0.952**
Maximum transverse diameter of the medulla / thickness of the cortex	0.806 ± 0.127	2.759 ± 0.550**
Maximum transverse diameter of the medulla / maximum transverse diameter of the tubule	0.261 ± 0.024	0.363 ± 0.021**
Thickness of the cortex / maximum transverse diameter of the tubule	0.368 ± 0.012	0.318 ± 0.011**
Total surface area of the medulla	198.255 ± 25.930	533.622 ± 86.719**
Total surface area of the tubule	3591.503 ± 407.41	3483.866 ± 212.44
Medullary surface area / tubular surface area	0.078 ± 0.014	0.179 ± 0.023**
Surface density	39076.6 ± 5609.2	35193.7 ± 2145.1

The histometric parameters in the hoof wall at different times from the onset of laminitis revealed a significant decrease ($p<0.05$) in the number of horny tubules, thickness of the cortex and surface density which starting at 2 and 6 days after appearance of signs of laminitis (table 2 and figures 7,8 and 9). The maximum transverse diameter of the medulla, total surface area of the medulla and the tubule, the ratio of medullary diameter / tubular diameter, medullary diameter / cortex thickness and medullary surface area / tubular surface area were significantly ($p< 0.05$) increased at 2 and 6 days after appearance of signs of laminitis. Inconstant significant changes were observed in the histometric parameters from 10 to 75 days (table 2 and figures 10 and 11).

Discussion

Poor hoof horn quality generally manifests clinically as hoof affections (Eustace, 1994). The horn quality of the hoof is influenced by its microstructure which can be judged by histometric examination (Meyer, 1985). Many authors established certain parameters for evaluation of the horn quality (Dietz, 1976; Hofstetter, 1985 and El-Ghoul, 1991).

Histometric studies of normal equine hoof in this study revealed that the number of horny tubules is 11.634 tubules / mm^2. Whereas Tscherne (1910) counted 5.0 tubules / mm^2 and Rossner (1940) found 9.8 tubules / mm^2 in the normal hoof wall. This may be attributed to the breed differences which influence the number of tubules as mentioned by Sedlacek (1933) who stated that the number of horny tubules is higher in hot - blooded horses than in cold - blooded one.

During this study, histometric evaluation of the hoof microstructure showed that the number of horny tubules / mm^2, the maximum transverse diameter of the tubule, the thickness of the cortex, the quotient value of the thickness of the cortex / maximum transverse diameter of the tubule in laminitis were significantly ($p<0.01$) lower than normal horse.

The number of the horny tubules determines the hardness of the hoof. The higher the number of tubules the harder the hoof horn. A horn with low number of tubules, means more intertubular surface (Dietz, 1976 and Empel et al, 1982). The increase in the thickness of the cortex and the quotient value of the thickness of the cortex / maximum transverse diameter of the tubule means increase in hoof horn hardness. That is because the tubular cortex is formed of cells of high quality which surround the medulla and give the tubule the first rate stabilization character (Geyer, 1980). The maximum transverse diameter of the tubule determines the hardness of the hoof, the wider the diameter, the softer is the hoof horn (Distal et al, 1981).

Consequently our findings showed poor horn quality of the hoof during experimental laminitis. Defects in the hoof horn reduce the functional integrity and are a major cause of reduced performance of the horse (Kempson, 1990). Therefore, decrease in the number of the horny tubules and the thickness of the cortex and increase in the tubular diameter reduced the quality of the hoof horn during laminitis.

The results of the maximum transverse diameter of the medulla, the quotient value of maximum transverse diameter of the medulla / thickness of the cortex, the quotient value of maximum transverse diameter of the medulla / maximum transverse diameter of the tubule, total surface area of the medulla, the quotient value of medullary surface area / tubular surface area in laminitis were significantly higher ($p< 0.01$) than the normal horse.

Distal et al (1981), Hofstetter (1985) and Zincker (1995) concluded that the increase in the maximum transverse diameter of the medulla , the total surface area of the medulla and tubule and their ratios indicates the increase in hoof horn softness. That is because the medulla is formed from decayed cells of poor quality.

The obtained results revealed a significant decrease in the number of horny tubules, surface density and thickness of the cortex, meanwhile a significant increase in the diameter and surface area of the medulla starting at 2 and 6 days after appearance of signs of laminitis. The results of the histometric parameters indicated that, the changes in the hoof microstructure require a time for appearance than the changes in the microcirculation of the hoof corium which occur directly following induction of laminitis (Nilsson, 1963).

There is association between the hoof affected with laminitis and poor hoof horn quality. As weak, poor quality hoof horn appears to lose its natural water - proofing properties, more prone to environmental influences, cannot protect against concussion and generally manifests clinically as hoof affections (Kempson, 1990; Eustace, 1994 and Zincker, 1995).

The findings of histometric parameters determined in this study indicated that, laminitis leads to formation of poor quality horn which make the hoof more susceptible to affections as mentioned by Kempson (1990). Furthermore, these results potientiate the hypothesis that laminitis is associated with changes in the epidermal parts of the hoof (Obel, 1948).

In conclusion histometric studies of the microstructure of the hoof during laminitis are accompanied with changes in the quality of horn material. The decreased in the number of the horny tubules and the thickness of the cortex coincide with the increase in diameter and surface area of the medulla and tubules will produce poor hoof horn quality.

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