

Original Communications**LIGHT MICROSCOPIC CHANGES IN THE VILLI HEIGHT OF THE SMALL INTESTINE OF LABORATORY RAT (RATTUS NORVEGICUS) FOLLOWING MEBENDAZOLE ADMINISTRATION****Thomas M. Amuti, Anne Pulei, Paul Odula, Hassan Saidi***Department of Human Anatomy, University of Nairobi, Nairobi, Kenya***ABSTRACT**

Through tubulin degradation, mebendazole interferes with glucose uptake into helminth intestinal cells. Exhaustion of glycogen reserves then leads to parasite death. When used in humans, the drug may mediate mucosal changes responsible for early reported side effects including diarrhea. Earlier studies have shown changes to the goblet and crypt cells in mammalian intestines. These changes may, nonetheless, not fully explain the unwanted effects. They may, however, be explained by changes on the villi height. However the effects of mebendazole on the intestinal villi height have not yet been determined. Forty laboratory rats were used; ten rats, for determination of baseline features. Three experimental groups of five rats received 1.6ml of MBZ for three, eight, fifteen and thirty days respectively. Once sacrificed, the rats from the interventional and control groups were dissected to harvest 5mm sections from duodenum, jejunum and ileum. These were processed, sectioned and stained for light microscopy to demonstrate light microscopic features. Villi were measured and data was represented in medians. Mann Whitney U test was done to check for differences between controls versus experimental groups at specific time points; Kruskal Wallis test, for differences among experimental groups or control groups alone. Mebendazole administration was associated with initial reduction in the villi height. The lowest value recorded was after 3 days in the duodenum and 8 days in the jejunum/ileum, beyond which continued MBZ administration was associated with increase in villus height until day thirty. Findings of this study suggest that MBZ has early effects on the villi height, hence, the diarrhea, but does not have effect on the villus height when used for prolonged periods. This may explain why patients who use the drug for long-term periods tolerate it.

Keywords: *Villi height, intestinal light microscopy*

RESUMEN

Mediante la degradación de la tubulina, el mebendazole (MBZ) interfiere con la entrada de glucosa a las células intestinales de helminto. Y el agotamiento de las reservas de glucógeno causa la muerte de parásitos. Al ser utilizada en seres humanos, el medicamento puede mediar como intermediario de cambios en las mucosas que pueden causar reportados efectos tempranos secundarios, incluyendo diarrea. Estudios previos han demostrado cambios en los intestinos de mamíferos, los cuales afectan las células

caliciformes y células de las criptas de Lieberkuhn. Sin embargo, estos cambios pueden no explicar completamente los efectos no deseados. Sin embargo, pueden explicarse por los cambios en la altura de las vellosidades. Desafortunadamente, los efectos del mebendazol en la altura de las vellosidades intestinales no se han determinado. Se utilizaron cuarenta ratas de laboratorio; diez ratas para determinar características básicas, mientras que tres grupos experimentales de cinco ratas recibieron 1,6 mL de MBZ por tres, ocho, quince y treinta días, respectivamente. Una vez que fueron sacrificadas, las ratas de los grupos de intervención y de control fueron disecadas para obtener secciones de 5 mm del duodeno, yeyuno e íleon. Estos segmentos fueron procesados, seccionados y teñidos para microscopía de la luz, para determinar su arquitectura celular bajo congelación. Se midieron las vellosidades y se utilizaron las medias como datos. Se realizó la prueba de Mann-Whitney para determinar si existían diferencias entre los grupos de control y experimentales tratados por plazos específicos de tiempo, y se realizó la prueba de Kruskal-Wallis para identificar sólo las diferencias entre grupos experimentales y grupos de control. La administración de mebendazole estuvo asociada con la reducción inicial en la altura de las vellosidades. El valor registrado más bajo ocurrió después de 3 días en el duodeno, y 8 días en el yeyuno y el íleon. Después de este tiempo, la continuación de la administración de MBZ fue asociada con un aumento en la altura de las vellosidades hasta el día treinta. Los hallazgos de este estudio sugieren que el MBZ tiene efectos tempranos en la altura de las vellosidades, por lo tanto, la diarrea, pero no surte efecto alguno en la altura de las vellosidades intestinales si se utiliza por plazos largos de tiempo. Esto explica por qué los pacientes que utilizan el medicamento a largo plazo llegan a tolerarlo.

Palabras clave: *Altura de las vellosidades intestinales, microscopía de luz intestinal*

* Correspondence to: **Mr Thomas M. Amuti.**
tomamuti@gmail.com

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INTRODUCTION

Mebendazole (MBZ) is a drug that eliminates helminth infestation in the human small intestines (Keystone and Murdoch, 1979). Its use, though, has been linked to unwanted gastrointestinal symptoms such as diarrhea, which has a high prevalence of 20% (Samappito et al, 2012). Basis of these effects remain partly elucidated. In the helminths, MBZ has been shown to bind and diffuse into the simple columnar cells where it causes tubulin degradation further interfering with intracellular transport and organelle structure (Blagov et al, 1991). Just as it affects the helminths, MBZ alters mammalian tubulin structure. A study on the effect of MBZ on the mucosa of the small intestine of the rat has shown that it damages the crypts and goblet cells (Blagov et al, 1991). These changes, nonetheless, may not fully explain the side effects experienced. Such may however be explained by changes on the villi.

Villi are folds of the small intestines (Nightingale, 2015). They are important in food absorption, and any changes on their structure, physically by heat stress (Marriot, 1993) or chemically by drugs (Samappito et al, 2012) might manifest as gastrointestinal signs and symptoms such as diarrhea. This is because villus changes may affect food absorption and this may result in a high concentration gradient in the intestinal lumen. This might lead to osmotic diarrhea, which has also been reported with MBZ use (Friedman et al., 2012; Samappito et al, 2012).

Villi functioning depends on their height. Their height, in turn, depends on the integrity of its simple columnar cells (Hasan et al, 1981). Since mebendazole has been shown to cause helminthic intestinal epithelial cell death (Blagov et al, 1991), it is possible that human intestinal epithelial cells are affected in the same way. This may lead to epithelial cell death and a subsequent villus shortening and resultant diarrhea. This study therefore aims to determine the light microscopic changes on the villi height of the small intestine of laboratory rats (*Rattus norvegicus*) following mebendazole administration.

MATERIALS AND METHODS

In this study, a quasi-experimental study design was employed, where 40 four-month old laboratory rats (*Rattus norvegicus*) (DeSesso and Jacobson, 2001; Dell et al, 2002) of male gender were used. At this age, the rat's gastrointestinal

system had attained maturity (Sangild et al, 2014).

The rats were randomly grouped and marked with a permanent marker for individual identification. They were then housed in 8 standard laboratory cages measuring 125mm by 300mm by 130mm, which were cleaned every three days. During the study, the rats were exposed to artificial 12 hours light–dark cycle and unlimited supply of clean tap water and standard animal pellet diet. The temperature of the room was kept at 22^o Celsius (OECD guidelines, 2001). Animals were handled by trained animal care personnel during transfer from one place to another, cleaning and feeding and protective gears were worn during the handling of animals.

Ten rats were used for determination of baseline features whereas three experimental groups of five rats received 1.6ml of MBZ twice daily (Dayan, 2003), echoing its administration in humans. The mebendazole preparation was made by mixing ground mebendazole (measured in grams) and distilled water. It was then administered using a 3-inch long gavage (Kari, 2015) for three, eight, fifteen and thirty days respectively. These days were selected because they are the common duration for which MBZ is administered to treat helminths.

On the sacrificial days, the tissue processing was performed in a well-ventilated laboratory in the Department of Human Anatomy, University of Nairobi. Firstly, the rats were euthanized using diethyl ether according to the ethical code of the animal house, perfused and incised from the anus to the tip of the xiphoid process. The abdominal skin was reflected using forceps following which the diaphragm and the anterior abdominal wall were identified. Using a pair of scissors, the anterior abdominal wall cut to expose the small intestine. The small intestines was then cut proximally at the gastro duodenal junction and distally, at the ileocecal junction. Once released, the duodenum was obtained by cutting sections at a distance 2 cm posterior to the gastro-duodenal junction, the ileum, 2 cm anterior to the ileocecal junction and the jejunum, 2 cm anterior to the Meckel's diverticulum. Five-millimeter sections from these segments were then cut, placed in separate specimen bottles and stored in 10% formalin for at least 24 hours for fixation. Following fixation, the specimens were dehydrated in ascending grades of ethyl alcohol, starting with 70% alcohol to absolute alcohol. They were then placed in 50% alcohol – 50% toluene following which clearing was done in toluene for 2 hours, and wax impregnation for 12 hours at 58^o Celsius.

Embedding was done using metal moulds in fresh molten paraffin wax (Paraplast, McCormick

Scientific LLC, USA). The embedded specimen blocks were allowed to cool for at least 2 hours from where they were fixed on wooden blocks to facilitate cutting on a sledge microtome. This was done by melting the base of the block, then tightly fixing this base on the upper grooved surface of the wooden block. A Lenz Wezlar (Germany) microtome was used in sectioning to produce 7–micron thick sections. By placing the section on a clean slide, the section was floated in a warm water bath at 45°C to enhance spreading. A clean slide was then smeared with egg albumin to enhance adherence and this slide was used to float out the sections. Following floating, the slides were left to dry in an oven at 38°C for

12 hours. From each embedded block, 10 slides each were obtained during sectioning.

De-waxing was done in three changes of xylene, each 5 minutes. This was followed by rehydration starting from 50:50 xylol followed by descending grades of alcohol for three minutes each. The slides were then stained.

For staining, they were first dipped in iron hematoxylin for 15 minutes then washed in running water for 2 minutes to remove excess stain. They were stained in 1% eosin solution for 3 minutes and dipped in ascending grades of ethanol from 70% to absolute alcohol for dehydration. The sections were then cleared in two changes of xylene before mounting.



Figure 1: High power photomicrograph showing the ileum at baseline. Note the well arranged villi comprising well arranged simple columnar cells. Additionally note the submucosa (SbM) and muscularis externa (ME) (Hematoxylin and Eosin *400)



Figure 2: High power photomicrograph showing the ileum of the experimental group 8 days after MBZ administration. Note the atrophied villi comprising poorly arranged simple columnar cells. Additionally note the submucosa (SbM) and muscularis externa (ME). (Hematoxylin and Eosin *400).

Once mounted, sections were examined and photographed at a magnification of *400. The height of the villi was measured by measuring the distance between a point taken at the base of the crypt adjacent to the villus and another at the tip of the villus. The distance between these two points served as the height of the villus. Three

villi heights were measured within the same macrograph and their average was used as the villus height. Five different macrographs from each slide were used to measure the respective villus height.

These data was recorded and represented in medians. Mann Whitney U test was done to

check for differences between controls versus experimental groups at specific time points; Kruskal Wallis test, for differences among experimental groups or control groups alone. P value was taken at 0.05.

RESULTS

Morphologically, the villi in the duodenum among the experimental groups appeared shorter when compared to those of the controls. This was noted at all-time points (p value = 0.009, 0.016, 0.009, 0.028 for days 3, 8, 15, 30 respectively). Comparison of the control groups at all-time points showed no observable differences in the villi height, p value = 0.102; however, differences were seen among the experimental groups, p value=0.002. The villi at day 3 appeared shorter than that of day 0. Additionally, the epithelial layer appeared sloughed off with absent simple columnar cells. At day 8, though, the villi appeared taller than that of day 3. Unlike the lining epithelium at day 3, the epithelium at day 8 comprised simple columnar cells arranged in a distinct manner. The simple columnar cells, however, appeared to be fewer in number when

compared to those of the control group and day 0, but more when compared to those of day 3. No observable differences were noted between the lining epithelium of the duodenum between days 8 and 30.

In the jejunum and ileum, the villi of the experimental group were seen to be shorter than that of the control at all-time points (p value = 0.117, 0.009, 0.009, 0.009 for days 3, 8, 15, 30 respectively in the jejunum) and (p value = 0.008, 0.008, 0.008, 0.005 for days 3, 8, 15, 30 respectively in the ileum). No observable differences were noted in the control groups at all-time points (p value = 0.193, p value = 0.189, in the jejunum and ileum respectively) but a trend was observed among the experimental group (p value = 0.001, p value = 0.001) in the jejunum and ileum respectively. At day 0 (Figure 1), the villi appeared taller than that of day 8 (Figure 2) comprising a well arranged single columnar epithelial layer. At day 8, though, villus atrophy was noted. Additionally, the epithelial layer appeared sloughed off with absent simple columnar cells. From day 15 to day 30, however, the epithelium comprised simple columnar cells arranged in a distinct manner (Figure 3). These changes were echoed morphometrically (Graphics 1-3).

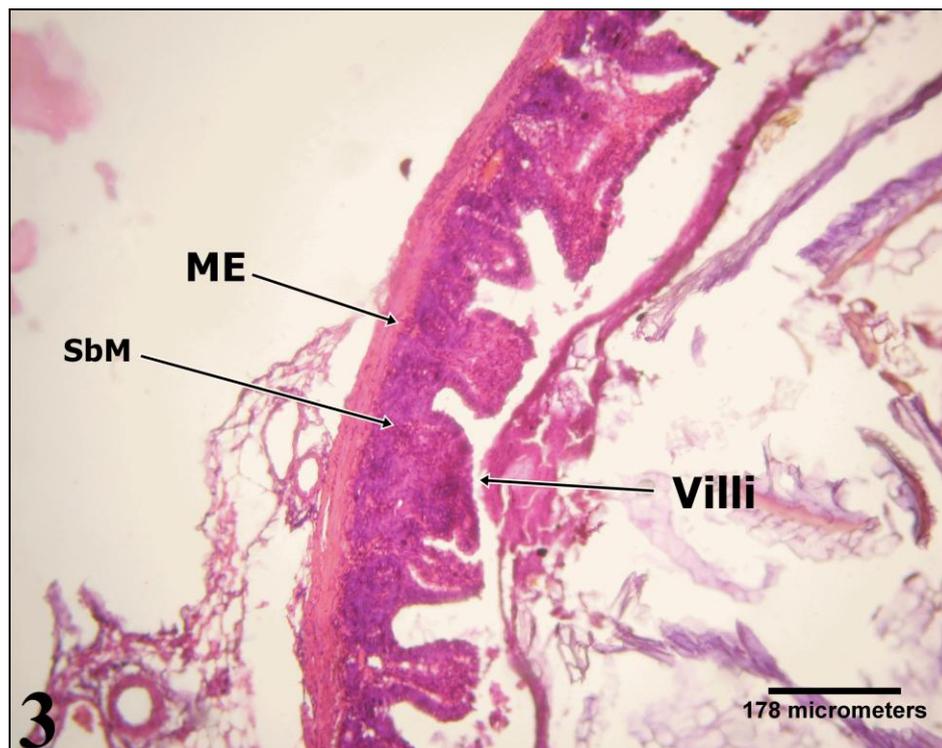
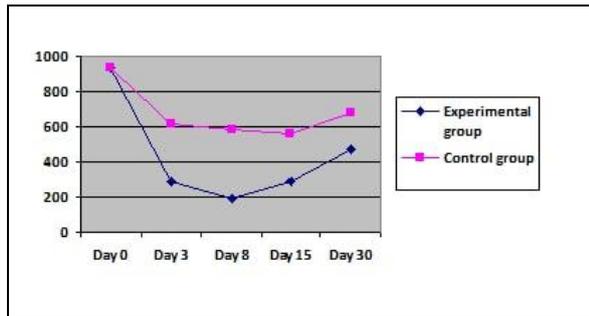
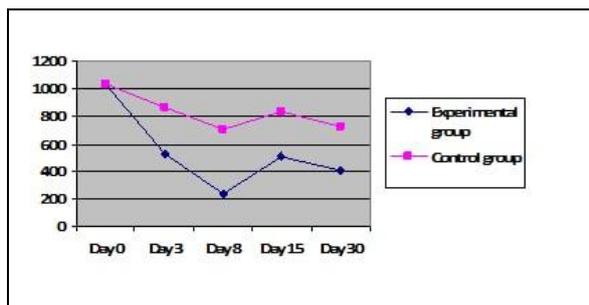


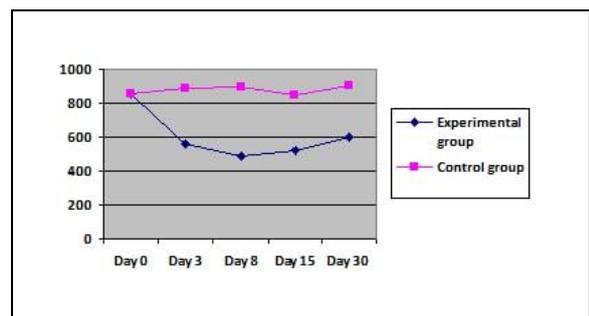
Figure 3: High power photomicrograph showing the ileum of the experimental group 30 days after MBZ administration. Note the short villi comprising the well arranged simple columnar cells. Additionally note the Submucosa (SbM) and muscularis externa (ME). (Hematoxylin and Eosin *400).



Graphic 1: Line graph showing the duodenal villus height (micrometers) at various time intervals (days).



Graphic 2: Line graph showing the jejunal villus height (micrometers) at various time intervals (days).



Graphic 3: Line graph showing the ileum villus height (micrometers) at various time intervals (days).

DISCUSSION

The decrease in villus height may be attributed to MBZ mode of action. Mebendazole binds and diffuses into the simple columnar cells where it causes helminth tubulin degradation, interfering with intracellular transport and organelle structure (Blagov et al, 1991). This leads to epithelial cell death. Just as it affects the helminths, MBZ might cause epithelial cell death in the same manner in humans and this might explain the reduction in epithelial count as well as villus height.

Other than tubulin degradation, MBZ might cause villus height reduction by inducing tumor necrosis factor (TNF) release (Mizuno et al, 2011). The released TNF further activates apoptotic pathways. These pathways lead to the activation of death domains and involvement of apoptotic factors that lead to cell death (Negroni et al, 2015). Endothelial death, in this case, may lead to reduction in villus height as observed.

In as much as MBZ induces cell death, there is production of anti-apoptotic factors during intestinal insults e.g. survivin (Martini et al, 2016). Survivin is expressed by normal intestinal mucosa and has been attributed to intestinal tissue healing (Martini et al, 2016). Its secretion has been shown to increase in cases of tissue injury, e.g. ischemia (Scheer et al, 2017). Hence, the more the intestinal damage caused by MBZ administration, for example, the more the production of survivin. Production of survivin might allow for growth and normal functioning of the simple columnar cells which manifests as an increase in villus height as noted from days 3 in the duodenum or 8 in the jejunum and ileum till day 30. The sequence of the changes noted, were observed in the duodenum first, possibly because the drug is metabolized or absorbed by the time it gets to the jejunum and ileum (Allan and Watson, 1983). As a result, its effects in the jejunum and ileum do not occur as fast as that in the duodenum. Therefore, as has been observed, mebendazole leads to early light microscopic changes in the small intestines. These changes though, revert back to normal during prolonged use of MBZ. This may explain why patients who use the drug for long term periods are able to tolerate it without gastrointestinal effects.

Conflict of Interests

The authors declare no competing interests

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Ethical Approval

Approval was sort from the Animal Welfare Biosafety, Animal care and Use Committee of the Faculty of Veterinary Medicine, University of Nairobi.

Informed consent

This was purely an experimental study. Consent was sort from the Animal Welfare Biosafety, Animal care and Use Committee of the Faculty of Veterinary Medicine, University of Nairobi.

Authors' contributions

Each author of this manuscript made the following contributions: T.A and H.S: Substantial contribution to concept design, acquisition and analysis of data; A.P: drafting of the article, critical appraisal of the intellectual content; P.O: drafting of the article and critical appraisal of content.

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