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Research Article

The Effects of Cimetidine and Ranitidine on Kidney Cortex of *Mus musculus albinus*

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Abstract: In this study, 4.3 µg/g cimetidine and 17 µg/g ranitidine were orally given to mice for two months. The effects of cimetidine and ranitidine on kidney cortical tissue were examined at light microscopical level. In both groups, it was detected that the tubules were separated by their lumen containing darkly stained remnant material. Vacuolization, destruction of apical membrane and decreased PAS positive reaction were observed in the apical membrane of proximal tubule epithelial cells. The nuclei of proximal and distal tubule cells were cylindrical. More excessively in the ranitidine treated group, widening of the tubule lumens and limited proximal tubule lobulation were observed. On the other hand, invagination of some distal tubules towards the lumen was detected. In conclusion, both drugs were found to be effective on kidney cortical tissue.

Key Words: Cimetidine, ranitidine, kidney, mouse.

Simetidin ve Ranitidinin *Mus musculus albinus*'un Böbrek Korteksi Üzerine Olan Etkileri

Özet: Bu çalışmada, farelere 4.3 µg/kg simetidin ve 17 µg/kg ranitidin, iki ay süreyle, oral yolla verilerek, bu maddelerin böbrek korteks dokusu üzerindeki etkileri ışık mikroskobu düzeyinde incelendi. Her iki deney grubunda da tubullerin birbirlerinden uzaklaştıkları, tubul lümenlerinin koyu boyanmış kalıntı materyal içerdikleri tesbit edildi. Proksimal tubul epitel hücrelerinde vakuolizasyon, apikal membranda parçalanma ve apikal membrandaki PAS pozitif reaksiyonda azalma görüldü. Her iki tubul hücrelerinin nukleuslarının çoğunun silindirik şekilli olduğu gözlendi. Ranitidin uygulanan grupta daha fazla olmak üzere, daha çok proksimal tubullerin sınırladığı loblanma, tubul lümenlerinde genişleme tesbit edildi. Ayrıca, bazı distal tubullerin lümene doğru invagine oldukları görüldü. Sonuç olarak, her iki ilaç da böbrek korteksi üzerinde etkili olmuştur.

Anahtar Sözcükler: Simetidin, ranitidin, böbrek, fare.

Introduction

Cimetidine is an H_2-receptor blocker used in the treatment of peptic ulcers (1). Like some organic cations (procainamide, ranitidine, triamterene, metformin, amiloride), cimetidine is an important factor in renal tubular secretion (2-4). While cimetidine is used in the treatment of primary or secondary peptic ulcers, various studies have been reported concerning its side effects (1,5-12). These side effects were shown to depend on the dose (13). The most important of these effects is acute renal failure (14). Several articles have reported that cimetidine caused in liver and kidney functional defects (15-23).

Cimetidine was reported to cause small but important changes in renal functions. The renal proximal tubule is responsible for the removal of organic cations. The brush border membrane vesicle studies of proximal tubule cells showed that an organic cation-proton carrier mediates active organic cation transport (24,25). In marsupial rats, the organic cation transporter in the apical membrane of the proximal tubule cells of the kidney was detected to be glycosylated (24). The organic cation transporter system is not developed completely after birth in pigs and mice. It is developed gradually from the early neonatal stage to mature capacity (25).

Recently, ranitidine, instead of cimetidine, is being used for the treatment of peptic ulcers. However studies on the side effects of ranitidine are few. The aim of this study was to determine the effects of cimetidine and ranitidine, H_2-receptor antagonists, on kidney cortical tissue.

Materials and Methods

Three groups, each containing 6 male and 6 female *Mus musculus albinus*, were used. All the animals were fed with pellet fodder. The first group was the control group. These animals were mouthfed 1ml of tap water with the help of group drill every morning for 60 days. 4.3 µg/g cimetidine and 17 µg/g ranitidine were given to the second and third groups, respectively, for 60 days in the same way mentioned above. The kidney cortex pieces were fixed in 10% formalin at the end of the experiment period. The tissue pieces were embedded in paraffin and 6-µm-thick sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS) and hematoxylin (26). The sections were photographed with a Carl Zeiss Ultraphote II light microscope. The microscopical magnification of all the figures is three times.

Results

First group (Control group): In the kidney sections of the control group, the proximal and distal tubules were of normal appearance (Figures 1,2). A PAS positive reaction was seen in the apical and basal membranes of the proximal tubule cells. Spherical nuclei were lightly stained in general and had a granular appearance. Distal tubule cells were more lightly stained and had wider lumen than did proximal tubules. The basal membrane of the parietal sheet of the Bowman capsules showed a PAS positive reaction. The capsular space was narrow. Endothelial nuclei, which compose the glomerulus, were granular and of normal appearance (Figure 2).

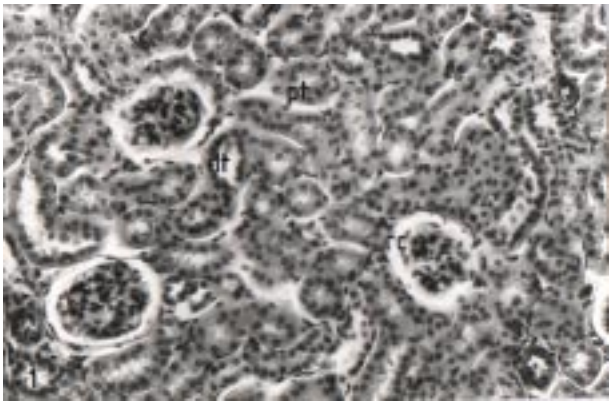

Figure 1. Kidney cortex of control group. Proximal tubules (pt) distal tubules (dt) and renal corpuscules (rc). Hematoxylin-Eosin, X 400.

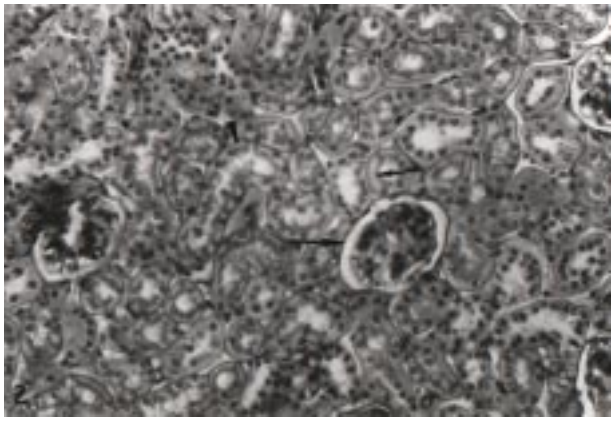

Figure 2. PAS (+) reaction in brush-border (fine arrow) and basal membrane of proximal cells (arrow head), in basal membrane of parietal cells (thick arrow). PAS-Hematoxylin, X 400.

Second group (Cimetidine group): Most of the proximal tubules were degenerated and the distal and proximal tubules in the cortical region became distant from each other (Figures 3,4). This degeneration was excessive in some tubules and only the basal membrane was distinctive. Tubule cells were stained more lightly than those in the control group and, in most of them, destruction of the apical membranes was observed (Figures 4,5). As a result, a brush border was observed regionally in some tubules while not detected in others. The PAS positive reaction was decreased in the apical membrane, but was strong in the basal membrane (Figures 4,5). There were invaginations across the lumen of some tubules (Figures 5,6). There were darkly stained remnant material and nuclei in the tubule lumen (data not

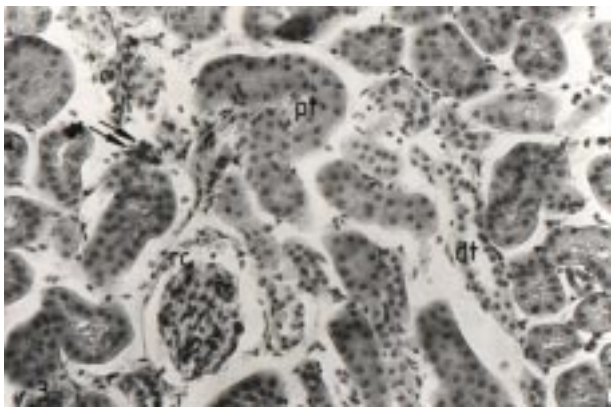

Figure 3. Proximal tubules (pt), distal tubules (dt), renal corpuscules (rc), interstitial nuclei (double arrow) in kidney cortex of cimetidine administered group. Hematoxylin-Eosin, X 400.

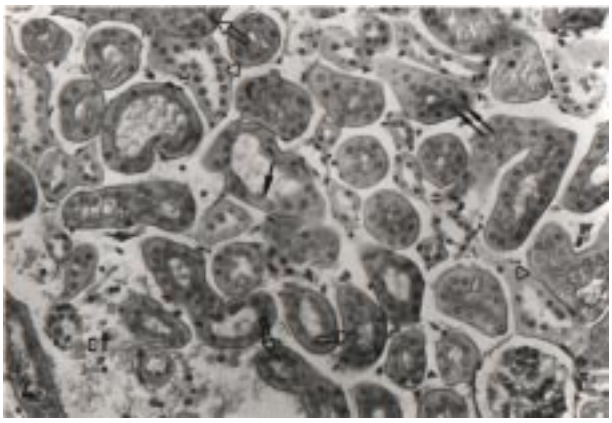

Figure 4. Cimetidin-administered rupturing in the brush-border (dark arrow) and distal tubules (light arrow head), cylindrical nuclei (light arrow), pyknotic nuclei (double arrows), connective tissue (ct). PAS - Hematoxylin, X 400.

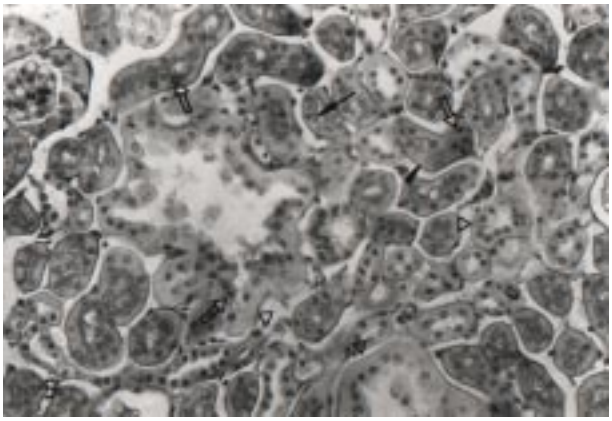

Figure 5. PAS (+) reaction in brush-border (dark arrow) and basal membrane (dark arrow head) of proximal tubules, pyknotic nuclei (light arrow), disruption and invagination of the distal tubules (light arrow head). PAS - Hematoxylin, X 400.

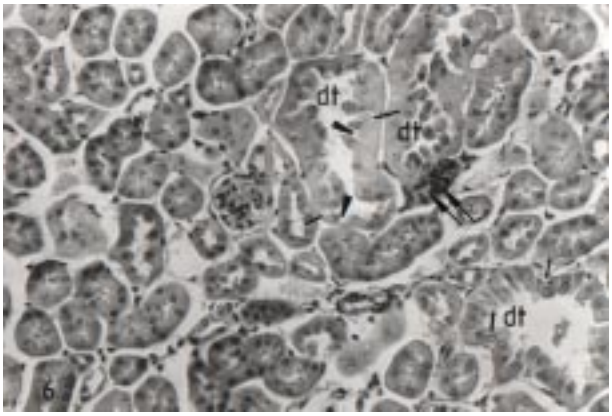

Figure 6. Disruption and residue material and nuclei in the lumen of distal tubules (dt), rupturing of the apical membrane of distal tubule cells (arrow head), vacuoles (line), interstitial nuclei (double arrows). Hematoxylin-Eosin, X 400.

shown). Tubule cells contained cylindrical nuclei and a large number of vacuoles (Figures 4,6). In most cells, large nuclei, which nearly filled the whole cell, were observed, and at various points the nuclei formed clusters. Kidney tissue was separated into lobes by blood vessels and tubules. The connective (Figure 4) and adipose tissues of the kidney capsule were increased.

The lightly stained distal tubules, compared to those in the control group, were separated from each other. The degenerations in the distal tubules were lower than in the proximal ones (Figures 5,6). The apical membranes of some distal tubule cells were shattered, and the vacuoles were seen in the cytoplasm of these cells. Tubule numbers were decreased in general. Some material giving a PAS positive reaction in the lumen of the tubule was found. While some distal tubules showed an excess amount of invagination (Figures 5,6) others were enlarged to cover large spaces. The basal membranes of many tubules were shattered and the traced ones gave a PAS positive reaction. The nuclei of distal tubule cells were cylindrical in various places (Figure 4). Most cells contained pyknotic nuclei but the nuclei of some cells were large enough to fill the cytoplasm entirely.

The renal corpuscules were large in number, and it has been observed that some renal corpuscules were enlarged, while the shape of renal corpuscules was degenerated and destroyed. Sometimes the urinary spaces of the Bowman capsule were expanded, but they were too narrow to be observed in the Bowman capsule of the renal corpuscules, where the glomeruli were very large. The pyknotic nucleus number was increased in the glomerulus.

Third group (Ranitidine group): In this group, it was observed that the proximal tubules were separated from each other (data not shown), the lumens were widened, and most tubules were destroyed. Also nuclei and the remnant material were observed in the widened lumen of such tubules (Figures 7,8). Because of the destruction of the apical membranes of proximal tubule cells, the continuity of the brush border could not be followed (Figures 8,9). There was a decrease in the PAS positive reaction in the apical end while the same reaction in the basal membrane was strong (Figures 7,8,10). Tubule cells contained many vacuoles of different sizes (Figure 8). While the nuclei of some cells were cylindrical, others were flattened (Figure 8). The lobulation consisting of entirely disrupted proximal tubules was mostly seen in

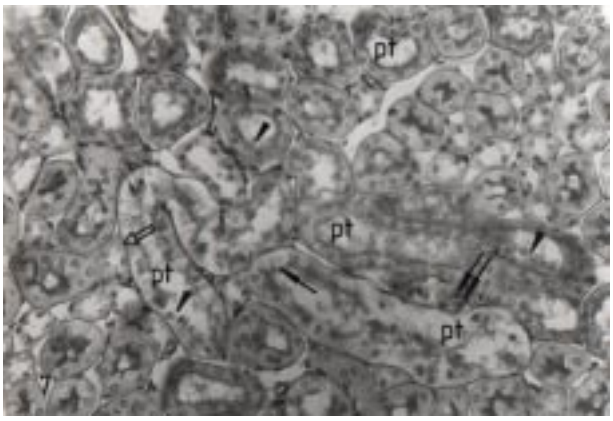

Figure 7. Dilatation and residue material in the lumen of proximal tubules (pt), rupturing of the apical membrane of their cells (arrow head), PAS (+) reaction in brush-border (dark arrow) and basale membrane of tubules (light arrow), increased interstitial nucleus (double arrows) in ranitidine administered group. PAS- Hematoxylin, X 400.

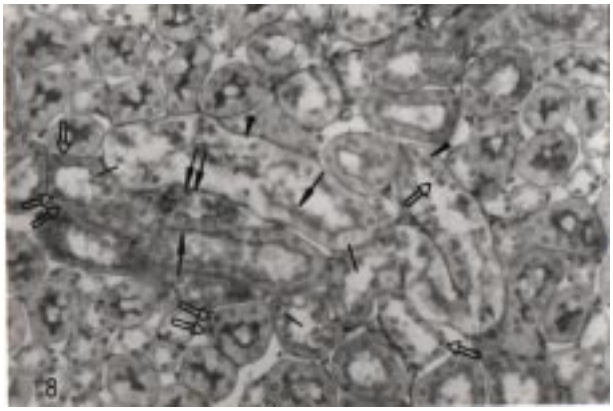

Figure 8. . PAS (+) reaction in brush-border (dark arrow) and basal membrane (arrow head) of proximal tubules. In proximal tubule cells vacuoles (line), pyknotic nuclei (light double arrows), cylindiric nuclei (light arrow) and interstitial nuclei (dark double arrows). PAS - Hematoxylin, X 400.

the inner cortex near the medullar region (Figures 9,10). The connective and adipose tissues were increased more in the sections of this group than in the control group. Some of the distal tubules which were separated from each other were degenerated. Because of the high number of vacuoles on tubule cells, only the nuclei were distinctive (Figures 8,10). The apical membrane of most cells was shattered. The number of pyknotic nuclei was increased and the nuclei could not be distinguished in some cells (Figures 8,10). Some tubule cells were spherical.

The parietal sheet of the Bowman capsule was degenerated. The capsular space was different in

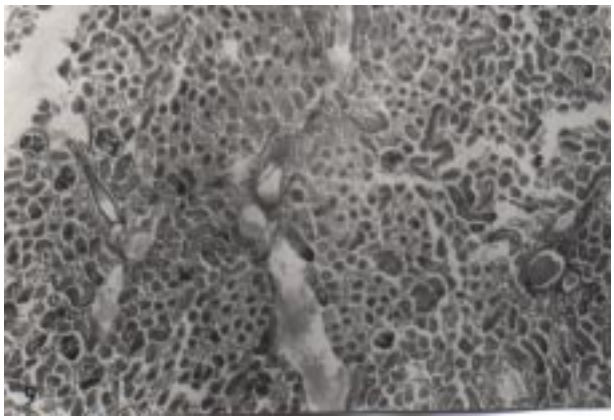

Figure 9. Lobulation in kidney cortex in ranitidine administered group. PAS- Hematoxylin, X 100.

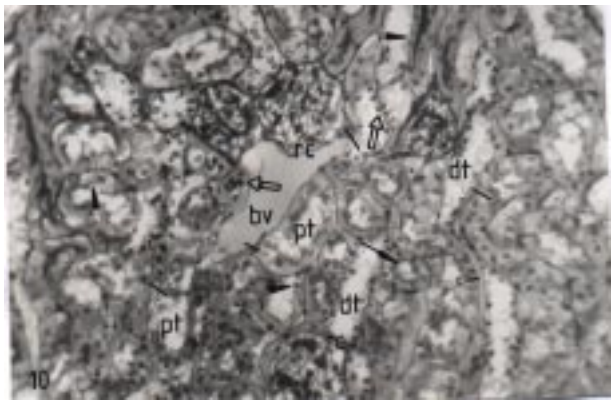

Figure 10. Distruption in proximal (pt) and distal tubules (dt) is shown. Vacuoles in tubule cells (line), rupturing (arrow head) and PAS (+) reaction (dark arrow) in the apical membrane of tubule cells and basal membrane (light arrow head) of proximal tubules, pyknotic nuclei (light arrow), renal corpuscules (rc), blood vessels (bv) in the cortex of ranitidine administered group. PAS-Hematoxylin, X 400.

appaerance that of the control group. The blood vessels involved in the glomeruli were widened and their shapes were degenerated. Some glomeruli were very large. The number of darkly stained nuclei was increased in the glomerulus.

Discussion

The effects of the antiulcerogenic drugs cimetidine and ranitidine, administered orally, on the kidney cortex were investigated after an experiment period of 60 days.

Cimetidine and ranitidine are histamine H_2-receptor antagonists and are widely used in acid-peptic diseases. There is not enough literature on the stimulation of

ranitidine in interstitial nephritis (27), while there are lots of articles on the acute interstitial nephritis cases stimulated by cimetidine (13,16-23). Studies have shown the development of interstitial nephritis after two weeks of cimetidine treatment (17,23,27). We saw some changes as observed in nephritis after the test animals were treated with ranitidine and cimetidine. According to several studies in which was cimetidine applied, these findings were dependent on drug symptoms (16,19,21,23) and this shows the hypersensivity reaction with cimetidine. However, the number of studies on ranitidine is limited (20). We also saw similar results, when we administered cimetidine and ranitidine, in the individual. Human lymphocytes are known to possess receptors for cimetidine but not for ranitidine (20). The effect mechanism of ranitidine has not been completely determined. Possibly, ranitidine exerts its effects in a different way. In this study, the weak formation of nephritis may be due to the hypersensitivity reaction associated with the use of both cimetidine and ranitidine.

It is reported that lobulation, focal increase in glomerular mesangial cells and hyalinized glomeruli were seen in the kidney biopsy of a patient who had been given 300 mg/day cimetidine (21). In this study, we determined lobulation that was limiting tubules and vessels following treatment with ranitidine and cimetidine, and ranitidine was markedly more effective than cimetidine. The results are consistent with the results of the literature cited. Extended and widened tubules were shown in the kidney biopsy material of cimetidine-treated patients (21,23). Similar findings obtained in this study are consistent with others (21,23).

It was stated that interstitial fibrosis was found in several areas and ruptured tubular wholeness (22). It has been suggested that inflammatory cells cause these ruptures in the tubular lumen (21,22,27). The results of this study showed that tubules were separated from each other due to the increasing connective tissue. Additionally, we determined that residue material and nuclei in the lumen of tubules represent infiltrated blood cells, which may have immigrated from the damaged glomerular structure.

Mechanisms exist in renal proximal tubules for the mediated transepithelial secretion or reabsorption of endogenous and exogenous organic cations (28). The results of studies indicate that the basolateral membrane of a clone of cells derived from the porcine renal epithelium cells has one or more transport processes for the mediated uptake of organic cations (29). In another study oral administration of cimetidine revealed significant reduction in brush-border-membrane-associated enzymes (30). In vitro addition of cimetidine to the brush-border-membrane also inhibited the enzyme activity (30). Transport by apical membranes is driven by an electroneutral proton/organic cation exchange system that is higly cimetidine sensitive (31-33), whereas basolateral membrane transport is stimulated by an inside-negative membrane potential and or countertransport (34,35). The weak PAS positive reaction corresponding to the ruptures in the apical membrane of proximal tubule cells is evidence of the pathological mechanisms associated with transport processes.

Several studies conducted with organic cations are case reports. The findings have been determined in the experimental studies for proximal tubules and glomeruli. In this study, degeneration in distal tubules, a decrease in their number and the presence of residue in the lumen were determined. The histological changes observed in the distal tubules indicate that distal tubules were not regenerated. In addition, we detected invagination of some distal tubules towards the lumen. Unfortunately, we cannot discuss these findings because of a lack of literature on this subject.

In the experimental group in this study, it was observed that the renal corpuscules were large and the number of picnotic nuclei was increased. This diagnosis may be due to the glomerular degeneration.

The PAS reaction is characteristic in showing carbohydrates on the tissue (36). The intensity of the reaction is due to the amount of glucose in carbohydrates. In this study, it was found that the reaction of PAS was decreased on the apical membrane of the proximal tubule cells in both experimental groups when compared to the control group. These results can be interpreted to show that components of the apical membrane of the cells have decreased or their chemical structure has been degenerated.

It was determined that tubule cells contain many vacuoles different in size and nuclei situated in the empty space. The kidney insufficiency may be due to toxic effects of the drugs. In experimental groups, pyknotic nuclei

have been observed in tubule cells. A pyknotic nucleus is a sign of nucleus degeneration (37). These nuclei resulted from excessive function of the cells in which they exist.

In conclusion, similar histological changes observed in kidney cortex in both experimental groups indicate that these drugs should be used more carefully.

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