Histological study of isoniazid-rifampicin related nephrotoxicity in Wistar rats

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Submitted: 22.12.2015. / Accepted: 25.03.2016.

**Abstract**

**Introduction:** Nephrotoxicity is a severe adverse effect of antituberculotics with acute renal failure as the most frequent clinical presentation. Associated pathohistological feature is tubular necrosis or interstitial nephritis with or without glomerular lesions. The incidence of rifampicin induced kidney damage varies from 1.8% to 16% of all acute renal failures and well-defined clinical, pathohistological and pathophysiological features have not yet been determined. The aim of this study is to perform qualitative histological analysis of Wistar rat kidney after 21 days coadministration of rifampicin and isoniazid.

**Methods:** Twenty-one adult male Wistar rats (210-280g) were divided into two groups: control group (seven rats) and RIF+INH (rifampicin + isoniazid) group (14 rats). The control group received 4mL/kg isotonic saline solution. Rifampicin and isoniazid were coadministered to RIF+INH group, each substance in the dose of 50mg/kg, dissolved in 4mL/kg of isotonic saline. Dosing was performed intraperitoneally, daily. After 21 days animals were euthanized to obtain kidney tissue for the histotechnological procedure. Kidney slides were stained with HE and PAS method and analysed using light microscopy.

**Results:** The most important findings of our study were glomerular lesions while renal tubules were generally preserved. Lesions had a patchy distribution, renal corpuscles were enlarged with possible mild mesangial hypertrophy. Glomerular capillary tufts showed shrinkage resulting in noticeably dilated Bowman’s space and sporadical lobulation of capillary loops.

**Conclusion:** Intraperitoneal coadministration of rifampicin and isoniazid for 21 days was associated with mild to moderate histological changes of kidney tissue in Wistar rats.

**Keywords:** Rifampicin, Isoniazid, Kidney, Toxicity, Histology

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**Introduction**

Nephrotoxicity is an adverse effect of many drugs, mainly nonsteroidal anti-inflammatory drugs, antihypertensives and antibiotics, including those used in antituberculosis treatment [1]. Rifampicin and isoniazid are widely used antimicrobial agents in standard antituberculosis treatment. Also, rifampicin, alone or in coadministration, is important for management of non-tuberculosis infections, especially those caused by multi-drug resistant Staphylococcal strains [2,3]. According to a literature review, the incidence of rifampicin induced kidney damage varies from 1.8% to 16% of all acute renal failures [1]. Some authors report that overall mortality is 18% [1]. Severe renal injury is associated with thrombocytopenia, immune haemolytic anaemia and intravascular haemolysis [4]. Withdrawal of therapy leads to recovery in most of the cases, but even if reversible, adverse effects often require a change of treatment, affect the compliance and have negative consequences for the outcome, e.g. relapse or the emergence of drug-resistance [1,2,5].

There are many studies suggesting that rifampicin is responsible for kidney damage while the role of isoniazid is yet not elucidated [1,4]. Isoniazid causes liver damage through the free radical formation and may potentiate nephrotoxicity caused by other agents through liver enzyme induction [6]. On the other hand, isoniazid is used for chemoprophylaxis in renal transplant recipients [7].

Many human and animal studies suggest that rifampicin-induced kidney damage appears because of oxidative stress [8]. Through conversion to electrophiles, nucleophiles and redox-active reactants, rifampicin has a direct toxic effects and causes cellular dysfunction [9]. Apart from direct damage, oxidative stress leads to the formation of vasoactive mediators that affect glomerular filtration rate through vasoconstriction. This further leads to the morphological changes [8].
Another proposed mechanism of antituberculotic drug-induced kidney damage is an immune reaction of hypersensitivity. Rifampicin-dependent antibodies are directed against the I-antigen which is present on erythrocytes and also on tubular epithelial cells [10]. Immune complexes found in the vessels are related to vascular constriction and consequent tubular ischaemia while interstitial deposits indicate interstitial nephritis [11,12]. However, circulating rifampicin-dependent antibodies have been observed in patients on treatment with rifampicin without any evidence of renal disease [13].

Nephrotoxicity is a severe adverse effect of antituberculotics with acute renal failure as the most frequent clinical presentation. Associated pathohistological feature is tubular necrosis or interstitial nephritis with or without glomerular lesions, although different descriptions are found in the literature [1,13]. Other forms include interstitial nephritis with or without mild glomerular lesions and rapidly progressive glomerulonephritis [4,14]. Some authors also described isolated or superimposed glomerular injury while several studies have reported cases of rifampicin-induced minimal change disease [15].

A well-defined clinical, pathohistological and pathophysiological features of rifampicin-induced acute renal failure have not yet been determined and are generally missing [4]. It is important to obtain the samples before the injury is severe and to elucidate the early stages of kidney morphological and functional alterations. The aim of this study is to perform qualitative histological analysis of Wistar rat kidney tissue after 21 days coadministration of rifampicin and isoniazid.

Materials and methods

Animals

Twenty-one adult male Wistar rats (210-280g) were obtained from the Faculty of Veterinary Medicine in Sarajevo. One week acclimatization was provided before the animals were used in the experiment. The rats were housed in polypropylene cages under a 12-hr light-dark cycle, temperature 23±2°C and 45±50% relative humidity. Standard diet and water were provided ad libitum.

Experimental design

The experiment lasted 21 days. Animals were divided into two groups: control group and RIF+INH (rifampicin + isoniazid) group. We administered 4mL/kg isotonic saline solution to seven rats in the control group, and 50mg/kg of each rifampicin and isoniazid, dissolved in 4mL/kg of isotonic saline to 14 rats in RIF+INH group. Rifampicin and isoniazid were of the highest purity commercially available. Each substance was administered intraperitoneally (IP), on daily basis. Dosing was performed at the same time of day throughout the study to avoid diurnal variability. Body weight and water consumption were measured and monitored continuously, from the start till the end of the study.

Histological analysis

All rats were euthanized with ketamine at the end of the experiment (24 hours after the last IP treatment). Representative samples of kidney tissue were taken and fixed in 10% buffered formalin. Paraffin-embedded tissue blocks were cut into 5 micrometer thick transverse sections and stained with hematoxylin and eosin (HE) and periodic acid - Schiff (PAS). Histological examination of slides was made by the light microscope with a digital camera (Eclipse E400, Nikon) with 100X and 400X magnification. Histotechnology tissue processing and the analysis were carried out at the Department of Histology and Embryology, Faculty of Medicine, University of Sarajevo.

Statistical analysis

Numerical values were expressed as mean±SD. Statistical differences between groups were determined using Student’s t-test and were considered statistically significant if p<0.05. Analysis was performed using MedCalc statistical software for Windows.

Results

There was no mortality in any of the groups. No noticeable change was observed in the usual appearance and behavior. During the experiment, all animals ate and drank regularly and gained weight constantly. On the first day of the experiment, body weight of the rats in the control group was 244.85±9.58g while in RIF+INH group it was 247.57±23.09g. The difference between the groups was not significant (Student’s t-test: mean difference 2.7, 95% CI: -21.93, 16.51, p=0.77). In the end of the experiment the difference remained statistically insignificant (Student’s t-test: mean difference 7.00, 95% CI: -31.00, 17.00, p=0.55). Body weight of the control group changed from 244.85±9.58g at the baseline to 252.85±13.75g at the end of the experiment (Paired t-test: mean difference 8.00, 95% CI: -5.04, 21.00, p=0.18), and from 247.57±22.25g at the baseline to 259.86±28.45g in RIF+INH group (Paired t-test: mean difference 12.29, 95% CI: 6.55, 18.01, p=0.0005).

Control group

We did not notice any lesion or abnormality on macroscopic examination of kidneys, even in their longitudinal section. Light microscopic analysis of HE slides showed a normal structure of renal parenchyma. The glomeruli were normocellular and glomerular capillary loops easily visible, open, some containing red blood cells (Fig. 1-A, Fig. 2-A). The tubules, closely arranged,
were lined by a single layer of cells with well-organized nuclei and homogenous tinctorial properties of cytoplasm (Fig. 3-A). The lumina of distal tubules commonly appeared more open and clear than those of proximal tubules. Interstitium, located between the tubules, was generally inconspicuous. PAS stain revealed normal capillary basement membranes, with no thickening or wrinkling, and preserved brush border on proximal nephrocytes.

**RIF+INH group**

Macroscopically the kidneys in RIF+INH group of rats showed no visible hemorrhage, edema or any other lesion. Longitudinal section showed well-organized cortex and medulla with usual width. However, a qualitative histologic study revealed structural alteration in kidney sections.

HE stained kidney slides showed several alterations and the most prominent findings were glomerular changes. Glomerular lesions had a patchy distribution. Involved renal corpuscles appeared enlarged with possible mild mesangial hypertrophy. However, glomerular capillary tufts revealed shrinkage resulting in noticeably dilated Bowman’s space (Fig. 1-B). Interestingly, some of the glomeruli showed lobulation of capillary loops (Fig. 2-B). Noninvolved glomeruli were normocellular. Interstitial oedema was mild, visible mostly around larger blood vessels and accompanied with peritubular capillary dilatation. Renal tubules were mainly preserved or appeared mildly affected. Their lumina sporadically contained acidophilic cellular debris and seemed widened, while cytoplasm vacuolation was noticed in proximal nephrocytes (Fig. 3-B). PAS staining showed no thickening of glomerular basement membrane neither tubular basement membranes. It approved that brush border of proximal nephrocytes appeared sporadically ruptured.

Figure 1. Photomicrographs of the kidney (HE, X 100):
A Normal appearance of the cortical labyrinth in the control group;
B Enlarged renal corpuscles with dilated Bowman’s space surrounded by generally preserved renal tubules in the RIF+INH group

Figure 2. Photomicrographs of the kidney (HE, X 400):
A The normocellular glomerulus, regular glomerular capillary loop and Bowman’s space, accompanied with proximal convoluted tubule of normal appearance in the control group;
B Noticeably dilated Bowman’s space and lobulation of glomerular capillary loops in the renal corpuscle with preserved distal convoluted tubule in the RIF+INH group
Discussion

In our study, histological slides of kidneys obtained from the animals treated with rifampicin and isoniazid showed mild to moderate alterations. The main findings were changes of renal corpuscles while renal tubules were mainly preserved.

Renal corpuscles of rifampicin-isoniazid treated group of animals seemed enlarged with relative shrinkage of glomerular capillary loops and dilated Bowman’s space in comparison to those of untreated, control group of animals. Kohno et al. reported no interstitial changes and minor glomerular lesions showed by the light microscopy [14]. Similar to our results, and apart from tubular damage, Shabana et al. found frequent shrinkage of glomerular capillaries associated with Bowman’s space dilatation and cellular proliferation in a mesangial area [17]. Peters et al. found inflammatory changes in the glomeruli of albino rats after 60 days of rifampicin treatment [18].

Our results referring to glomerular changes are similar to those found in the study of Perico et al. [19]. Cyclosporine administration to rats for 5 months was associated with the appearance of a subset of glomeruli that became larger than normal. These mildly damaged glomeruli were characterized by ischemic lesions and increased mesangial matrix [19]. Topical application of p-phenylene diamine to female rats caused histological changes in glomeruli including lobulation of glomerular tufts with glomerular cell vacuolation, as well as alterations in glomerular capillaries [20]. Hashmi et al. [21] found severe infiltration in the glomerulus in kidneys of rifampicin-isoniazid treated rabbits.

Park et al. demonstrated that histological changes of glomeruli in their study were so subtle and consequently not visible on the HE slides using light microscopy [15]. The authors found diffusely effaced podocytes processes on electron microscopy. Min et al. presented a case of rifampicin-associated tubulointerstitial nephritis while electron microscopy showed electron-dense deposits in the subendothelial and mesangial space [22].

According to the literature review, histological changes of kidney related to use of rifampicin or the isoniazid-rifampicin combination are generally mild and less extensive [17,21,23]. Tubular necrosis and interstitial nephritis are the most frequent histological findings while acute renal failure is the main clinical feature of rifampicin nephrotoxicity [14]. Other nephrotoxic antibiotics, such as gentamicin, cause clearly visible, extensive fields of necrosis and complete destruction of tubules [24].

Our study revealed the presence of mild interstitial edema. Renal tubules were mainly normal, only some showed alterations as cytoplasm vacuolation and cellular debris in lumina. Also, in the present study PAS staining showed loss of brush border in proximal nephrocytes of some tubules which is in accordance with other studies [17,21,23].

In contrary to our results, many authors reported tubular necrosis of different severity in animal as well as in human biopsy specimens after exposure to antituberculosis. Proximal and distal tubules differently respond to toxic injury because of their functional differences. Morphological alterations in proximal tubules are expected to be more noticeable [25]. Interstitial nephritis, which is a frequent finding in some studies, is considered as the result of an allergic response to the drug. It involves activation of antigen-presenting cells in renal interstitium and tubular cells itself [26]. Shabana et al. found degeneration of tubules including lysis of cytoplasm and vacuolation in rifampicin treated Wistar rats [17]. Rosati et al. found severe tubulointerstitial nephritis with segmental glomerulosclerosis similarly as Chiba et al. who reported plasma cells, neutrophils, lymphocytes and slight eosinophil infiltration.
in the interstitium and no specific damage on renal corpuscles [13,23]. There was mild to moderate congestion in the renal parenchyma and mild to moderate necrosis in albino rabbits after isoniazid-rifampicin administration [21].

Study has some limitations. The qualitative histological analysis in the present study was done on the basis of light microscopy. The use of electron microscopy or special techniques could improve understanding of the morphological changes.

**CONCLUSION**

Intraperitoneal administration of rifampicin-isoniazid combination for 21 days, caused mild to moderate histological changes of kidney tissue in Wistar rats. Glomerular lesions were the most prominent findings while renal tubules seemed generally preserved.

**DECLARATION OF INTEREST**

Authors declare no conflict of interest.

**REFERENCES**


