Original Article

Pathological Changes of Rifampicin Toxicity on Some Organs in Rats

Dalia Amer Khudhair
Department of Pathology, College of Medicine, University of Babylon, Babylon, Iraq

Abstract

Background: Rifampin, also known as rifampicin (RFP), is one kind of rifamycin group of antibiotics and it is used to treat nontuberculous *Mycobacterium* infections including leprosy (Hansen's disease). The purpose of this study was to determine the pathological alterations caused by rifampin poisoning in several organs in rats **Materials and Methods:** We chose 20 albino rats, both sexes, these were divided into two groups (10 rats per group): a treated group and a control group. In the control group, 1 mL/kg/day of dimethyl sulfoxide was given intraperitoneally for 14 days. Group of drugs, RFP 120 mg/kg/day administered intraperitoneally for 14 days. After the organs, including the liver, kidneys, and spleen, were removed, a tissue slide was prepared, and a histology technique was performed. **Results:** The results revealed that the liver sections from the treated group exhibited thickening of the capsule, moderate inflammation, and congestion between the interstitial spaces, absence of fibrosis, and small clusters of neutrophils inside the parenchyma. The kidney showed distal and proximal renal tubule degeneration, leading to cytoplasm lysis and vacuolation. The glomerulus also often contracts. Additionally, Bowman's gap dilation is caused by glomerular capillary atrophy. Casts are also seen in some renal tubules. In the mesangial region (M), cells start to proliferate. Atrophy of glomerular tufts was also seen. Reduced red pulp size and splenic white pulp disarray were seen. The red pulp and white pulp were not separated because the marginal zone was less evident. Compared with the typical rat, the red pulp tended to swell. Not only did sinusoidal gaps sufficiently enlarge, but white blood cell growth was also seen in a few instances. Severe congestion of blood sinuses with the proliferation of megakaryocytes is also seen in the RFP-treated group. It was noted that the toxicant group had greater levels of all changes when compared with the control group. **Conclusion:** This study concludes that RFP treatment at a dose of 120 mg/kg/day for 14 days causes pathological effects on the liver, kidney, and as well as splenic tissues.

Keywords: Histopathology, kidney, liver, rifampicin, spleen

Introduction

A well-known antibiotic, rifampicin (RFP) is used to treat leprosy, meningococcal infections, and tuberculosis (TB); it also significantly shortens the time of therapy for these conditions.[1,2] In sensitive cells, RFP inhibits protein synthesis by binding to the bacterial ribonucleic acid (RNA) polymerase component and preventing transcription from starting.[3] One recognized aim of clinically evident acute liver illness is the elevation of serum bilirubin and aminotransferase levels, which RFP is known to affinitize with. Tragically, this increase in levels may be deadly.[4] The chance of this occurring is increased when additional medications, such as isoniazid or pyrazinamide, are used in combination therapy regimens.[5] There is a lack of chain polymerization in RNA structure because RFP inhibits deoxyribose nucleic acid-dependent RNA polymerase of microbes by creating a consistent enzyme–drug combination.[6,7]

Rapid and almost total oral absorption of RFP occurs in nearly all bodily fluids and areas (except for the brain).[8] The majority of its excretion occurs in the bile, with trace levels being present in the urine.[9] Hepatitis is one of the

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Address for correspondence: Dr. Dalia Amer Khudhair, Department of Pathology, College of Medicine, University of Babylon, Babylon P-51001, Iraq.
E-mail: med.dalia.khudhair@uobabylon.edu.iq

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documented side effects of RFP, which also includes nausea, vomiting, lack of appetite, stomach cramps, and diarrhea. Other reported adverse effects, such as vertigo, headache, exhaustion, disorientation, and menstruation disruption.[10] RFP toxicities primarily manifest as an immunoallergic reaction or a dose-dependent hepatic type. Symptoms of the immunoallergic variant include a course that is, both interrupted and protracted. Because of an issue with hepatocyte absorption and excretion, RFP produced cholestasis at sinusoids and additional at hepatic canaliculi. Hepatitis often occurs in persons with pre-existing liver disease and affects fewer than 1% of the population.[11]

Its metabolite, desacetylRFP is responsible for the hypersensitivity response that underlies nephrotoxicity, which usually manifests itself during interrupted or intermittent treatment.[12] Elevations of alanine aminotransferase (ALT) and/or alkaline phosphatase over the upper normal range by two to three times and/or two times, respectively, indicate cholestatic, hepatocellular, or mixed hepatic damage.[13-15] Administering a variety of drugs can cause liver injury due to the biotransformation of drug metabolites, which can produce harmful or reactive substances, such as electrophilic chemicals or free radicals. This, in turn, can cause structural damage, programmed death, or even necrosis.[16,17]

As shown before in rat experiments, RFP causes hepatotoxicity by way of oxidative doping.[18] The rapid breakdown of hepatocytes is directly correlated with the amount of oxidation of acetyl hydrazine, which is why RFP significantly increases cytochrome P450 activity and promotes the oxidation of reactive metabolites of acetyl hydrazine, turning them into macromolecules that are specific to liver cells.[19,20] Officially recognized as a genesis of liver fibrosis, the activity of glucose 6 phosphatase—the trigger of lipid oxidation—is significantly reduced by long-term exposure to RFP.[21,22] In drug-induced liver injuries, phospholipids membrane degeneration is accelerated by phospholipase A2 activity, which is stimulated by an increase in intracellular calcium concentration.[23,24] Additionally, there is evidence that the accumulation of fatty acids in the liver and the upregulation of CYP2E1 are caused by an excess of lipid supply to the liver.[25-27]

Much of this work involves the association of these toxic effects with RFP in various organs, namely the liver,[28] kidney,[29] testes,[30] ovaries,[31] blood,[32] and bone.[33]

Materials and Methods

Experimental design

In the investigation, 20 albino rats, both sexes, were divided into two groups (10 rats per group): a treated group and a control group were used. These are control groups: 1 mL/kg/day of dimethyl sulfoxide was given intraperitoneally for 14 days.

Treated group: RFP 120 mg/kg/day administered intraperitoneally for 14 days. For every animal, an insulin syringe was used to inject dissolved RFP, which was given at a dosage of 120 mg/kg/day. Every day, the animal was confined in its cage to monitor its clinical signs. After the experiment was over, the animals were put to sleep by inhaling chloroform, and the organs, including the liver, kidneys, and spleen, were removed. These organs were quickly dissected and removed, washed in saline solution, and then chopped into 1–2 cm^3 pieces before being preserved in neutral buffered formaldehyde solution (10%) for at least 48 h and, then, tissue sectioning including:

1. Drying the section: The slides were kept in a hot oven to get dry. The oven temperature was slightly more than the melting point of the paraffin.

It was immersed paraffin wax. Glasss slides stained with haemtoxylin and eosine stain following the production of 5-micron -thick slices in compliance with 12 for histopathological analysis.[34]

Ethical consideration

Following the moral guidelines established by the Helsinki Declaration, the study was conducted. On October 22, 2023, the study protocol was examined and accepted by a local ethics committee under reference number 4-1.

Results

Normal hepatocytes and a central vein were shown by a qualitative test for histological abnormalities in the control group [Figure 1]. The liver sections from the treated group exhibited thickening of the capsule, moderate inflammation, congestion between the interstitial spaces, absence of fibrosis, and small clusters of neutrophils inside the parenchyma [Figure 2].

Figure 3 exhibited the normal architecture of the kidney in the control group. While in the treated group, distal and proximal renal tubules degenerate in the kidney, leading to cytoplasm lysis and vacuolation. The glomerulus also often contracts. Additionally, Bowman's gap dilation is caused by glomerular capillary atrophy. Casts are also seen in some renal tubules. In the mesangial region (M), cells start to proliferate. Renal casts and atrophy of glomerular tufts were also seen [Figure 4].

The spleens from the control group showed typical histoarchitecture, including red and white pulp, a well-defined peripheral zone, and a capsule encircled by trabeculae. Central artery, periarterial lymphatic sheath, and tightly packed tiny lymphocytes made up the white pulp. A layer of marginal zone had a well-developed marginal sinus that divided the red pulp from the white pulp. It showed the red pulp meshwork, which contains normally distributed splenic cords, venous sinuses, and reticular cells [Figure 5].

Reduced red pulp size and splenic white pulp disarray were shown. The red pulp and white pulp were not separated because the marginal zone was less evident. Compared with the typical rat, the red pulp tended to swell. Not only did sinusoidal gaps sufficiently enlarge, but white blood cell growth was also seen in a few instances. Severe congestion of blood sinuses with the proliferation of megakaryocytes was also seen in RFP treated group [Figure 6]. It was noted that the toxicant group had greater levels of all changes when compared with the control group.

Table 1 revealed the histological scores in the treated group of different organs.

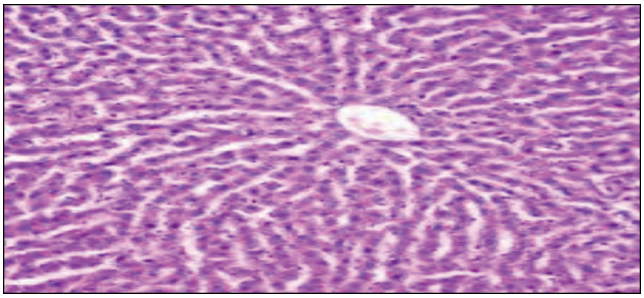

Figure 1: Normal appearance of liver histology of rat in the control group (H&E,100×)

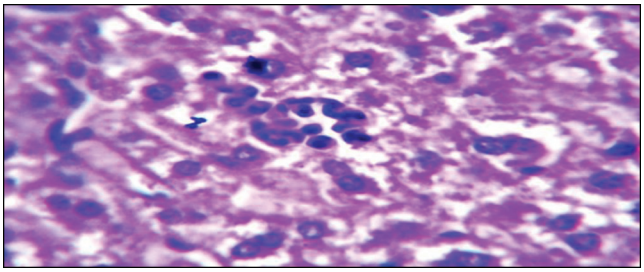

Figure 2: Liver of rats treated with RFP showing tiny aggregation of neutrophils in the parenchyma (H&E, 400×)

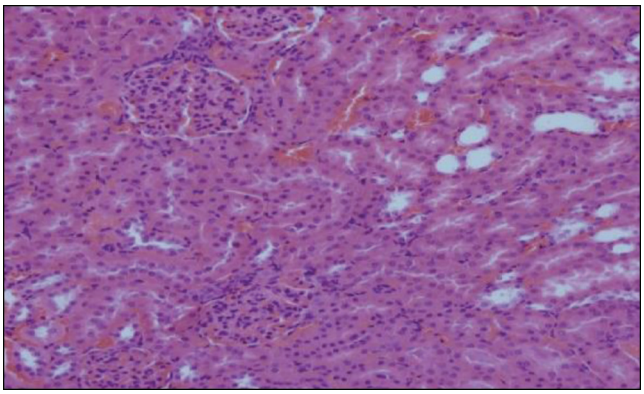

Figure 3: Normal appearance of kidney histology of rat in the control group (H&E, 100×)

Discussion

RFP is one of the first-line drugs used to treat TB.[35,36] The most serious side effect of RFP is liver damage.[37,38] found that RFP, by agonizing pregnane X receptor activity, might modulate drug metabolism and transport enzyme expression, potentially increasing them caused liver damage. However, there is little evidence that RFP causes liver damage by elevating ALT and aspartate transaminase levels in the blood.[39]

According to the study Plumb *et al.*[40] nephritis and hepatitis are among the rare but serious side effects of RFP. Acute renal failure has been documented in a small number of instances after RFP treatment.[41] The Romanian review study states that 0.05% of the 45-year-old patients given RFP suffered acute kidney injury (AKI), which was defined as a serum creatinine level of more than 44.2 μmol/L or 20% of the baseline level within 2 weeks.[42] The exact way that rifampin causes AKI is still not fully understood. Depending on the study, it could be a type II or type III hypersensitivity reaction to rifampin antigens, where immune complexes formed by anti-rifampin antibodies end up in renal vessels, the glomerular endothelium, and the interstitial area.[43]

The current study showed reduced red pulp size and splenic white pulp disarray was shown. The red pulp and white pulp were not separated because the marginal zone was less evident. Compared with the typical rat, the red pulp tended to swell. Not only did sinusoidal gaps sufficiently enlarge, but white blood cell growth was

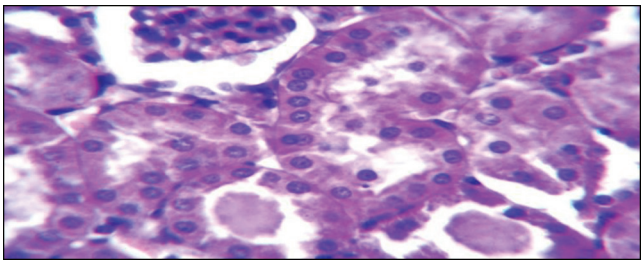

Figure 4: Kidney of rat treated with RFP showing renal casts and atrophy of glomerular tufts (H&E, 400×)

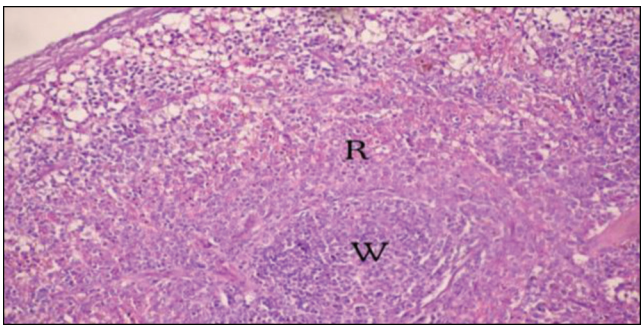

Figure 5: Section of control rat spleen stained with H&E at 400x reveals typical appearance of red pulp (R) and white pulp (W)

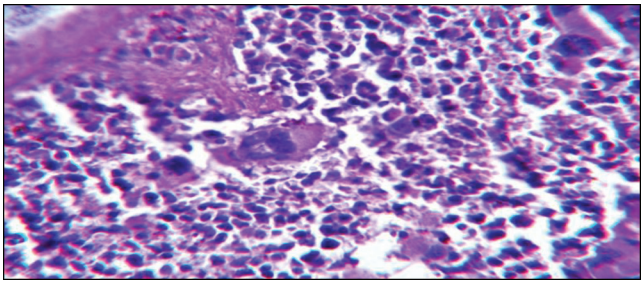

Figure 6: Spleen of rat treated with rifampin showing severe congestion of blood sinuses with proliferation of megakaryocytes (H&E 400×).

Table 1: Histological score of different organs

Histological findings	Liver	Kidney	Spleen
Congestion	+++	+++	+++
Necrosis	+++	++	+
Neutrophilic infiltrations	+++	+++	++

also seen in a few instances. Severe congestion of blood sinuses with the proliferation of megakaryocytes was also seen in RFP treated group, these were in agreement with the study of Sharma *et al.*[44] showed the same results, whereas another study showed no changes in splenic architecture after RFP treatment[45] which disagreed with our findings.

Conclusion

RFP treatment at a dose of 120 mg/kg/day for 14 days causes pathological effects on the liver, kidney, and as well as splenic tissues.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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