Original Article

Histopathological effects of sub-chronic lamivudine-artesunate co-administration on the liver of diseased adult Wistar rats

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Abstract

Background: Lamivudine and artesunate are sometimes co administered in HIV-malaria co morbidity. Both drugs are used concurrently in presumptive malaria treatment and simultaneous HIV post exposure prophylaxis. **Aim:** The aim of this study was to investigate the effect of lamivudine-artesunate co administration on the histology of the liver of diseased adult Wistar rats. **Materials and Methods:** Five groups of rats of both sexes were used for the study and placed on feed and water *ad libitum*. Disease state consisted of immunosuppression with cyclophosphamide, and infection with *Plasmodium berghei*. Group 1 animals served as vehicle control, while group 2 were the diseased controls. Group 3 animals received 20 mg/kg lamivudine for three weeks, while group 4 similarly received 20 mg/kg Lamivudine but also received 10 mg/kg artesunate from day 12. Animals in group 5 received 10 mg/kg artesunate from day 12. All drugs were administered intraperitoneally. The animals were treated for twenty-one days, at the end of which they were sacrificed and their livers fixed in 10% formalin for histological studies. **Result:** Results from the study show the presence of regions of focal necrosis and perivascular cuffing with animals that received artesunate. Hemosiderosis was a common feature in all the parasitized groups, while fatty degeneration was observed in the group that received artesunate alone. **Conclusion:** Concurrent lamivudine-artesunate administration resulted in some histopathological changes in the liver. This study suggests there may be considerable histological changes with repeated occurrence of malaria and immunosuppression that may warrant intermittent lamivudine-artesunate administration, and may require evaluation as well as monitoring of liver function during such therapeutic interventions.

Keywords: Drug interaction, lamivudine, artesunate, liver, histopathology.

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Introduction

Malaria is a parasitic disease of global importance, with more than 3000 million people in over 100 malaria endemic countries being at risk [1], and is responsible for the death of approximately a million people annually. Over 90% of yearly deaths resulting from malaria occur in sub Saharan Africa [2]. Poverty and poor sanitary conditions have made this situation more challenging. Drugs such as chloroquine and sulphadoxine-pyrimethamine which had been useful against the lethal falciparum malaria infection for over fifty years, are no longer effective due to wide spread resistance in all malaria endemic regions [3, 4]. In addressing the problem of drug resistance, the World Health Organization (WHO) has advocated the use of artemisinin and its derivatives in combination for the treatment of malaria [5]. Artesunate is one of the most extensively used artemisinin derivatives and is employed both as monotherapy and in combination with many of the

older anti malarial medications. The WHO has advised the use of presumptive diagnosis as basis for treatment of uncomplicated malaria where microscopy based diagnosis is unavailable or cannot be conducted [6]. In many of such instances, artesunate and other artemisinin derivatives are used in combination or as monotherapy.

As a result of the geographic overlap in malaria and HIV/AIDS especially in Africa, concurrent drug therapy is essential. The co morbid state of malaria and HIV result in serious concerns all around the globe and both diseases are known to affect each other negatively [7]. Malaria appears to reduce $CD4^{+}$, while an increase in the incidence and severity of malaria in the HIV population may also result in worsening of the HIV burden [8]. HIV/malaria co-infection thus presents the challenges of not only multiple drug therapy, but also possible adverse events that can alter haematological recovery following co infection [9]. The integrity and function of other important organs such as the liver may also be affected.

The liver is the second largest organ in the body involved in a host of functions including synthesis of clotting factors, detoxification and metabolism of lipids and carbohydrates [10]. It is also a major organ involved in digestive activities within the digestive system. Substantial disruption in its anatomy or function may result in severe alteration in its metabolic roles, and this may adversely affect physiological functions. The co existence of malaria and other conditions that necessitate the concurrent administration of lamivudine and artesunate may result in adverse effects, leading to possible structural and functional alterations of the liver. The objective of this study was to investigate the possible effects of lamivudine-artesunate co administration on the histopathology of the liver of rats in the diseased state of rodent malaria with concurrent immunosuppression.

Materials and Methods

Drugs

Drugs used for this study were lamivudine (Evans), artesunate (Tuyil Pharmaceuticals) cyclophosphamide (KLab), sodium bicarbonate (Martindale). Both test drugs were of 99.9% analytical grade.

Animals

Animals used in this study were adult Wistar rats of both sexes. The animals were obtained from the Animal Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria and were used according to the NIH animal care guidelines with approval of the Departmental Animal Committee (DAC/IW-OT/1-07). Animals were placed on food and public water supply *ad libitum* for the entire duration of the experiment. The animals were allowed to acclimatize with the experimental room for a period of two weeks prior to the commencement of the study.

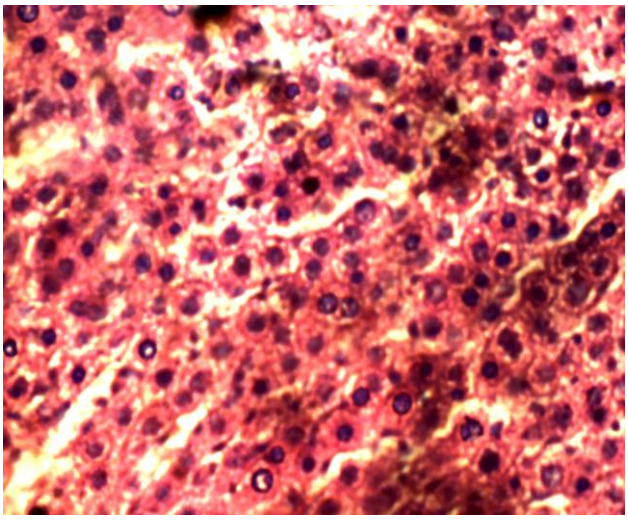

Fig. 1 Control Section of the liver of a vehicle control (H & E). (Mag. X 400)

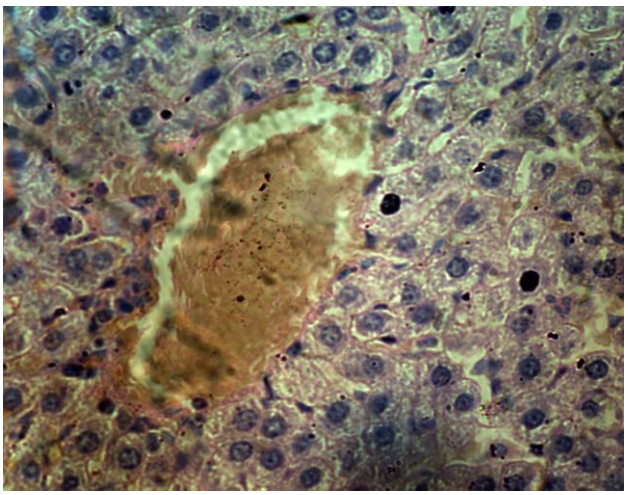

Fig. 2 Section of liver from a rat immunosuppressed with cyclophosphamide and infected with *P. berghei*. H & E. (Mag. x 400). The section shows areas of mild haemosiderosis and areas of focal necrosis.

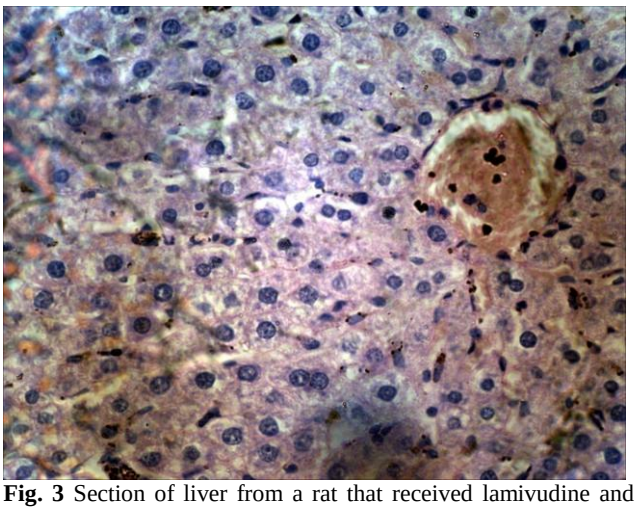

Fig. 3 Section of liver from a rat that received lamivudine and was immunosuppressed with cylcophosphamide, and infected with *P. berghei*. H & E.(Mag. x 400). The section shows mild haemosiderosis and focal necrosis and some congested sinusoids.

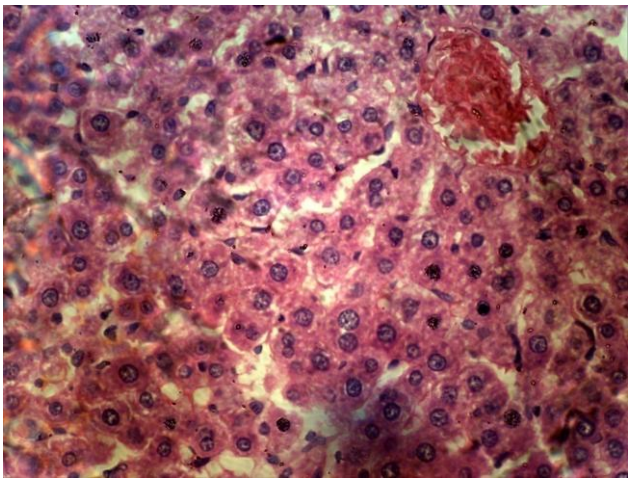

Fig. 4 Section of the liver of a rat that received lamivudine and artesunate treatment, and was immunosuppressed with cylcophosphamide, and infected with *P. berghei*. H & E. (Mag. x 400). The section shows congestion in the liver, intact hepatocytes with few necrotic hepatocytes and well defined hepatic cords and sinosoidal spaces.

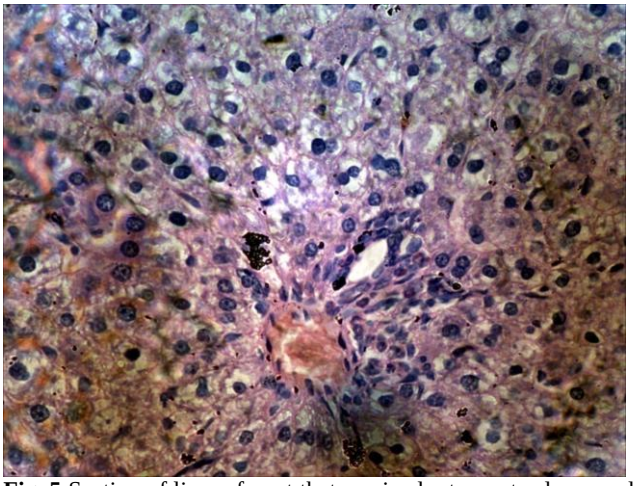

Fig. 5 Section of liver of a rat that received artesunate alone, and was immunosuppressed with cylcophosphamide, and infected with *P. berghei*. H & E. (Mag. x 400). The section reveals heamosiderosis, perivascular cuffing and slight fatty degenaration.

Preparation of Drugs

Both cyclophosphamide and lamivudine are very soluble in distilled water and were prepared using distilled water as vehicle. Artesunate was prepared by dissolving 60 mg of the powder in 1 ml of 5% sodium bicarbonate, and this was made up to 6 ml with distilled water. Required concentrations for administration were prepared from initial stock solutions.

Grouping of animals

Animals were grouped into five groups of six animals (equal distribution of male and females). Animals in the first group served as vehicle control. The animals in the second group served as diseased control. Animals in the third group received lamivudine (20 mg/kg) alone while the animals in the fourth group received a combination of lamivudine (20 mg/kg) and artesunate (10 mg/kg). Animals in the fifth group received 10 mg/kg artesunate alone. All animals belonging to groups 2-5 were immunosuppresed with an intraperitoneal stat dose of 100 mg/kg cyclophosphamide [11], followed by a booster dose of 50 mg/kg on day 8. Malaria parasite was inoculated into the rats by intraperitoneal injection of approximately $1x10^6$ parasites [12] on day 12. Animals that were treated with lamivudine received 20 mg/kg daily for 21 days while those that received artesunate were treated with 10 mg/kg of artesunate starting from day 15 of the study. The total length of the study was 21 days. At the end of the 21 days, animals were sacrificed following chloroform anaesthesia [13]. The rats were dissected and their livers were then removed after which they were fixed in 10 % formalin solution. Thereafter, Heamatoxylin and Eosin stained 6 microns sections were prepared as previously described [14]. Representative images of the sections as observed are herein presented.

Results

The liver from the healthy controls that were neither immunosuppressed nor infected with *P berghei* showed no pathology and had distinct hepatic cords and sinusoids. Animals that received immunosuppressive therapy and were simultaneously infected with *P berghei* showed different degrees of haemosiderosis and pathologic involvement ranging from focal necrosis to some congestion in sinusoidal spaces, while some perivascular cuffing was also observed in the group that received artesunate alone (Figures 1-5).

Discussion

Many xenobiotics, drugs and chemicals are able to result in diverse forms of liver injury [15], and this may result in distortion in liver histology. The histological picture of the liver in the diseased control animals and animals that received only lamivudine appeared largely similar, showing mild haemosiderosis and some focal areas of necrosis and congestion. That of the group that received both drugs also showed similar tissue architecture. Studies reported earlier have also shown artesunate treatment resulting in congestion of hepatic sinusoids [16] in healthy rats. However, in the current study, the liver of animals that received artesunate alone also showed haemosiderosis, perivascular cuffing and some fatty degeneration. Fatty degeneration is often seen with severe weight loss as was the case in this study with most of the groups. Artesunate has shown diuretic effect [17] and may account for the loss in weight with reduction in food intake as a result of the signs of malaria which includes anorexia. The majority of the altered hepatic architecture associated with the diseased animals in all of the groups derive from the pathophysiology of malaria infection, which affects several major organs in the body. The increase level of circulating iron due to haemolysis accounts for the associated haemosiderin. Previous work in healthy animals have shown no toxicity with low oral doses of artesunate in rats [18], while 15 mg/kg doses have been reported to result in focal necrosis in rats [19].

The nature of hepatic involvement in severe malaria has long been described with necrosis of the liver which is particularly a more pronounced problem in children or non immune individuals [20]. This is the case with the animals in this study which were not previously exposed to the parasite thus making them exhibit atypical response to malaria parasite as against what obtains in semi immune individuals. The histological observations from this study has not shown any significant histological effects that could be directly attributed to the combination of lamivudine and artesunate as most of the observed effects appear to be consequence of the disease condition. However, there is consistent data to support the histopathological changes that are a result of artesunate administration in other studies, which appear not to differ in the disease model used.

Conclusion

The outcome of lamivudine and artesunate co administration in the presence of immunosuppression and plasmodiasis does not appear to show clearly distinct differences in hepatic histology in comparison with the immunosuppressed and parasitized controls. However there remains need for caution and urgency of treatment of malaria in the presence of immunosuppression due to the possible consequences of recurrent malaria infection and its possible attendant damage on the liver. The histopathological consequences of artesunate which have been previously reported in healthy animals and confirmed in this disease model, may suggest the need for caution and monitoring of hepatic parameters that may be possible markers of histopathological consequences of the drug treatment.

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