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Research Article

SINUSOIDAL DILATATIONS AND SPLENOMEGALY AS FEATURE SPECIFICATIONS OF DRUG INDUCED THROMBOCYTOPENIA IN OXALIPLATIN TREATED WISTAR RATS.

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ABSTRACT

The clinical character of oxaliplatin induced thrombocytopenia is marked by severe and acute drop in platelet count associated with bleeding and bruising within a few hours up to 48 hrs after oxaliplatin administration. The nadir of platelet count can be as low value of $2x10^9$/L. The dose intensity of oxaliplatin is associated with an increase in spleen size and both of these aspects correlate with the degree of thrombocytopenia. This study is conducted on Albino Wistar rats treated with 0.8mg/kg of oxaliplatin, twice a week for two months. Thrombocytopenia is markedly assessed and platelet count is compared among the treatment and control groups. Abnormalities in liver tissues i.e. pyknosis, erythrocyte filled sinusoidal dilatation and fibrosis is observed in the treatment group with spleen enlargement. This study indicates that oxaliplatin induced thrombocytopenia in rats is associated with splenomegaly and sinusoidal dilatations indicative of splenic sequestration leading to a fall in platelet count.

Keywords: Sinusoidal dilatation, splenomegaly, oxaliplatin, rats, thrombocytopenia

INTRODUCTION

The quest of a novel Platinum complex is fulfilled by the development of oxaliplatin [trans-L-dach (1R, 2R-diaminocyclo hexane) oxalatoplatinum, L-OHP], which has a similar mode of action to the other platinum derivatives but the spectrum of antineoplastic activity varies in different tumors in comparison [1]. The cytotoxic action of oxaliplatin is by formation of DNA adducts that prevents the replication and transcription [2,3,4]. Oxaliplatin has antitumor activity in not only platinum sensitive tumors such as ovarian or lung cancer, but also has substantial therapeutic efficacy against colorectal and breast carcinoma, in which cisplatin is not an established treatment preference [5]. Thrombocytopenia induced by oxaliplatin is mainly due to bone marrow suppression in a similar manner like the rest of the compounds in the platinum family, however some unique aspects of oxaliplatin induced thrombocytopenia such as myelosuppression, immune mediation and splenic sequestration are documented with due considerations of specific treatment strategy [6,7]. Oxaliplatin induced prolonged thrombocytopenia is associated with enlargement of the spleen which is reported in some cases [7].The use of oxaliplatin prior to hepatectomy in liver metastases patients is known to be associated with sinusoidal injury, portal hypertension and thrombocytopenia [8].Varying degrees of hepatic sinusoidal injury is reported in 78% of patients being treated with oxaliplatin, whereas other chemotherapeutic agents such as 5FU is poorly associated with sinusoidal toxicity. The chemotherapy induced veno occlusive lesion and perisinusoidal fibrosis leads to portal hypertension in the normal liver as well ascitis, splenomegaly, esophageal and hemorrhoidal bleeding associated with thrombocytopenia [9].This study is designed to comparatively assess the intensity of drug induced thrombocytopenia in oxaliplatin treated rats as induced toxic manifestations. The gross and histological pathology (biopsy) of the spleen and liver respectively is reported to correlate the pathogenesis with the biochemical assessment of thrombocytopenia in the experimental model.

MATERIAL AND METHOD

Animal protocols

The experimental design was granted ethical and institutional approval by Dow University of Health Sciences (DUHS) and the study was conducted in the Rat Animal House of DUHS. Virgin male albino Wistar rats of the species *Rattus Norvegicus* (a stock of inbreeding rats), weighing between 230-280 g were used. The animals were adapted to the environment (temperature 23$\pm^{o}$C, relative humidity 65-75%, light: dark cycle 10:14) and assigned a rest period of seven days with standard feed (protein content 40%) and water *ad libitum*.

The animals were housed in standard polypropylene cages with appropriate bedding in two groups (A, B) of six animals each. Group A was treated with 0.9% NS (served as control group 0.5 ml/rat).The Group B was treated with oxaliplatin (0.8 mg/kg), intra peritoneal bolus injection -relative volume 500 µl/rat of 250 g, twice a week for 8 weeks. After completion of the dosing the rats were assigned a rest period of seven days. On the eight day (d 8), animals from each group were euthanized by ether and blood was sampled by cardiac puncture, collected in EDTA. K3 tubes and labeled for biochemical assessment of platelet count. The spleen and liver was excised and processed for morphological assessment. All the procedures involving animals were in strict adherence to the ethical considerations and standard protocols.

Hematological parameter

Blood (2 ml) was collected in EDTA. K3 tubes for blood hematological examination, platelet count (PLT) [10] on automatic Humacount plus (3 part differential with histogram. Hematology analyzer. Model # 16400/S). (Human Germany).

Gross morphology

The spleen is blotted on filter paper and weighed. The gross morphology of the spleen was assessed for color, remarkable surface and size. The weight of the spleen was comparatively assessed between the two groups.

Light microscopy

The liver samples were fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in molten paraplast wax blocks at 57-61^{o}C, 4-5 micron thick section cut were stained by H&E (Hematoxylin & Eosin) and Periodic Acid Schiff (PAS).The prepared slides were assessed for structural evaluation under a bright field light microscope by a trained pathologist unaware of the treatments (blinded assessment).

RESULTS

The spleen weights of the rats (mean value) in the control and treatment group are shown in Table 1.The mean spleen weight of oxaliplatin treated group of rat is more than the mean spleen weight of the rats in the control group. The difference between the spleen weights of the two groups is highly significant (p=0.015). The gross

pathology of the rat spleen is shown in Figure 1A .The spleen is reddish brown with unremarkable surface. The spleen of the oxaliplatin treated rat is enlarged shown in Figure 1B, which is indicative of drug induced splenomegaly. The surface of the spleen is unremarkable with a higher tint of reddish brown. The section of the rat liver tissue in the control group is shown in Figure 2 A, which shows normal morphology with no structural loss. The section of rat liver in oxaliplatin treated group shown in Figure 2 B is marked by abnormal hepatocytes, faded cytoplasm, nucleus of variable size (pleomorphism) and pyknosis. Wide blood sinusoids are seen. Numerous sinusoids are filled with erythrocytes. Liver fibrosis can be suspected due to spots of focal collected granulomatous lesions. Table 2 shows the difference in platelet levels of the rats in the control and treatment group which is highly significant (p=0.001).The mean value of platelet levels in Oxaliplatin treatment group (287x10^9/l) is indicative of severe thrombocytopenia.

Table 1:Mean spleen weight in control and treatment group

Treatment Group	Number of rats	Mean spleen weight (gm)	Mean Difference	Significance (p value)
Control group (Normal Saline 0.9%)	6	0.4		
Treatment group (Oxaliplatin 0.8mg kg^{-1})	6	0.8	0.37+0.12	0.015

Table 2:Mean platelet count in control and treatment group

Treatment Group	Mean Platelet Count(x10^9/L)	Std. Deviation	Mean Difference	t	Significance (p value)
Control Group (Normal Saline0.9%)	669.1324	74.42			
Treatment Group (Oxaliplatin 0.8mg kg^{-1})	287.2015	87.34	381.9309	3.540	0.001

Figure 1A: Rat spleen control group (2.5x0.6 cm)

Figure 1B: Rat spleen treatment group (3.5x1 cm)

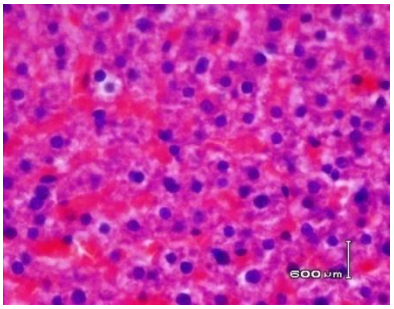

Figure 2 A :(H&E × 40) Liver rat (Control group 0.9%NS), No structural loss & no hemorrhage b/w sinusoids.

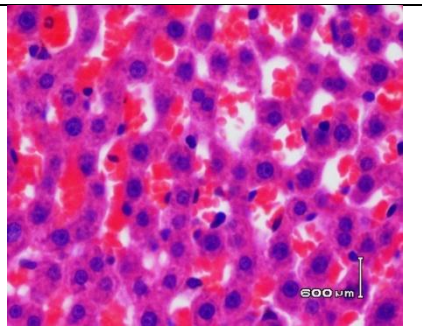

Figure 2 B: (H&E X40) Liver rat (Treatment group), Sinusoidal dilatation filled with erythrocytes, periportal inflammation with hepatocytes degeneration.

DISCUSSION

The oxaliplatin induced hepatic sinusoidal damage is by deposition of collagen in the perisinusoidal space ,fibrosis, veno occlusive lesions and disruption of the sinusoidal barrier [9,11,12].The chemotherapy induced veno occlusive lesion and perisinusoidal fibrosis leads to portal hypertension in the normal liver as well ascitis, splenomegaly, esophageal and hemorrhoidal bleeding associated with thrombocytopenia.[9] The investigations made in this study clearly indicate that oxaliplatin treatment has caused a severe fall in the platelet count. The spleen weight and size (splenomegaly) is markedly increased in these rats, which indicates that the mechanism of thrombocytopenia in these rats is by splenic sequestration. The histopathological assessment of the rat liver is indicative of periportal damage. Pathological features of the spleen and the liver tissues can be easily correlated to the fall in the platelet levels assessed comparatively in the control and treatment groups. Splenomegaly associated with thrombocytopenia due to the unique aspect of splenic sequestration in oxaliplatin therapy has been reported in many clinical studies. The interesting correlation exist between the dose intensity of oxaliplatin and increase in the spleen size, whereas, the degree of thrombocytopenia is significantly related to the increase in the spleen size. In some cases of Oxaliplatin induced thrombocytopenia, I.V immune globulin has lead to steady rise in platelet count [13]. Furthermore the increase in the spleen size is correlated with the grade of sinusoidal injury, and hence splenomegaly is the marker for oxaliplatin induced portal hypertension due to sinusoidal injury associated with thrombocytopenia due to splenic sequestration of platelets [11].

It is reported earlier that the splenic index (SI) can serve effectively as a surrogate marker for portal hypertension as splenomegaly is more frequently associated with oxaliplatin based chemotherapeutic

regimens as compared to 5FU/LV based chemotherapy [14]. The rate of spleen size increase in oxaliplatin treatment is reported to be 86% and the mean increase in SI is 47.15% [11, 14]. The incidence rate of thrombocytopenia in patients of splenomegaly is 28% as compared to the incidence rate of 5%in patients without increase in the size of the spleen.[7]. Moderate prolonged thrombocytopenia due to splenic sequestration is evident after a median time of 18 weeks following treatment with oxaliplatin [7].The platelet count is nearly $81x10^9$/l, although heamorrhagic complications in the patients is rare [15].Immune mediated acute thrombocytopenia can be fatal [16]. Splenomegaly, so frequently associated with thrombocytopenia should be assessed by imaging techniques, and when ruled out, other etiological factors for thrombocytopenia such as bone marrow suppression or drug induced immune thrombocytopenia (DIIT), should be taken into account. In the same context, it is reported that platelet recovery after cessation of therapy with oxaliplatin ensues but is rather slow if associated thrombocytopenia is associated with splenomegaly [11]. Use of thrombopoietic agents is limited due to the narrow therapeutic index however; treatment with recombinant thrombopoietins have shown enhanced platelet recovery in patients with platinum drugs induced thrompocytopenia [17].

CONCLUSION

Oxaliplatin induced thrombocytopenia in rats is due to splenic sequestration associated with sinusoidal dilatation and erythrocyte filling in the liver tissues. Since splenomegaly is clearly associated with thrombocytopenia, the splenic index (SI) can serve as a surrogate marker for Oxaliplatin induced thrombocytopenia.

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