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Research Article

Effect of cyclophosphamide on the microanatomy of liver of albino rats

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ABSTRACT

Background: Cyclophosphamide is widely used in the treatment of various neoplastic diseases and diseases associated with altered immunity. Higher doses used for longer duration effects many organs like urinary bladder, lungs, liver, heart and male reproductive organs.
Methods: To study the effect of cyclophosphamide on the micro anatomy of liver, sixty eight Albino rats were taken and divided into three groups, group A (control group) of 20 animals, were fed with routine diet, group B (low dose group) of 24 animals, were given cyclophosphamide at the dose of 0.5 mg/100 gms in addition to the routine diet and group C (high dose group) of 24 animals, were given high dose of cyclophosphamide at the dose of 0.7 mg/100 gms of weight of animal in addition to the routine diet. The animals were sacrificed at intervals of 3, 6, 9 and 12 weeks, 5 microns sections of the tissue were prepared and stained with Haematoxylin and Eosin stain.
Results: Microscopic changes in liver were apparent in the drug treated animals. In group B the changes appeared after 6 weeks while in group C they started appearing after 3 weeks of drug treatment. The changes were in the form of fatty changes, hemorrhages and central vein congestion.
Conclusion: Cyclophosphamide induces histological changes like fatty infiltration and central vein congestion in the liver. These changes are with low doses given for longer durations and manifest earlier when larger doses are used. Thus it is advised that patients receiving cyclophosphamide should be periodically evaluated for liver dysfunction.

Keywords: Cyclophosphamide, Fatty infiltration, Central vein congestion

INTRODUCTION

Cyclophosphamide an alkylating agent is widely used in the treatment of various neoplastic diseases and disorders associated with altered immunity. It was first synthesized in 1958 by Arnold and Bourseaux[1] and was approved for use in United States in 1959. It is given orally as well as parenterally. IV route is preferred for parenteral use while orally it is given in the form of tablets and solutions. Cyclophosphamide is well absorbed orally. The drug is activated by hepatic cytochrome P 450 system.[2] The active metabolite 4-hydroxycyclophosphamide is in steady state with the cyclic tautomer aldophosphamide.[2] The active Cyclophosphamide metabolite such as 4-hydroxycyclophosphamide and its tautomer are carried in circulation to tumor cells where aldophosphamide cleaves spontaneously, generating stochiometric amounts phospharamide mustard and acrolein. Phospharamide is responsible for anti tumor effects, while acrolein causes hemorrhagic cystitis. Cyclophosphamide acts by

modifying and cross linking purine bases in DNA, thus inhibiting DNA, RNA and protein synthesis and death of rapidly dividing cells.

For routine clinical use ample fluid intake is recommended and vigorous intravenous hydration is required during high dose treatment. Brisk haematuria in a patient receiving daily oral therapy should lead to immediate drug discontinuation; refractory bladder haemorrhage may require cystectomy for control of bleeding.

Cyclophosphamide has been found to cause many toxic effects[3] These include haemorrhagic cystitis, pulmonary fibrosis, irreversible azospermia in man, gastrointestinal bleeding etc.[2] Toxic effects also include micro vascular fatty changes in liver.[4] Secondary malignancies are seen with use of high doses cyclophosphamide. These include bladder malignancies and lymphoproliferative or myeloprolipherative malignancies.

Cyclophosphamide is routinely used in the treatment of various autoimmune and certain neoplastic diseases. It is known to cause various side effects. The target organs are lungs, heart, liver, urinary bladder and reproductive system. Since the drug is known to cause adverse effects on liver so the present study is aimed to study the histological effects of this drug on the liver of albino rats and for correlation of these findings to human beings.

METHODS

68 Albino rats weighing on an average 100 grams were taken from animal house department of our college. Approval was sought from institutional animal ethics committee. The animals were housed under uniform husbandry conditions and were divided in 3 groups: group A (control group) of 20 rats were fed with routine diet and water, group b (low dose group) of 24 albino rats were given cyclophosphamide at the dose of 0.5 mg/100gms of weight of rat besides the routine diet. Group c (high dose group) of 24 rats was given high dose of cyclophosphamide the dose of 0.7mg/100gms weight of rat besides the routine normal diet. The drug was given by mixing it with pellets of flour. The animals were kept in different cages labeled as (A), (B) and (C).

Dose of the drug: since the daily oral therapeutic dose of cyclophosphamide for human beings is 5mg/kg body weight so from this dose the dose for albino rats was calculated as 0.5mg/100gms weight of albino rat as low dose group and 0.7mg/100 grams weight of albino rat as high dose. The process of administration was continued up to 12 weeks regularly.

To study the effects of the drug, the animals were sacrificed in intervals of 3, 6, 9 and 12 weeks. In each sitting 5 rats from group A and 6 rats each from group B and C were taken. The animals were anaesthetized with chloroform, a mid line abdominal incision was given, liver was identified and excised. The organ was put in between blocking papers. Standard histological techniques were used for processing the tissues, 5 to 7 micrometer thick sections of the tissues were made, stained with haematoxylin and eosin, observed under compound light microscope and observations were recorded.

RESULTS

Control group: microscopic examination of the sections of this group showed normal histological structure like hepatic lobes containing cords of hepatocytes with sinusoids between these cords. The central vein was also normal and the portal triads also appeared normal.

Cyclophosphamide treated group: histological changes started appearing after 6 week in the low dose group and after 3 weeks in high dose group. In the low dose group at 6 weeks slight fatty changes were seen in some areas, the portal triads and central vein was normal. In the low dose group at 9 weeks the fatty changes were more pronounced, central vein was congested and the loss of architecture of hepatocyte. The changes were more pronounced in the tissues if animals treated with high dose of cyclophosphamide. There was diffuse fatty infiltration, central vein congestion, loss of hepatocyte architecture, at 12 weeks in Group C rats besides there was damage to endothelium of sinusoids with sinusoidal congestion.

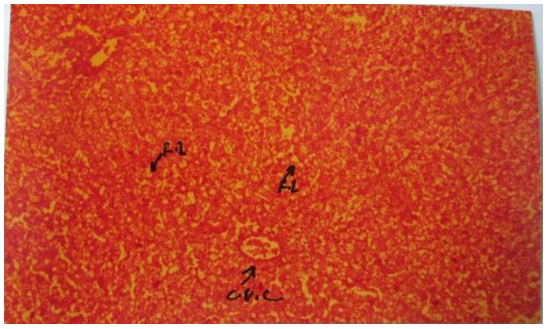

Figure 1: Shows Fatty Infiltration and Central Venous Congestion in Group B at 9 Weeks.

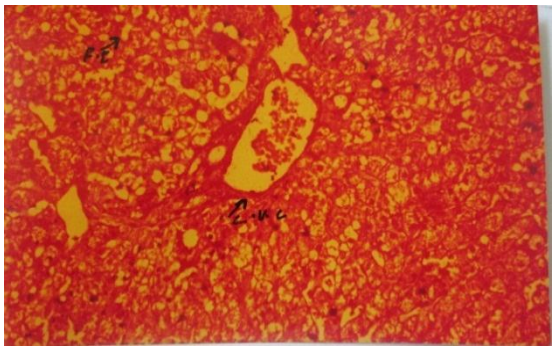

Figure 2: Shows Pronounced Changes of Fatty Infiltration and Central Venous Congestion with Disruption of Hepatic Architecture in Group B at 12 Weeks.

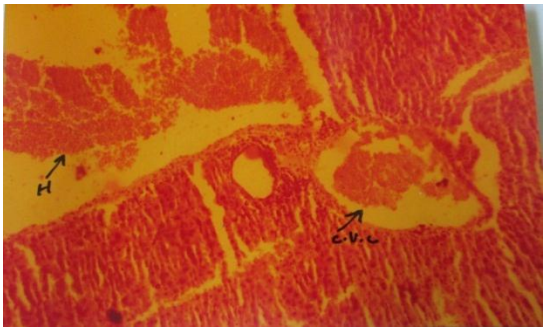

Figure 3: Shows Areas of Hemorrhage and Central Venous Congestion in Group C at 6 Weeks.

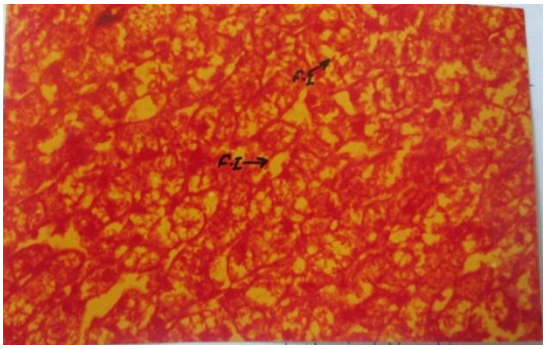

Figure 4: Shows Massive Fatty Infiltration and Loss of Hepatic Architecture in Group C at 9 Weeks.

DISCUSSION

Cyclophosphamide is routinely used for treatment of various neoplastic diseases and diseases associated with altered immunity. The drug is metabolized in liver by hepatic cytochrome P450 system. Not much work has been done on the effects of cyclophosphamide on liver of animals; however LD Delve (1996)[5] worked on cellular target of cyclophosphamide in murine liver. He observed that cyclophosphamide was involved in hepatic venooclusive disease. The target site was sinusoidal endothelial cells. In co-culture, sinusoidal endothelial cells are significantly more susceptible to cyclophosphamide toxicity and this is likely caused by acrolein generated by hepatocyte. As seen with other toxins implicated in venooclusive disease the profound depletion of sinusoidal endothelial cells precedes the onset of toxic effects. Akay H etal (2006)[6] observed acute liver injury with jaundice during cyclophosphamide therapy. Auberry DA. (1970)[7] observed that a 61 year old woman with breast cancer developed jaundice after starting cyclophosphamide (50mg) daily, with progressive hepatic failure and death. On autopsy massive necrosis of hepatocytes was observed. In our study the observations like damage to sinusoidal endothelium, with sinusoidal congestion correlate with the observations made by the earlier workers.

D-Bissell etal (2001)[4] while studying the effect of cyclophosphamide in exporters who remove toxic cyclophosphamide, observed micro vascular fatty changes in liver of the exporters. In the present study the observations in the form of fatty changes in liver of the rats correlate with the observations of the earlier workers.

George B etal,[8] while studying the Cyclophosphamide on liver, following haemopoietic stem cell transplantation observed that patients developed sinusoidal obstruction syndrome. Similar changes were observed in the liver of the animals in the present study.

The most common hepatic abnormality associated with cyclophosphamide use is sinusoidal obstruction syndrome. The hepatotoxicity is mainly due to metabolites of cyclophosphamide. The mechanism of injury is probably due to toxic effect of cyclophosphamide on the sinusoidal endothelium of liver causing their necrosis, obstruction and obliteration of hepatic veins. Mild and transient elevation in serum aminotransferase levels are found in up to 43% of patients who are treated with cyclophosphamide. Clinically apparent liver injury from standard doses of cyclophosphamide is not much common nevertheless there are reports of acute liver injury with jaundice in patients treated with cyclophosphamide. The onset is within 2 to 8 weeks of starting cyclophosphamide and the pattern of serum enzyme elevation is hepatocellular. The injury in most cases is self limited and resolves within 1 to 3 months of stopping; however, fatal instances have been reported.

In some clinical trials it has been observed that liver toxicity caused by high dose myeloablative therapy leads to significant morbidity after hematopoietic cell transplantation. Cyclophosphamide was infused at 60mg/kg over 1to 2 hours on each of two consecutive days, followed by total body irradiation. Plasma was analyzed for cyclophosphamide. Of 147 patients, 23 (16%) developed moderate or severe sinusoidal obstruction syndrome.[9] It is observed that metabolism of cyclophosphamide is highly variable particularly for metabolite O-Carboxyl ethyl-phospharamide mustard. Exposure to this metabolite is statistically significantly related to sinusoidal obstruction syndrome, bilirubin elevation, non relapse mortality, and survival, after adjusting for age and irradiation. Acute liver failure and death can occur when high doses of Cyclophosphamide are used as chemotherapy in cancer or as myeloablation therapy in combination with total body irradiation or busulphan, in preparation for haemopoietic cell transplantation. The onset of injury is usually within 10 to 20 days of myeloablation, and characterized by a sudden onset of abdominal pain, weight gain, ascites increased levels of aminotransferase, subsequent jaundice and liver dysfunction. The severity of injury varies from self limiting liver injury to acute hepatic failure. The clinical features like weight gain, hepatic enlargement and tenderness, ascites and jaundice help the diagnosis of the toxicity. Liver biopsy can confirm the diagnosis but it is contraindicated because of severe thrombocytopenia after haemopoietic cell transplantation.

CONCLUSION

From the present study, it is concluded that cyclophosphamide given to albino rats at varying doses over variable period causes fatty infiltration of liver, hepatic sinusoidal endothelial damage and central vein congestion. Thus it is concluded that before prescribing cyclophosphamide, patients be screened for liver diseases and further it is advised that patients receiving cyclophosphamide be routinely monitored for liver toxicity.

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Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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