Hepatic Changes Induced by Leflunomide in Swiss Albino Mice Fetuses

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ABSTRACT

Introduction: Rheumatoid arthritis is an autoimmune disorder commonly affecting the females and pregnancy with this is a challenge for gynaecologists. Drugs used for treating rheumatoid arthritis during pregnancy should have its detailed teratological influences on the developing foetuses. The present study was carried out with the aim to know its teratogenicity specially on hepatic cells of mice foetuses.

Material and Methods: The present teratological study was conducted in the Teratology laboratory of the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Results: Examined multiple slides of treated group fetuses and observed various histological changes. The consistent findings were dilated central vein, hepatocytes showing enlargement, cell division and nuclear degeneration kupffer cells were also increased in number.

Conclusion: when given as 50mg/kg in single dose, It is producing microscopic changes in liver and at 15mg/kg as a continuous dosing it is embryolethal producing resorptions. Changes in liver architecture is more pronounced in early phases of gestation.

Keywords: Leflunomide, Teratology, Hepatocytes, Kupffer Cells

INTRODUCTION

Leflunomide (Arava) is an immunomodulating and disease-modifying antirheumatic drug with anti-inflammatory and immunosuppressive activity¹. It is used for the management of signs and symptoms of rheumatoid arthritis to improve physical function and to retard structural damage associated with the disease in adults with moderate to severe active rheumatoid arthritis. Pregnancy outcomes from women reporting to a North American network of teratology centres pregnancy counselling service, the number of major malformations in the Leflunomide group was not significantly greater than that in either of control group and was similar to that expected in the general population 3-4%. Because the drug has been successful in the treatment of rheumatoid arthritis, it is being widely prescribed.² Leflunomide is the only orally administered drug for rheumatoid arthritis. The aim of our study is to elaborate its teratogenic effects on Liver and To know about its teratogenic effects in early and late phases of gestation.

MATERIAL AND METHODS

Study was done in The Teratology Lab of Department of Anatomy, IMS, BHU during July 2009 to June 2011. Ethical Clearance was obtained from the Central Ethical committee of BHU, Varanasi.

48 female albino mice of an average weight of 25-30g and an average age of 80-100 days were used in our study. They were housed in separate plastic cages in animal house on a light dark 12:12 cycle of 12:12 Fed on diet pellets and water ad libitum. Female mice were transferred in the evening to the cages containing male mice of the same stock in the ratio of 3:1.

The presence of vaginal plug on the following morning indicated pregnancy and was considered as day 0 of gestation. The pregnant mice were weighed and kept individually in separate cages. The pregnant mice were divided into groups as follows

Control (Group A) mice were given equal volumes of distilled water

Single dose treated group (B-G) given leflunomide 50mg/kg body weight on particular gestation day

Continuous dose treated (group H) – given leflunomide 15mg/kg body from 6th -11th day of gestation.

Pregnant mice were sacrificed with overdose of ether anaesthesia on day 19 of pregnancy. Uterine horns were exteriorized after opening abdomen by midline incision, the sacs were inspected for sites of resorption and viable fetuses and then Fetuses were removed and examined for gross abnormalities and dissected to remove the livers, which was fixed in 10% formaline for 48 hours, processed and slides were prepared to see microscopic changes. The slides thus prepared were stained with haematoxylin and eosin.

STATISTICAL ANALYSIS

SPSS version 21 was used for the statistical analysis. Descriptive statistics like mean and percentages were used for the analysis.

RESULTS

Resorption rate was maximum in continuously treated (GD6-11) group H (100%) (Table-1). Apart from gross reduction in the size of treated fetus liver there were no gross morphological changes. Among 154 single dose treated fetuses we removed the liver of all fetuses and fixed in formalin and made slides to observe any histological changes examined and compared it with control group liver histology (Figure 1 & 2).

The consistent findings were dilated central vein, hepatocytes showing enlargement, cell division and nuclear degeneration kupffer cells were also increased in number. The degeneration of hepatocytes was hallmarkd by nuclear condensation and nuclear fragmentation (Figure-2).

Leflunomide when given as 50mg/kg in single dosing schedule produces microscopic changes in liver and at 15mg/kg as a continuous dosing it is embryolethal producing resorption of gestation sacs, Changes in liver architecture is more pronounced in early phases of gestation.

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The disease may occur via inhibition of dihydroorotate dehydrogenase (DHODH) and tyrosine kinases. Leflunomide has significant toxic effects on fetal liver cells. The above mentioned findings on hepatocytes suggests that Leflunomide has significant toxic effects on fetal liver cells. Effects are more pronounced in early phases of gestation. The use of this drug should be avoided in pregnancy.

**DISCUSSION**

Pregnancy is a physiological condition in which majority of immunological conditions have varied presentations and it is very challenging to treat because limited therapeutic options are available.

RA is less common in women of child-bearing age than in older women (0.1–0.2% vs 2–5%), but its occurrence in pregnancy is increasing as women delay child-bearing. The disease may be severe enough to use the drug even though the safety data is unavailable, so prescribing physician should know about its effects and in particular effects according to gestation days.

Mossalam H. H. Et al (2013), in their study on newborn albino rats showed similar histological picture having Pyknotic and Karyolysed Liver cells on H and E staining. In our study we also found hepatocyte with nuclear degeneration. Similar findings on human patients were reported by Sevilla-Mantilla et al.

In the present study, all the fetuses were resorbed when the group; Figure-2: (H and E stain under 400X magnification) (Yellow) Showing increased number of Kupffer cells (White )Showing dilated Central Vein (orange arrow) Showing increased mitotic figures, (Black) arrow showing enlarged hepatocytes.

**REFERENCES**


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