Hepatic Changes Induced by Leflunomide in Swiss Albino Mice Fetuses

Krishna Pandey[1], Badal Singh[1], C Mohanty[2], SN Shamal[2], Nishtha Singh[1]

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 activity[1]. 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 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 6^{th} -11^{th} 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 hallmarked 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

[1]Assistant Professor, Department of Anatomy, MLN, Medical College, Allahabad), [2]Professor, Department of Anatomy, IMS, BHU, Varanasi, India

Corresponding author: Badal Singh, Department of Anatomy, MLN, Medical College Allahabad, India

How to cite this article: Krishna Pandey, Badal Singh, C Mohanty, SN Shamal, Nishtha Singh. Hepatic changes induced by leflunomide in swiss albino mice fetuses. International Journal of Contemporary Medical Research 2017;4(6):1309-1310.

Groups	Number of fetuses Showing changes	Percentage of fetuses with degenerative changes in liver
Control group A	00/40	0
Treated group B	20/24	83.33%
Treated group C	15/30	50%
Treated group C	12/32	37.5%
Treated group D	10/35	28.57
Treated group-E	08/40	20%
Treated group F	08/40	20%

Table-1: Comparison in the degenerative changes in the liver of the fetuses among treated group

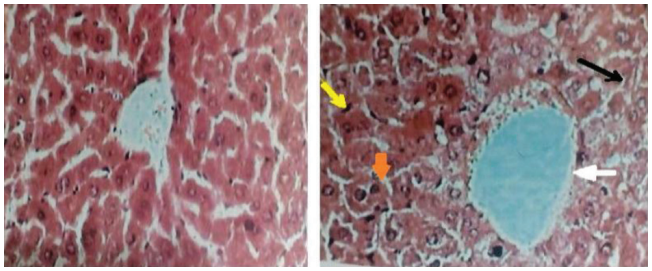

Figure-1: Photomicrograph of Hepatocytes of control and treated groups sacrificed on 19th day of gestation showing Changes in treated group; **Figure-2:** (H and E stain under 400X magnification) (Yellow↑) Showing increased number of Kupffer cells (White ↑)Showing dilated Central Vein (orange arrow) Showing increased mitotic figures, (Black) arrow showing enlarged hepatocytes.

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.[3] 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),[4] 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[5].

In the present study, all the fetuses were resorbed when the pregnant mice were exposed to Leflunomide continuously from GD 6 to11. Fukushima et al(2007)[6], observed that at 70mg/kg dose all embryos were resorbed and at 30mg/kg dose Leflunomide reduced fetal viability and increased the incidence of multiple external malformations.

Leflunomide toxicity may be attributed to the active open ring metabolite, the malononitrilamide, A77 1726 [(2- cyano-3-hydroxy-N-(4-trifluoromethylphenyl) butenamide)] as a result of conversion of the drug in the gastrointestinal tract and plasma and inhibit de novo pyrimidine synthesis[7].

Leflunomide is an immunosuppressant drug displaying teratogenicity in mice, rats, and rabbits. Its immunosuppressive effect occurs via inhibition of dihydroorotate dehydrogenase (DHODH) and tyrosine kinases[8].

It is evident that rats and rabbits are more sensitive to leflunomide inhibition of pyrimidine synthesis[9].

Leflunomide increases the risk of silent liver fibrosis in patients with rheumatoid arthritis receiving methotrexate.[10]

The active metabolite has a long half-life (1-4 weeks), which may contribute to adverse effects that persist, worsen or even appear after leflunomide has been stopped[11].

CONCLUSION

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

REFERENCES

1. Fox R I, Herrmann ML, Frangou CG, Wahl GM, Morris R E, Strand V, and Kirschbaum B J, Short Analytical Review Mechanism of Action for Leflunomide in Rheumatoid Arthritis, 93:198–208.
2. Chambers CD, Johnson DL, Robinson LK, Braddock SR, Xu R, Lopez-Jimenez J, et al. Birth outcomes sin women who have taken leflunomide during pregnancy. Arthritis Rheum. 2010;62:1494–503.
3. Symmons D, Turner G, Webb R et al. The prevalence of rheumatoid arthritis in the United Kingdom: new estimates for a new century. Rheumatology. 2002;41:793-800.
4. Mossalam H H. Hepatotoxic potential of leflunomide drug in adult male albino rats. AAMJ. 2013;11:284–309.
5. Sevilla-Mantilla, C., Ortega, L., Agúndez, J. A. G., Fernández-Gutiérrez, B., Ladero, J. M., and Díaz-Rubio, M. Leflunomide-induced acute hepatitis. Digestive and Liver Disease. 2004;36:82–84.
6. Fukushima R, Kanamori S, Hirashiba M, Hishikawa A, Muranaka R Kaneto M, Nakamura K and Kato I. Teratogenicity study of the dihydroorotatedehydrogenase inhibitor and protein tyrosine kinase inhibitor Leflunomide in mice. Reprod. Toxicol. 2007;24:310–316.
7. Breedveld F, Dayer J. Leflunomide: mode of action in the treatment of rheumatoid arthritis. Ann. Rheum. Dis. 2000; 59:841-849.
8. Temprano K, Bandlamudi R, Moore T. Antirheumatic drugs in pregnancy and lactation. Semin. Arthritis Rheu. 2005;35:112-121.
9. Brent, R. L. Teratogen update: Rrproductive risks of leflunomide (Arava); A pyrimidine synthesis inhibitor: Counseling women taking leflunomide before or during pregnancy and men taking leflunomide who are contemplating fathering a child. Teratology. 2001;63:106-112.
10. Lee et al.: Leflunomide increases the risk of silent liver fibrosis in patients with rheumatoid arthritis receiving methotrexate. Arthritis Research and Therapy. 2012;14:R232.
11. Aventis Pharmaceuticals. Arava tablets data sheet. 21 September 2003. www.medsafe.govt.nz/profs/Datasheet/a/aravatab.htm

Source of Support: Nil; **Conflict of Interest:** None

Submitted: 16-05-2017; **Accepted:** 27-06-2017; **Published:** 06-07-2017