EFFECTS OF DEXAMETHASONE ON LIVER HISTOLOGY OF ADULT ALBINO MICE

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ABSTRACT

Objective: In this study the effects of dexamethasone on liver histology in animal model were evaluated.

Design: This study comprised of 120 albino adult mice which were divided in four groups; one control and three experimental groups (on ascending doses of dexamethasone) administered for twelve weeks. The mice were sacrificed at 4, 8 and 12 weeks and liver histology was examined. The liver was serially sectioned and stained with Haematoxylin and Eosin stains. A comparison of any dose-related changes of dexamethasone on the liver was made with the control group. One way ANOVA was applied to compare weight of liver, size of hepatocyte and nuclear changes in all groups.

Results: Histological analysis showed that dexamethasone induced increase in weight of liver, an increase in hepatocyte size in relation to the dose given. Nuclear changes showed pyknosis with heightened staining. Cellular infiltration and portal vein congestion.

Conclusion: These findings suggest thatdexamethasone has deleterious effects on liver histology.

Key Words: Dexamethasone, Liver histology.

INTRODUCTION

The liver is the largest gland of the body. It is the principal metabolic organ and detoxification center for drugs and toxins[1]. The mammalian liver is invested by peritoneum except at the bare area. Beneath the peritoneum it is invested by a fibrous capsule called the Glisson's capsule. The capsule sends in septa at the porta hepatic that divide the liver into lobules. Three types of hepatic lobules are there. 1) The classical hepatic lobule. 2) The hepatic portal lobule. 3) The hepatic acinar lobule[2].

Dexamethasone is the synthetic analogue of betamethasone. It is identical in chemical structure to betamethasone except for configuration of the methyl group at C16[3]. Most of the known effects of the glucocorticoids are mediated by widely distributed glucocorticoid receptors. These proteins are members of the superfamily of nuclear receptors that includes steroid, sterol (vitamin D), thyroid, retinoic acid, and many other receptors with unknown or nonexistent ligands (orphan receptors), All these receptors interact with the promoter region and regulate the transcription of target genes[4]. They have broad actions on the regulation of growth factors, proinflammatory cytokines and to a great extent mediate the anti-growth, anti-inflammatory, and immunosuppressive effects of glucocorticoids[5].

Kanazuin 2010 observed in a study that dexamethasone treated female rats had reduced cytochrome p450 activity in liver microsomes and metabolism of these rats decreased[6]. Salje in 2012 observed in a study on adult Wister pregnant rats that dexamethasone induced and promoted Abcb1 in fetal brain and liver indicating that dexamethasone when used clinically effects liver metabolism at the genetic level[7].Steissin 1989 in a study observed and concluded that pregnant rats when given dexamethasone in a dose of 1mg/kg subcutaneously produced deleterious effects on skeletal muscle weight, types of fibers and DNA of muscle. For this purpose soleus muscle was used. The prenatally exposed rats also showed overall decrease in weight as compared to control[8].

To diminish or regulate the side effects of synthetic glucocorticoids, pulse therapy as high as 1.5gm per day of methylprednisolone for 3 days is also given. However the benefits of pulse therapy remain undefined[9].

MATERIALS & METHODS

120 sexually mature adult Wister albino mice of either sex, weighing approximately 20-30 gm were obtained from the animal house of our institute (PGMI. The mice were housed at the animal house of Post Graduate Medical Institute, Lahore under optimum conditions of temperature 24±2° C, humidity 50 ± 10 %, and in 12

hours light and 12 hours dark cycles. Each animal was fed on daily maintenance diet of 20gm of chick feed no. 13, water was given *adlibitum*.

Dexamethasone being used in this research was a product Merck Pharmaceutical Products®, Karachi, Pakistan. After initial acclimatization of 2 weeks, the mice were divided into 4 groups by using random number table.

Group I: (30) control mice were kept on food and water *adlibitum*.

Group II: (30) mice were given Dexamethasone 5mg/kg of body weight 24 hourly by intra muscular injection.

Group III: (30) mice were given Dexamethasone 10 mg/kg of body weight 24 hourly by intramuscular injection.

Group IV: (30) mice were given Dexamethasone 15mg/kg body weight by intramuscular injection.

Tissue Sampling

The mice were sacrificed at 4, 8 and 12 weeks and liver histology was examined. At the time of sacrifice the mice were anaesthetized by placing them in a glass container with cotton balls with chloroform and the lid of container was closed lightly for 2-3 minutes. Then animal was placed on dissecting board with extremities nailed to it. The abdomen and thorax were opened by a longitudinal incision given through anterior abdominal wall cutting both skin and muscles, extending from lower lip to public symphysis, then to expose abdominal contents the incision was extended laterally in form of letter "T" at the same time a nick was given in right atrium. This was carried on till almost clear fluid was coming out of atrium. Liver was identified and carefully dissected out. It was cut and placed in a single tissue cassette after labeling its identification. The tissue pieces were processed for 18 hours. The wax blocks containing samples were kept in refrigerator for approximately 15 minutes prior to sectioning. Blocks were mounted on rotary microtome ("Jung Histocut 820" Leica) and sagittal serial sections of thickness 3-5µm were obtained. Slides were stained using haematoxylin and eosin stains[10].

Histological examination and Micrometry

Slides were studied under light microscope (Olympus CH2) with 4X, 10X and 40Xmagnifications to see the histological architecture of the liver parenchyma and stroma changes in the cellular elements of hepatocyte size + shape and stromal elements (including hemorrhages, cellular infiltration and fibrosis) were studied in comparison with controls. Histomorphometric measurements were performed for each section by occulomicrometer.

RESULTS

Comparison of weights of mice.

The weights of different groups were taken at 4, 8 and 12 weeks. At each interval, ten animals of each group were sacrificed. At four weeks, the mean weight of control group was 20.3 ± 0.5. Group II showed mean weight of 21.8 ± 0.6. Group III had mean weight of 25.8 ± 1.0 and group IV had mean weight of 26.3 ± 1.4. (Table 1)

Comparison of weight of liver.

Average weight of liver showed increase in ea[ch successive group. Mean weight of liver of control group was 1.56 ± 0.09, group II 1.83± 0.11, g[roup III 1.96 ± 0.2 and group IV 2.06 ± 1.17. This shows that animals who were exposed to dexamethasone in higher doses showed more increase in weight of liver. (Table 2)

Comparison of size of hepatocytes (µm) of mouse liver.

Mean and S.D values of mouse liver in different groups were 11.5 ± 0.16, 12.3 ± 0.3, 3.3 ± 0.4 and 14.7 ± 0.2 with ranges of 11.3 – 12.0, 11.3 – 13.0, 12.5 – 15.0 and 14.0 – 15.3 respectively. This shows an increase in hepatocyte size in relation to the dose given. (Table 3)

Comparison of presence of nuclear changes, cellular infiltration and portal vein congestion.

Hepatocytes showed a change in shape with disrupted borders and cell vacuolization. Out of 10 samples in each group, 4 showed cell changes at 4 and 8 weeks and 8 samples showed change at 12 weeks in group II. Group III samples showed a change of 3 in 4 weeks, 7 in 8 weeks and 8 slides showed cell change at 12 weeks. Similarly group IV ad 5 slides out of ten with hepatocyte disruption, 7 samples at 8 weeks and 9 samples at 12 weeks. Nuclear changes showed pyknosis with heightened staining. Cellular infiltration and portal vein congestion increased in groups of III and IV with perivascular cuffing and portal vein congestion. (Table 4).

Table 1: Comparison of animal weight (gm) in different groups at 4, 8 and 12 weeks

Week of sacrifice	n	Group I	Group II	Group III	Group IV	P value
0	10	18.5±0.5	18.8±0.8	18.8±0.7	18.7±0.9	>0.05
4	10	19.1±05	19.8±0.6	20.1±1	22.2±1.4	<0.05
0	10	18,4±0.6	18.7±0.6	18.8±1	19±1.4	>0.05
8	10	21±0.6	22±0.53	22.8±1.8	23.5±1.1	<0.05
0	10	18.6±0.5	18.8±0.6	18.8±1	18.9±0.8	>0.05
12	10	22.4±0.6	22.6±0.8	23±0.8	24.4±0.8	<0.05

Table 2: Comparison of liver weight (gm) in different groups at 4, 8 and 12 weeks

Week	n	Group I	Group II	Group III	Group IV	P value
4	10	1.52±0.08	1065±0.1	1.70±0.23	1.72±0.05	<0.05
8	10	105±0.1	1.68±1.1	1.78±0.2	2±0.2	<0.05
12	10	1.53±0.07	1.72±0.13	1.90±0.18	2.50±0.8	<0.05

Table 3: Comparison of hepatocyte size (µm) in different groups at 4, 8 and 12 weeks

Week	n	Group I	Group II	Group III	Group IV	P value
4	10	11.5±0.16	12.3±0.15	13.3±0.2	14.7±0.2	<0.05
8	10	11.5±0.2	12.4 ±1.18	13.5 ±0.18	15±0.16	<0.05
12	10	11.5±0.3	12.6 ±0.17	13.8 ±0.16	15.8±0.25	<0.05

Table 4: Comparison of qualitative changes in different groups at 4, 8 and 12 weeks

Time	n	Group I	Group II	Group III	Group IV	Change
4 weeks Hepatocyte size (µm)	10	0	4 40%	3 30%	5 50%	Moderate
Nuclear shape		0	2 20%	2 20%	0	Minimal
Cellular Infiltration		0	2 20%	0	1 10%	Minimal
Portal Vein congestion		0	0	2 20%	2 20%	Minimal
8 weeks Hepatocyte size (µm)	10	0	4 40%	7 70%	7 70%	Maximum
Nuclear shape		0	2 20%	6 60%	7 70%	Maximum
Cellular Infiltration		0	1 10%	2 20%	3 30%	Minimal
Portal Vein congestion		0	0	2 20%	3 30%	Minimal
12 weeks Hepatocyte size (µm)	10	0	8 80%	8 80%	9 90%	Maximum
Nuclear shape		0	7 70%	7 70%	3 30%	Moderate reversed
Cellular Infiltration		0	5 50%	5 50%	6 60%	Moderate
Portal Vein congestion		0	0	4 40%	5 50%	Moderate

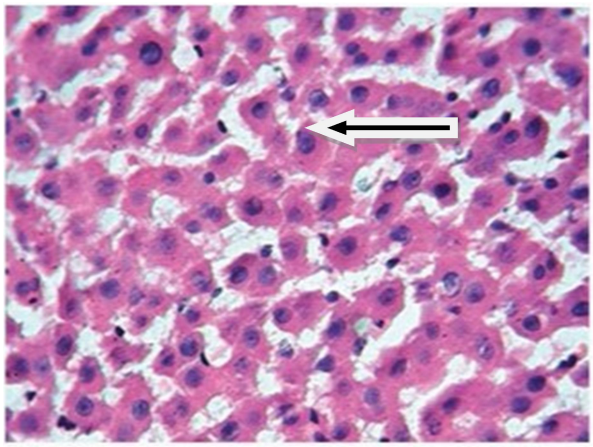

Figure 1: showing normal architecture of liver of control group. Arrow showing hepatocyte.

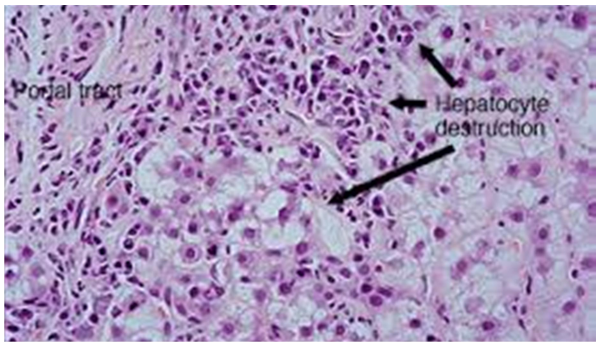

Figure 2: showing hepatocyte destruction and abnormal architecture of liver histology in experimental group C. (8 weeks)

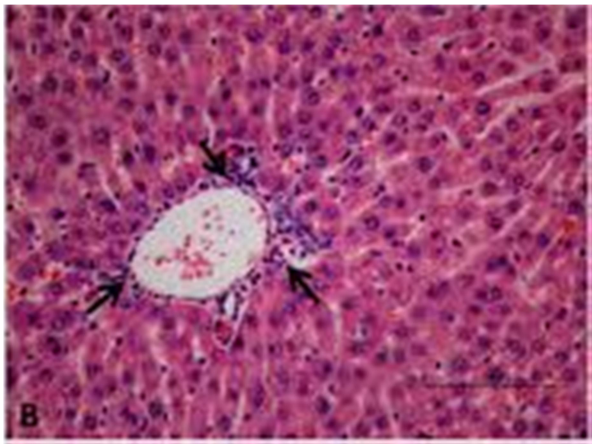

Figure 3: showing perivascular infiltrate of inflammatory cells around central vein in experimental group C.

DISCUSSION

In the present study 120 mice were divided into 4 groups. Group I was control and group II, III and IV were given incremental doses of dexamethasone by intramuscular injection. To assess the effect of high dose on liver histology of adult albino wistar mice, a time variable was added of 4, 8 and 12 weeks i.e. 10 animals of each group were sacrificed at these intervals. The liver histology was seen under light microscope.

In 1989 study it was seen by J.E Steiss et al., in USA which showed that pregnant rats when given dexamethasoneperinatal lost weight and also the muscle mass of soleus was reduced and type I muscle fibers were decreased. This means that dexamethasone can cause effects on different body tissues such as skeletal muscles and affect in pregnancy should be monitored carefully[8]. R. Ramachandran et al., published a research in the Journal of Clinical Pathology 2009 in USA that histological patterns of liver disease can bring about vacuolization in cells, hepatocyte derangement, pyknosis and perivascular cuffing. Nowadays light microscopy has been replaced by immunohistochemistry and electron microscope studies[11]. Barboro L. et al 1999 showed that dexa when given with albumin increased its uptake by (nonparenchymal cell) NPC of liver. These cells are active in combating acute and chronic inflammatory diseases and liver fibrosis¨. Dexa also has anti-inflammatory uses and when used wisely can help in recovery of liver tissue. On the other hand CYP 450 (cytochrome P450) was seen to be reduced in rats treated dexa and then given ERM (erythromycin). When erythromycin was given, the inhibitory effect on CYP 450 was reduced. This suggests that dexa effects CYP 450 and decreases its activity leading to slower metabolism and increase in half-life of drugs[6]. The basis of diseases is influenced by genes and recent studies are relating dexa with Abcb1 gene which regulates the distribution and absorption of xenobiotics. Salje K. et al. 2012 observed that dexa promotes Abcb1 in fetal brain and liver so it is safe to conclude that liver metabolism is affected by dexa at the genetic level[7].

Quantitative findings, such as that of animal weight, liver weight and hepatocyte size, showed no variation in control group I. Groups II, III and IV, showed significant increase in these parameters. Nuclear changes were noted in experimental groups but remained unchanged in control. The most remarkable change was pkynosis. Cellular infiltration and portal vein congestion were most prominent in group IV. Ramachandran et al. 2009, found that steroids produce drug induced liver injury (DILI) and produce

steatohepatitis with ballooning degeneration of hepatocytes, perivascular fibrosis and sinusoidal disruption. Although these conditions are reversible they may take years to recover. Repeated drug insults can lead to cirrhosis and necrosis[11].Ranta et al., 2006 syudied the effect of dexamethasone and found that it is hyperglycemic and causes cell death of insulin secreting cells[12].

Thébaudet al., 2001 studied infants with bronchopulmonary dysplasia (BPD). Treatment reduced lung complications but dexamethasone caused neuromotor , cardiovascular, gastrointestinal and pancreatic abnormalities.

CONCLUSION

These findings suggest that dexamethasone has deleterious effects on liver histology.

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