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Hepatic Tissues under the Effect of Dexamethasone: Histological Study, Dose and Duration Related Changes

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Abstract

Background Dexamethasone is a highly potent glucocorticoid. Treatment with dexamethasone results in several metabolic perturbations on nearly all organs of the body including the liver.

Objective This study had been carried out in order to investigate the effects of dexamethasone sodium phosphate as synthetic form of glucocorticoids on the rabbit liver as a model for human liver, by a light microscope, using two extreme of doses and two durations to show the dose and duration dependency.

Methods Liver specimens were obtained from rabbits treated with dexamethasone sodium phosphate and from control groups 1 and 2, the specimens were fixed and processed to evaluate the histological and histochemical changes**.**

Results Vacuolation and ballooning of hepatic cells were observed in the liver of the treated groups associated with degenerative changes of these cells, dilatation and congestion of central hepatic vein and sinusoidal capillaries were observed, positive periodic acid schiff's stain (PAS) reactions were noticed in the treated groups. All these changes were dose and duration related.

Conclusion Morphological changes induced in the liver by dexamethasone sodium phosphate could be accepted as side effects of these drugs.

Key words Liver, dexamethasone, histology, glycogen.

Introduction

The liver is a vital organ for processing nutrients absorbed from the gastrointestinal tract and for transforming them into materials needed by other tissues of the body [(1)]. It is under the influence of many hormonal actions such as insulin [(2)], glucagon [(3)] and the adrenal steroids [(4)]. The synthetic glucocorticoids are administered for a variety of disorders and illnesses, but their administration may be associated with development of multitude of complications involving almost all organ systems. The degree of complications depends on a number of factors, including length of treatment, time of day of administration, glucocorticoid preparation chosen, route of administration, dose administered and dosing intervals [(5)].

Dexamethasone is a potent glucocorticoid that is indicated for a wide range of diseases such as endocrine and non-endocrine diseases including rheumatoid arthritis, osteoarthritis and other connective tissue diseases and also indicated for inflammatory diseases such as respiratory disease, dermatological diseases etc. However, dexamethasone has wide spectrum of side effects on nearly all the body systems [(6)].

Serum corticosteroid-binding protein, transcortin, has been considered to be synthesized and secreted by liver cells [(7)]. Transcortin is involved in the selective transfer

of glucocorticoid across the plasma membrane and influences the intracellular entrance and transport to the nucleus of steroid bound to protein [8].

Glucocorticoids inhibit glucose and amino acid uptake in many instances and enhance lipolysis in adipose tissue [9]. In the liver, these steroids stimulate a number of enzymes and increase protein and glycogen content. There is an enhanced hepatic capacity for gluconeogenesis; which, with substrate from catabolism elsewhere, results in increased glucose production.

The integrated effects of glucocorticoids thus result in hyperglycemia, negative nitrogen balance and fat loss [9]. The general stimulatory glucocorticoid effect on the liver is in pattern of hypertrophy of hepatocytes, since the total protein content in the liver cell is increased [10].

The objective of this study was to explore the effects of dexamethasone sodium phosphate on the rabbit liver as a model for human liver, by a light microscope, using two extreme of doses and two durations to confirm the dose and duration dependency.

Methods

Healthy white New Zealand female rabbits weighing between 1000-1250 grams were kept in separate plastic cages, fed *ad-libitum* and used for scientific research from January to march 2012 in Al-Mustansiriyia College of Medicine Laboratories.

The animals were divided into six groups, seven animals in each. The first group was treated daily for 10 days with (0.5 mg/kg of body weight (b.w.) equal to 0.1 ml/kg b.w.) intramuscular injection of dexamethasone sodium phosphate as single injection every 24 hours (ZMC import-export GmbH Germany as 8 mg/2 ml ampoules) in the thigh muscle. The second group was treated with (1.5 mg/kg b.w. equal to 0.4 ml/kg b.w.) of the same reagent for 10 days. The third group was received (0.5 mg/kg b.w.) of dexamthasone for 15 days. The fourth one was treated with (1.5 mg/kg b.w.) of dexamethasone sodium phosphate for 15 days. The fifth group was considered as a control (1) animals, they received equal amounts of 0.9% saline solution as intramuscular injections for 10 days. The sixth group was received also 0.9% saline solution for 15 days and considered as the control (2) group [11].

Twenty- four hours after the last injection, the animals were anaesthetized with chloroform. After dissection of the abdomen, the liver were removed and they were fixed in 10% formaline solution for 24 hrs., dehydrated, cleared, and embedded in paraffin and the blocks obtained were sectioned and stained by:

1. Haematoxyllin and Eosin stain (H&E): routine slandered stains for general structure of liver [12].
2. Alcoholic periodic acid- Schiff's stain (PAS): for carbohydrates including glycogen, mucin, and most basement membranes [13]. Staining methods and techniques were done on the basis of Humason and Luna [12, 13].

Results

H&E sections show vacuolation and ballooning of hepatic cells, which started to appear more clearly in the second and fourth groups (as the dose and duration of treatment were increased) as seen in fig. 1.

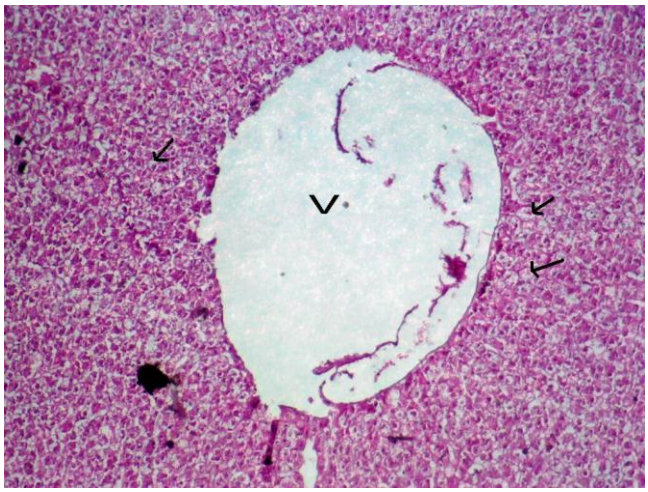

Fig. 1: Photomicrograph of liver cells of treated groups showing dilated and congested central vein (V), hepatic cells (arrows) H&E X100.

Degenerative changes of liver cells were noticed including; distortion of nuclei with distortion of

hepatic cell cytoplasm started to appear from the second group and further on till the fourth group where pyknotic nuclei were demonstrated (Fig. 2).

In some sections of first group, we noticed the appearance of hepatic cells with nuclei containing prominent nucleoli (Fig. 3). Dilatation and congestion of central hepatic vein were evident in all treated groups (Fig. 3). Sinusoidal dilatation and congestion also were demonstrated specially in the second and fourth groups (Fig. 3).

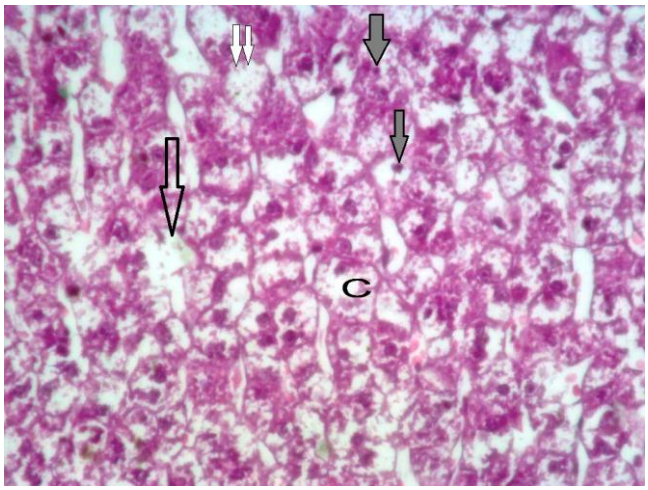

Fig. 2: Photomicrograph of treated liver cells (C) showing vacuolation (double white filled arrows), ballooning (single not filled arrow) and degenerative changes with pyknotic nuclei (gray filled single arrow) H&E X400.

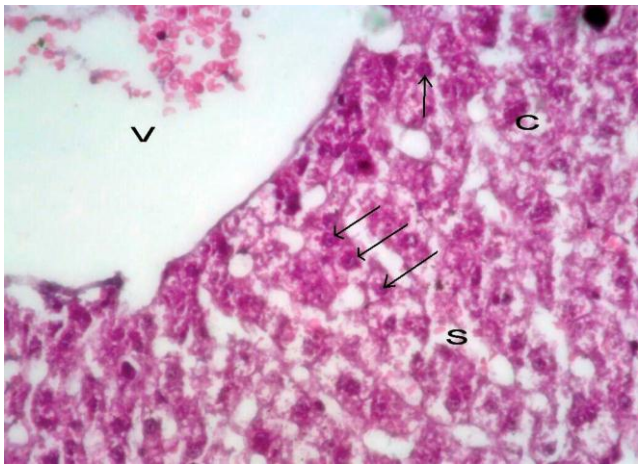

Fig. 3: Photomicrograph of treated liver cells (C) showing prominent nucleoli in some cells (arrows), dilated and congested sinusoids (S), dilated and congested central vein (V) H&E X400.

All the above changes were dose and duration related when compared with the control animals (Fig.4).

With PAS stain, the strength of PAS reaction depends on the pattern of distribution of the dye appeared in the tissue. We noticed that there were positive (+ve) PAS reactions in all of the treated groups, but with differences in the strength of positivity, with presence of dispersed staining that indicated a +ve reaction to PAS and gradually this staining became heterogenic and then appeared as clumped masses (Fig. 5, 6, 7 and 8) and summarized in table 1.

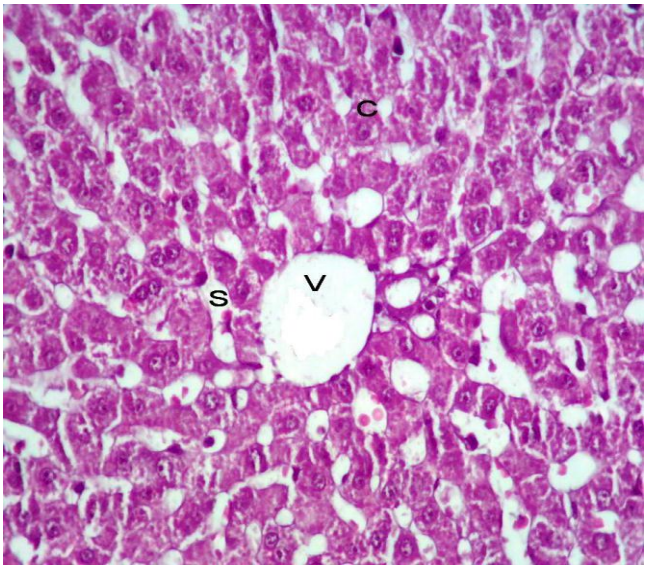

Fig. 4: Photomicrograph of control rabbit liver showing hepatic cells (C), central vein (V) and sinusoids (S) H&E X400.

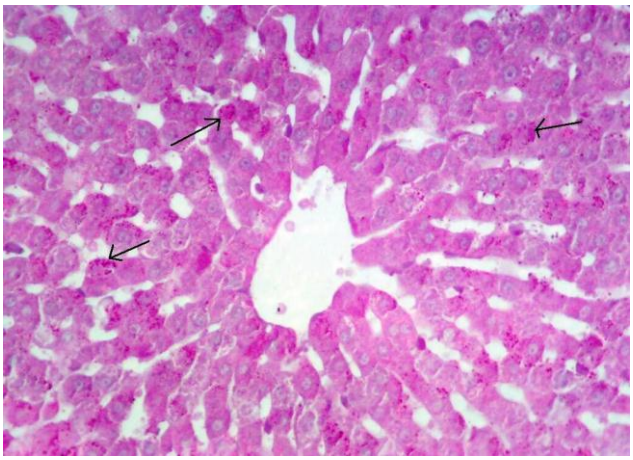

Fig.5: Photomicrograph of control rabbit liver with weak +ve PAS reaction (arrows) PAS X400.

Discussion

Drugs are an important cause of liver injury. Large number of drugs has been reported to cause liver injury. Drug-induced hepatic injury is the most common reason cited for withdrawal of approved drugs. Physicians must be vigilant in identifying drug-related liver injury because early detection can decrease the severity of hepatotoxicity if the drug is discontinued [6].

In the present study, the histological sections of treated rabbits with dexamethasone showed areas of ballooning and vacuolation of hepatocytes, which were directly proportional to the duration and dose of treatment.

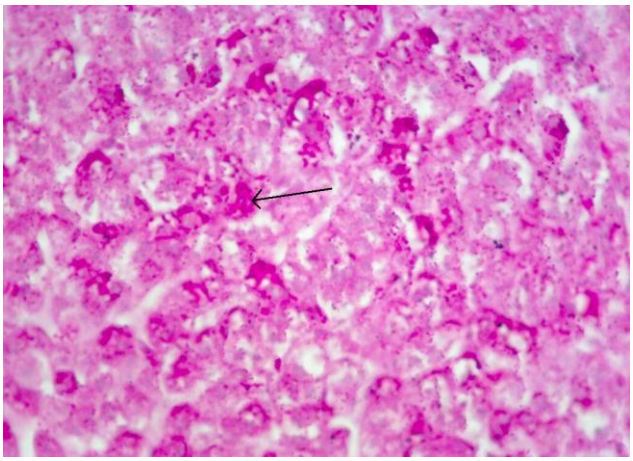

Fig. 6: Photomicrograph of treated rabbit liver cells with strong +ve PAS reaction (arrows) PAS X400.

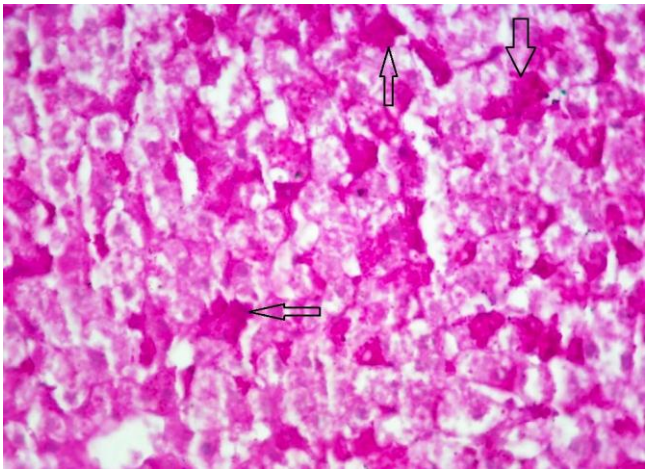

Fig. 7. Photomicrograph of treated rabbit liver cells with ++ve PAS reaction (arrows) PAS X400.

The histological sections stained with PAS showed that the vacuoles inside the hepatocytes contain glycogen inside their cytoplasm, there were positive (+ve) PAS reactions in all of the treated groups, but with differences in the strength of positivity, with presence of dispread staining that indicated a +ve reaction to PAS.

Gradually, this staining became heterogenic and then appeared as clumped masses which indicate strongest reaction of glycogen to PAS, that differ in the different doses and duration used in this study. Some researchers [14] stated that glycogen deposition was time and dose dependant and they indicated that repeated administration of dexamethasone increase liver weight and glycogen content and these changes were reduced by cessation of treatment.

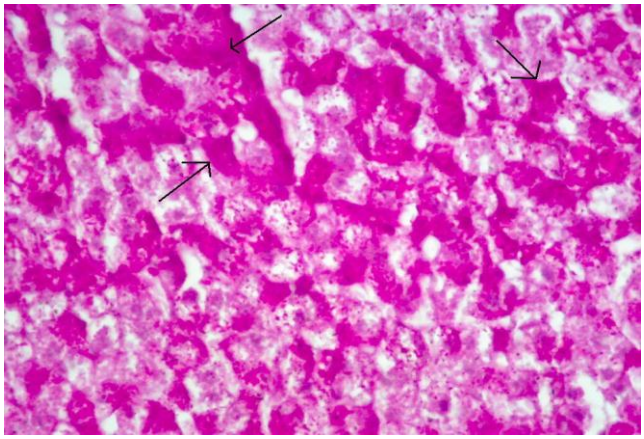

Fig. 8: Photomicrograph of treated rabbit liver cells with +++ve PAS reaction (arrows) PAS X400.

In the periphery (body organs outside the liver), glucocorticoids stimulate lipolysis and protein breakdown, releasing glycerol, fatty acids and amino acids, respectively, that act as substrates for gluconeogenesis[9].

Table 1: Periodic Acid Schiff's stain (PAS) reaction of liver cells in the study groups.

Study groups	PAS reaction
First group	Strong +ve
Second group	++ve
Third group	Strong +ve
Fourth group	+++ve
Fifth group (control (1))	Weak +ve
Sixth group (control (2))	Weak +ve

In the liver, glucocorticoids stimulate hepatic gluconeogenesis and increase the hepatic synthesis and storage of glycogen. Glucocorticoids also decrease glucose uptake in peripheral tissues, including adipose tissue, further contributing to increases in blood glucose. In response to elevated blood glucose, there is a compensatory increase in insulin secretion [6]. However, glucocorticoids inhibit the suppression of gluconeogenesis by insulin and cause insulin resistance in peripheral tissues, further contributing to hyperglycemia [9]. Treatment with dexamethasone causes time dependant changes in glucose and insulin levels, and increases the secretion of insulin, which makes the glycogen to be deposited, so that as the time increases, more glycogen deposited in the cytoplasm of hepatocytes [15]. Also dexamethasone causes up-regulation in insulin receptors in time dependant way due to stimulation of insulin receptors synthesis [16].

A number of biochemical processes in the liver, such as protein synthesis, glycogenesis, lipogenesis, certain mitochondrial functions, and the release of hydrolytic enzymes, are known to be affected by cortisone treatment. Many of these processes can be related to specific ultrastructural elements of the cytoplasm [17].

Some researchers [18] indicated that dexamethasone causes enhancement of smooth endoplasmic reticulum (SER), which is functionally associated with the increase in glycogen. The hepatocytes show increase in the amount of SER preceding glycogen deposition. In addition to that dexamethasone increases the activity of glycogen synthase which increases the glycogen accumulation [19] and inhibits activation of glycogen phosphorylase [20].

Degenerated hepatocytes ballooning is not due to glycogen deposition only, but also due to lipid accumulation. This is because glucocorticoids cause an increase in hepatic synthesis and secretion of VLDL [21]. Glucocorticoids cause time dependant accumulation in triglyceride within the cytoplasm of hepatocytes due to decrease in its secretion or due to increase synthesis and /or esterification of fatty acids [22].

There was evidence that glucocorticoids cause progressive increase in fragility of intracellular organelles, such as lysosomes with alteration in the plasma membrane properties [23] and decrease in the number with marked changes in the ultrastructure of mitochondria [17]. It is also known that dexamethasone reduces the number of mitochondria in treated hepatocytes and decreases the oxidative phosphorylation and their active respiration [24], this may lead to a disturbance in electrolyte balance through the "Sodium-Potassium pump". As this mechanism is energy dependent, the efflux of potassium ions may happen with the influx of sodium ions, increasing osmotic pressure in the cytoplasm beside the alteration in plasma membrane function; which attract water molecules. As a result, swelling of the cells occurs; also the leakage of hydrolytic enzymes may cause macromolecular crowding [25].

Dexamethasone, which is a synthetic form of glucocorticoid, inhibits the synthesis of arachidonic acid and prostaglandin which normally act as antiaggregant agents [26]. This, together with hypertension and polycythemia [27], caused the sinusoidal dilatation and congestion, which have been noticed in the treated groups of this study with the marked differences between them which indicate dose and duration dependency.

From our results, we concluded that the morphological changes induced in the liver by dexamethasone sodium phosphate were accepted to be side effects of these drugs.

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