

Evaluation The Effects of Betamethasone on A Leukocytes Count by Using the Hen's Egg Test – Chorioallantoic Membrane (HET- CAM) Test As A Novel In Vivo Model

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Abstract

The aim of this study was to examine the possibility effects of betamethasone on a differential white blood cell counts in the hen's Egg test – chorioallantoic membrane (HET- CAM) test as a novel in vivo model. As well as in this study was manifested this model had to all types of mature white blood cells of chick embryos in age 12-15 days of incubation period. Because of the short period of chick embryos were treated with betamethasone (only 120 minutes) did not showed significantly changes in leukocyte counts (Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils) in treated groups with different doses of betamethasone (3, 6, 12, 24, 48, 96, 192 µg / g of total egg weight, topically). We conclude in this study that, the (HET- CAM) test may be used as in vivo model to study the changes in deferential leukocyte counts, because of the blood smear have all types of leukocyte, and the work was appeared in this model have low cost accompanied with other laboratory animals.

Keywords: Betamethasone, leukocytes count, hens Egg test, chorioallantoic membrane.

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INTRODUCTION

Betamethasone is a glucocorticoids belonging to the synthetic corticosteroid group [1], which is clinically used in the field of medicine as an anti-inflammatory effect and in the case of allergic and skin infections [2-4]. Betamethasone has the inhibitory effects of the immune system because of changes in the blood smear, especially in relation to the reduction of lymphocytes, which plays a large role in the immune response resulting from long-term exposure to betamethasone to increase incidence of fungal and bacteriological diseases caused by the immune system [5, 4] from here it turns out the importance of studying the blood smear in animals treated with betamethasone to detect the inhibitory effects of the drug on the immune system and also because of the high costs spent on experimental animals of nutrition and breeding and care and provide the suitable environment for breeding when conducting research, so scientists looked for alternatives to these laboratory animals until they reached Biologically active tissue contains a dense network of blood vessels, transparent and Shine which is called the Hen's Egg Test –Chorioallantoic membrane test [6-13]. The objective of this study was possibility to study used of the Hen's Egg Test – Chorioallantoic membrane as an in-vivo model to study

the effect of betamethasone on the differential leukocyte count.

MATERIALS AND METHODS

In this study was used the Hen's Egg Test – Chorioallantoic membrane as an in-vivo model to study the effect of betamethasone (the General Company for drugs and Medical Supplies in Samarra, Iraq) on blood smear at doses (3, 6, 12, 24, 48, 96, 192 µg / g of total egg weight, topically).

Preparation of Hen's Egg Test –Chorioallantoic membrane to experiment

The rose chicken eggs 308 were obtained from hatched by egg hatching machine in the College of Veterinary Medicine, University of Duhok, Iraq. At five days from incubation. The incubation was followed in the incubator (Karl Kolb-Scientific Technical Supplies D.6072 Dreieich, Germany) until the embryo reaches the required age for the experiment, which is 12-15 days of incubation where the blood vessels of the chorionic mucous membrane have reached full maturity [14]. And it was confirmed that the egg contained the embryo by using candling. Then, identify the site of the chorionic membrane which under the air space and mark it by a pencil. Then removed the marked section

of the outer egg shell about 1 cm² using rotating dentist saw blade, the inner membrane of the egg shell was moisten by placing drops of distilled water at a temperature of 37° c and leave for a short period of time. Afterward, the internal membrane of the shell was carefully lifted using forceps to obtain directly of the chorionic membrane which containing a dense network of blood vessels [14]. All these steps were carried out in the hood culture under sterile conditions. The study was conducted in the laboratory of pharmacology / college of Veterinary Medicine / Duhok University.

Application of Chorioallantoic Membranes

Design of Experiment

The experimental groups were divided into 8 subgrouping, each containing 5 eggs per group

- Group I: Treated the chorioallantoic membrane with betamethasone at dose (3µg / g of total egg weight, topical).
- Group II: Treated the chorioallantoic membrane with betamethasone at dose (6 µg / g of total egg weight, topically).
- Group III: Treated the chorioallantoic membrane with betamethasone at dose (12 µg / g of total egg weight, topical).
- Group IV: Treated the chorioallantoic membrane with betamethasone at dose (24 µg / g of total egg weight, topical).
- Group V: Treated the chorioallantoic membrane with betamethasone at dose (48 µg / g of total egg weight, topical).
- Group VI: Treated the chorioallantoic membrane with betamethasone at dose (96 µg / g of total egg weight, topical).
- Group VII: Treated the chorioallantoic membrane with betamethasone at dose (192 µg / g of total egg weight, topical).
- Group VIII: Treated the chorioallantoic membrane with propylene glycol solution at dose size (0.01 ml / g of total egg weight, topical). This group was introduced into the experiment design because the propylene glycol solution was the best solvent of betamethasone.

Betamethasone or propylene glycol was added to largest blood vessel (1st order vessels) of the chorioallantoic membrane at dose size (0.01 ml / g of total egg weight) and then closed the hole with transparent adhesive and returned the treated egg to the incubator and after 120 minutes was removed egg from the incubator and withdrawn blood and blood smeared

and fixed by using methyl alcohol 70% and then soaked with gimza stain and then dried to be ready for the differential counting of white blood cells. The percentage of each type of white blood cell was calculated, which included Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil [15].

Statistical Analysis

The data was statistically analyzed using two way analysis of variance followed by the least significant difference (LSD) test and the moral difference at the probability level ($A < 0.05$) [16].

RESULTS AND DISCUSSION

The aim of the present study was the possibility of using the chorioallantoic membrane of chick embryo as in-vivo model to study the effect of betamethasone on the differential leukocyte count.

The results of our study showed the possibility of blood smeared from chick embryos at the age of 12-15 day of the incubation, where the blood smear contains all types of white blood cells (Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil). This was similar to adult chicks blood smear, and this reflecting the importance of this model in detecting the side effects of pharmaceutical compounds in the blood film. And due to the short period of incubation after betamethasone treated, which was only 120 minutes. Therefore, no significant differences were observed in the white blood cells counting (Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils), between doses of betamethasone (3, 6, 12, 24, 48, 96, 192 µg / g of total egg weight, topical). ("Table"), because it is impossible during this short period to make changes in the distribution of blood cells between the circulatory system and lymphatic tissue, where [17] mentioned the cause of changes induced by betamethasone in the white blood cells counting due to the re-distribution of cells from the circulation to the lymphatic tissue. This result was agree with the [18] showed no significant differences in the differential white blood cells count after 120 minutes of cortisol injection in horses. We conclude from our current study that it is possible to use the chorioallantoic membrane as a model within the in-vivo to study changes in the differential white blood cells count due to the blood smear contain all types of white blood cells, as well as that the cost of this model is low compared to laboratory animals.

Table-1: The effect of betamethasone on the differential white blood cells count of chick embryo

Compound Dose\gram of total weight of egg	Lymphocyte % after 90 min.	Heterophile % after 90 min	Monocyte % after 90 min	Esinophile % after 90 min	Basophile % after 90 min
Propylenglycol 0.01 ml/g	55.8 ± 0.23	35.8 ± 2.04	5.8 ± 0.39	1.4 ± 0.08	0.7 ± 0.03
192 µg / g	55.9 ± 1.85	36.1 ± 1.78	5.0 ± 0.30	2.0 ± 0.13	0.6 ± 0.04
96 µg / g	54.8 ± 2.21	36.2 ± 1.70	6.0 ± 0.27	1.8 ± 0.03	0.7 ± 0.04
48 µg / g	53.9 ± 2.71	38.0 ± 1.71	4.8 ± 0.30	1.9 ± 0.26	0.9 ± 0.11
24 µg / g	52.7 ± 2.30	39.3 ± 2.29	5.4 ± 0.29	1.9 ± 0.22	0.5 ± 0.04
12 µg / g	52.2 ± 2.43	40.0 ± 0.62	5.5 ± 0.29	1.4 ± 0.10	0.5 ± 0.07
6 µg / g	52.9 ± 1.35	38.5 ± 3.84	5.6 ± 0.91	1.8 ± 0.17	0.7 ± 0.03
3 µg / g	55.78 ± 0.44	36.9 ± 2.64	4.6 ± 0.27	1.6 ± 0.08	0.7 ± 0.01

Values represent the mean ± standard error (five fertilized eggs / dose).

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