



Research Article

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Dose response relationship of dexamethasone on insulin levels in wistar rats

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ABSTRACT

Glucocorticoids are commonly used in various clinical conditions. But its use is often associated with adverse effects, including insulin resistance, hyperinsulinemia and hyperglycemia. The objective of the present study was to measure the median effective dose (ED_{50}) of dexamethasone to induce hyperinsulinemia in wistar rats. Graded doses (0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16mg/kg) of dexamethasone were administered intraperitoneal (i.p.) to male wistar rats for 6 days ($n=6$). On the last day of experiment, serum insulin and glucose levels were measured. ED_{50} value was calculated by plotting log-dose response curve. The maximum effect of dexamethasone on insulin levels was observed in 8mg/kg and minimum effect was observed in 0.0625mg/kg dexamethasone. There was no further increase in response at 16mg/kg dexamethasone in rats. Significant increase in glucose levels were observed only with higher doses (0.5, 1, 2, 4, 8 and 16mg/kg) of dexamethasone. We concluded that median effective dose (ED_{50}) of dexamethasone was 1.8mg/kg or 1800 μ g/kg for inducing hyperinsulinemia. This dose is preferable for detecting the efficacy and potency of insulin sensitizers as well as to screen drug effects which may reduce or increase insulin resistance.

Key words: Glucocorticoids, Insulin sensitizers, Median effective dose, Hyperinsulinemia, Efficacy

INTRODUCTION

Type 2 diabetes mellitus is characterized by insulin resistance [1]. Insulin resistance is a condition in which peripheral tissues fail to respond to normal levels of circulating insulin, hence higher than normal concentrations of insulin are required in order to maintain normoglycemia [2]. It is due to an impaired effect of insulin mainly in muscle, fat and liver [3]. Insulin resistance and hyperinsulinemia are closely linked [4]. It has been documented that β cells compensate for insulin resistance by raising insulin levels and that results in hyperinsulinemia. However, there is increase in glucose levels as the β -cell function becomes altered which leads to insulin resistance and its complications like diabetes, dyslipidemia etc. [5]. Treatment of insulin resistance involves life style changes like increase in physical activity and high dietary fiber intake which can prevent the progression of disease. In those patients who fail to follow the life style changes, insulin sensitizers can help in the improvement of insulin sensitivity [6].

Hyperinsulinemia is a marker for cluster of abnormalities, including hypertension, dyslipidemia, impaired fibrinolysis and impaired insulin mediated glucose uptake [7,8]. High plasma insulin concentration may increase the risk of ischemic heart disease through alterations in metabolic processes [9]. Elevated insulin levels are also associated with insulin resistance, polycystic ovary syndrome, coronary artery disease, and some other health issues.

The major characteristics of insulin resistance are decreased uptake of glucose by muscle, uncontrolled lipolysis in adipose tissue and increased gluconeogenesis in the liver [10].

Glucocorticoids(GCs) are broadly used for various clinical disorders because of their anti-inflammatory, antiallergic and immunosuppressant properties. However, GCs can produce metabolic adverse effects, including hyperinsulinemia, hyperglycemia and hyperlipidemia [11,12]. Glucocorticoids are well known for their use in research to induce insulin resistance in experimental animals [13,14]. Although there is inadequate data on optimum dose of dexamethasone to produce hyperinsulinemia in rodents. The ED₅₀ of dexamethasone can be useful to study the efficacy of novel insulin sensitizers. And also to study the effect of drugs concurrently given along with dexamethasone in clinical practice on the insulin resistant state it induces. Hence, this present study was undertaken to find out the median effective dose (ED₅₀) of dexamethasone to induce hyperinsulinemia in wistar rats.

EXPERIMENTAL SECTION

Animals:

The present study was conducted on male albino Wistar rats (weight 230-300gms). Institutional animal ethics committee permission was taken prior to study. Animals were maintained under standard conditions, as prescribed by the Committee for the Purpose of Control and supervision on experimental animals (CPCSEA), at temperature (23±2)°C, humidity 50±5%, 12:12hr light-dark cycles. Animals were maintained in polypropylene cages (UN Shah Manufacturers), rat pellets (Hindustan lever limited, Mumbai) and water were given ad-libitum.

Drugs and Chemicals:

Dexamethasone injection was obtained from Zydus pharmaceuticals, Mumbai. Ketamine injection was obtained from Neom laboratories limited, Mumbai, India. Serum Insulin levels were estimated by ultra sensitive rat insulin ELISA kit purchased from Genxbio HealthSciences Private Limited, Delhi, India. Serum glucose levels were measured by using commercially available kits (Erba Mannheim, Transasia Biomedicals LTD., Germany).

Experimental design:

The animals were divided into 10 groups. Each group consisted of 6 rats. Control group rats (group-1) received normal saline (2ml/kg i.p). Rats from group 2-10 were treated with dexamethasone daily with graded doses (0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16mg/kg/i.p respectively) for a period of 6 days.

At the end of the experimental period, i.e on day 6, the overnight fasted rats were anaesthetized with ketamine and blood was collected by retro orbital sinus puncture method. Blood samples were centrifuged at 2000RPM and serum was separated. Serum insulin levels were measured by Enzyme Linked Immunosorbant Assay (ELISA) method by using ELISA reader. Serum glucose levels were estimated by Glucose oxidase and peroxidase (GOD-POD) method.

For determination of the median effective dose (ED₅₀) of dexamethasone to induce hyperinsulinemia, log dose-response curve was obtained by plotting log of doses of dexamethasone on the x-axis and insulin levels on the y-axis.

Statistical analysis: The data was presented in Mean ± SEM. Results were analyzed by one-way ANOVA followed by Scheffe's multiple comparison tests using SPSS software. Statistical significance was assumed if $p < 0.05$.

RESULTS AND DISCUSSION

Results: Daily intraperitoneal administration of dexamethasone (Group 4-10) for 6 days resulted in significant hyperinsulinemia when compared to control group rats ($P < 0.05$). However, lower doses (Group 2, 3) of dexamethasone did not show significant increase in serum insulin levels when compared with control group. The maximum effect of dexamethasone on serum insulin levels was observed in 8mg/kg dose (Group 9) and minimum effect was observed in 0.0625mg/kg dose (Group 2). There was no further increase in response at a dose of 16mg/kg (Group 10) dexamethasone (Table1)

Table 1

Group	Dexamethasone dose (mg/kg)	Insulin values (ng/ml)
1	Control	2.37± 0.1
2	0.0625	3.51±0.19
3	0.125	4.83±0.33
4	0.25	6.85±0.37*
5	0.5	8.12±0.13*

6	1	9.09±0.28*
7	2	11.05 ±0.59*
8	4	13.93 ±0.44*
9	8	18.71±0.72*
10	16	17.57±0.54*

Results are expressed in Mean±SEM. Data were analyzed by One-way ANOVA followed by scheffe's multiple comparison method ($P<0.05$)*

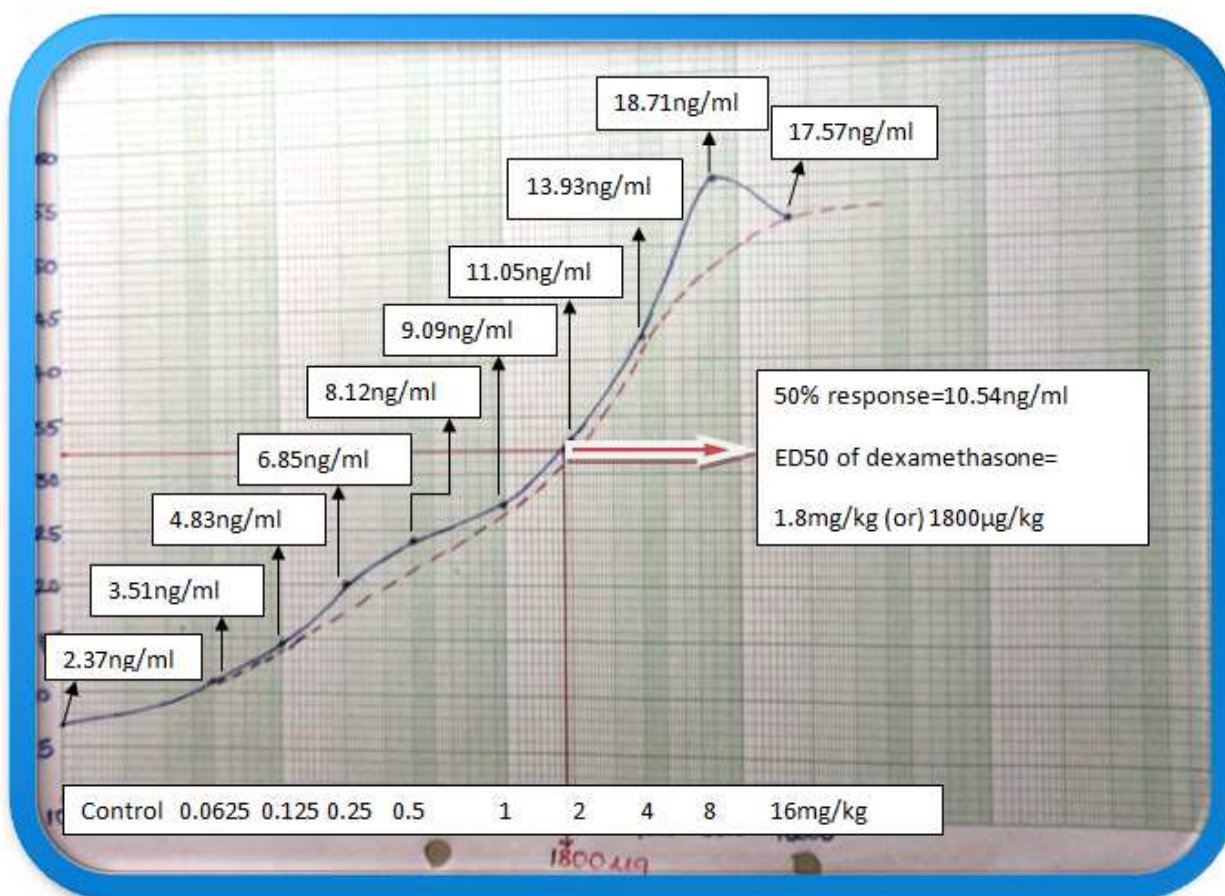
There was dose dependent increase in serum glucose levels, observed in all dexamethasone treated groups when compared to control group rats. Lower doses (Groups 2-4) of dexamethasone showed only slight increase in glucose levels. Higher doses (Groups 5-10) of dexamethasone showed significant increase in glucose levels. There was no further increase in serum glucose level at a dose of 16mg/kg (Group 10) dexamethasone. (Table 2)

Table 2

Group	Dexamethasone dose (mg/kg)	Glucose values (mg/dl)
1	Control	104.59±1.26
2	0.625	107.08±2.46
3	0.125	109.37±1.54
4	0.25	112.15±2.75
5	0.5	131.03±4.01*
6	1	147.23±1.69*
7	2	178.93±2.59*
8	4	205.42±4.02*
9	8	265.51±3.19*
10	16	262.19±1.47*

Results are expressed in Mean±SEM. Data were analyzed by One-way ANOVA followed by scheffe's multiple comparison method ($P<0.05$)*

Figure 1; Dose response curve of dexamethasone on insulin levels



Scale: 3 divisions equal to 1 unit of insulin on Y-axis

X axis: Dexamethasone doses

Y axis: Insulin values

The median effective dose or ED₅₀ is a dose which produces 50 per cent of the maximal response and this was obtained by plotting log dose-response curve. Dexamethasone dose was plotted in logarithmic scale on X-axis and serum insulin levels were plotted in arithmetic scale on Y-axis. A sigmoid shaped curve was obtained on joining the points. The degree of hyperinsulinemia was calculated by deducting the normal control value of insulin (2.37ng/ml) from the maximal response ((18.71ng/ml) and half of the maximum response was 8.17 ng/ml. The normal control insulin value was added to the maximum response to get final value (10.54ng/ml), since hyperinsulinemia starts above the normal value of 2.37ng/ml. The ED₅₀ of dexamethasone to induce hyperinsulinemia in wistar rats was found to be 1.8mg/kg or 1800µg/kg (10.54ng/ml). (Figure 1)

DISCUSSION

Metabolic syndrome is a cluster of abnormalities including glucose intolerance, dyslipidemia, hypertension and central obesity with insulin resistance [15]. Glucocorticoids (GCs) are involved in the regulation of glucose homeostasis and nutrient metabolism. It is well known that on administration of GCs there is possible reduction in peripheral insulin sensitivity. It is adaptively controlled by increased pancreatic β-cell function and mass which leads to hyperinsulinemia [16]. Dexamethasone was preferred to induce hyperinsulinemia and hyperglycemia because it lacks mineralocorticoid effect [17]. As dexamethasone is commonly used in large doses in clinical practice, it is desirable to prevent its metabolic adverse [18].

There is in-vitro and in-vivo models of glucocorticoid induced insulin resistance. But in-vitro methods require expensive setup. Through that one can screen a large number of compounds in quick time. In-vivo models are indispensable as that is the proof of efficacy of the compound tested [19,20]. Present study aimed to standardize the protocol for dexamethasone induced hyperinsulinemia in wistar rats.

In this study, we have evaluated the effect of graded doses (0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16mg/kg/i.p) of dexamethasone on serum insulin levels. Intraperitoneal administration of dexamethasone at different doses for 6 days resulted in significant hyperinsulinemia in a dose dependent fashion in all wistar rats. It supports the earlier studies in which the administration of glucocorticoids increases insulin concentration in blood, as well as induces changes in the insulin binding to its receptor [21,22]. A previous study suggests that the pharmacological doses of glucocorticoids induce gene expression in rat's adipocyte tissue within 24h. This leads to complex metabolic changes resulting in increase in insulin levels, decrease in bodyweight and food consumption, profound obesity often accompanied by diabetes and development of insulin resistance [23]. Glucocorticoid induced insulin resistance is attributed mainly to a postreceptor defect of insulin action [24].

The current study also demonstrated the effect of dexamethasone on serum glucose levels. It has shown significant increase in glucose levels dose dependently. But lower doses (0.0625, 0.125 and 0.25mg/kg) of dexamethasone have shown only mild increase in glucose levels in wistar rats. The possible mechanism of glucocorticoid induced hyperglycemia is mainly due to increased gluconeogenesis, decreased peripheral glucose uptake into muscle and adipose tissue, breakdown of muscle and fat to provide additional substrates for glucose production [25,26].

A log dose-response curve has a characteristic sigmoid or S-shape with linear portion in the middle part of the curve. This part is the most sensitive part of the curve and the working doses are therefore selected around this region for any study [27]. Hence, the present study determined the median effective dose (ED₅₀) of dexamethasone as 1.8mg/kg in wistar rats.

The idea of the right dose of dexamethasone to induce hyperinsulinemia arose because of the huge variation of the doses used seen in the review of literature [28,29,30,31,32]. The doses used varied mainly on the objective of the study which were physiological, biochemical or pathological. Hence it was necessary to choose the right dose for our objective. We decided to arrive at the median effective dose to induce hyperinsulinemia because it would help in comparing the efficacy, potency of the insulin sensitizers as well as whether the given drug reduces or increases insulin resistance. We found that when we used larger doses of dexamethasone, the severity of the illness produced was such that only insulin would produce statistically significant benefits and we would miss a potentially effective insulin sensitizer. When we used a smaller dose, we could not differentiate the efficacy between different insulin sensitizers. Our objective was to get hyperinsulinemia in vivo in quick time and hence we chose six days time. It is also possible to know the outcome of the effect of other drugs given along with dexamethasone in clinical practice on insulin resistance, which is not possible to know in clinical practice because of inter-individual variations as well as polypharmacy. Knowing the outcome of such interactions with other drugs, one can make the choice of right drug. That will be another use of this model.

CONCLUSION

We concluded that median effective dose (ED₅₀) of dexamethasone is 1.8mg/kg or 1800µg/kg for inducing hyperinsulinemia. This dose is suitable to screen the efficacy and potency of insulin sensitizers as well as whether the given drug reduces or increases insulin resistance.

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