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Original Research Article

To Study Effect of Topical Betamethasone Dipropionate on Blood Pressure, Blood Sugar and Blood Calcium in Healthy Volunteers

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ABSTRACT

Corticosteroids are mainly used to reduce inflammation and suppress the immune system. Corticosteroids are used in a variety of conditions, ranging from brain tumours to skin diseases. In the skin diseases, corticosteroids are used for few days to few weeks and use of different corticosteroids according to potency, depends on site (location) of application and severity of disease. Adverse effects of corticosteroids depend on potency of corticosteroids, duration of treatment and area of involvement. In this study we investigated the effect of topical high potent betamethasone dipropionate on blood pressure, blood sugar and blood calcium after four hours in healthy volunteers. Also, correlated the absorbed concentration of corticosteroids with blood pressure, blood sugar, blood calcium and affected body surface area. Four hours after betamethasone application there was no effect on blood pressure, blood sugar and blood calcium level in healthy volunteers.

INTRODUCTION

Corticosteroids are mainly used to reduce inflammation and suppress the immune system. Corticosteroids are used in a variety of conditions, ranging from brain tumours to skin diseases. Mainly corticosteroids are administered by oral, parenteral and topical route. [1]

Topical corticosteroids are often considered to have greater safety than oral corticosteroids. There are different topical corticosteroids used in skin diseases. e.g. clobetasol, mometasone, beclomethasone, betamethasone, desonide and fludrocortisone. Use of these steroids depends on strength of corticosteroids and anatomic location. In 2014, a study conducted in skin OPD in MGM medical college concluded that in skin outpatient department, maximum corticosteroids are prescribed by topical route and betamethsone was maximally prescribed corticosteroid than prednisolone, clobetasol and beclomethasone.[1]

Clinical effectiveness of topically applied medications depends on the ability of the active ingredient to leave its vehicle and penetrate into the epidermis. The stratum corneum is that layer of the epidermis which functionally is the most important in limiting percutaneous absorption, showing the characteristics of a composite semipermeable membrane. There are different factors that altering transport through skin e.g. hydration of epidermis, concentration of the molecules applied, keratolytics, particle size, regional variations etc.[2]

Short-term corticosteroid use is associated with generally mild side effects, including cutaneous effects, electrolyte abnormalities, hyperglycemia, pancreatitis, hematologic, immunologic, and neuropsychologic effects. Long-term corticosteroid use may be associated with more serious side effects including osteoporosis, hypertension, adrenal insufficiency, gastrointestinal, hepatic, and ophthalmologic effects, hyperlipidemia, growth

suppression, and possible congenital malformations.[3]

In the skin diseases, corticosteroids are used for few days to few weeks and use of different corticosteroids according to potency, depends on site (location) of application and severity of disease. Adverse effects of corticosteroids depend on potency of corticosteroids, duration of treatment and area of involvement. High potency corticosteroids are used for longer duration in the treatment of diseases like psoriasis, discoid lupus erythematous and other. In topically used corticosteroids, the extent of percutaneous absorption is determined by many criticalfactors such as formulation, vehicle, anatomical site of application, integrity of the epidermal barrier, use of occlusive dressing and concentration and frequency of application.[4] Thus, the study was undertaken to estimate the effect of betamethasone dipropionate on the blood pressure, blood calcium and blood sugar level and to estimate the blood concentration of topically applied betamethasone dipropionate in healthy volunteers and to correlate these effect with the concentration of betamethasone in blood.

OBJECTIVE

To study effect of Betamethasone dipropionate cream on blood pressure, blood sugar and blood calcium in healthy volunteers.

MATERIALS AND METHODS

This study was conducted in MGM Medical College and Hospital Kamothe, Navi Mumbai in healthy volunteers. Total number of 10 healthy volunteers who were not using corticosteroid treatment for more than one month prior to inclusion in the study was included. Study was carried out after the permission of Institutional Ethics Committee proper consents were taken from each healthy volunteer before enrolling in the study.

Before enrolment of healthy volunteers in the study, they were explained each and every information of study including use of study, effects and side effect of drug, procedure of blood pressure measurement, blood sugar and calcium level estimation, drug application and blood samples collection.

All details of healthy volunteers were taken while proceeding the study, name of volunteer, age, sex, disease history, drug history, general examination, blood pressure was measured before and four hours after drug application by sphygmomanometer, blood sugar was measured before and four hours after drug application by glucometer, blood calcium level was measured by biochemical method by collection of blood sample in plain tube before and four hours afterdrug application, blood collected in EDTA tube for estimation of corticosteroid in blood by HPLC method.

One FTU (fingertip unit) (0.5 gm) Betamethasone dipropionate cream 0.05 % w/w (Betamil cream) was applied on 4 cm × 4 cm area on medial superior side of forearm. Blood pressure, blood sugar and blood calcium level were measured before and four hours after drug application. Three ml of blood was collected in plain tube for calcium estimation before drug application and four hours after drug application two ml of blood was collected in EDTA tube for Betamethasone dipropionate estimation and three ml of blood was collected for calcium level estimation.

Inclusion criteria:

1. Human subjects who were willing to participate in the study.
2. Age 18 to 50 years.

Exclusion criteria:

1. Volunteer using oral or topical corticosteroid.
2. Hypertensive and hypotensive human subjects
3. Obese subjects.
4. Diabetic patients

Blood pressure measurement:

Blood pressure was measured before and four hours after drug application in sittingposition on right hand by using mercury sphygmomanometer.

Blood sugar measurement:

Blood sugar level were measured before and four hours after drug application by glucometer.

Blood calcium measurement:

Serum samples stored in plain tube were used for calcium investigation. Estimation of calcium done by OCPC method (O – cresolphthaleincomplexone)

Estimation of Betamethasone dipropionate concentration in the blood:

HPLC method standardization:

5 mg standard betamethasone dipropionate dissolved in 50 ml acetonitrite to get concentration of 100 µg/ml and then serially dilutions were made in the concentration of 10 ng/ml, 20ng/ml, 30ng/ml, 50 ng/ml, 70ng/ml and 100ng/ml.

0.4 ml of betamethasone dipropionate from each dilution was added in 1 ml plasma in separate test tube and mixed properly.

Extraction procedure:

Liquid liquid extraction: 100 µl of plasma added in 1200ul of ethyl acetate (extracting solvent) in eppendorf tube and kept in vertex for proper mixing, then the eppendorf tube centrifuged at 6000 rpm at -6 0c for 10 minutes. The supernatant liquid was removed from eppendorf tube transferred into small test tube. Then small test tubes were kept on simple heater at 40 0c for evaporation of volatile solvent. Then remaining solid residue dissolved in 400 µl of acetonitrite to run in HPLC.

HPLC parameter:

Mobile Phase: Methanol : Water (80:20)
Flow Rate: 1ml/min
Retention time of drug: 5.1 min
Column: C18
Wavelength: 254nm

After running different dilutions of betamethasone dipropionate in HPLC at given HPLC parameter gives reading [area under curve (AUC)]. We plotted graph AUC Vs concentration, drown straight line.

Serum samples:

Extraction procedure:

Liquid liquid extraction: 100ul of serum sample added in 1200µl of ethyl acetate (extracting solvent) in eppendorf tube and kept in vertex for proper mixing, then the eppendorf tube centrifuged at 6000 rpm at -6 0c for 10 minutes. The supernatant liquid was removed from eppendorf tube transferred into small test tube. Then small test tubes were kept on simple heater at 40°C for evaporation of volatile solvent. Then remaining solid residue dissolved in 400 µl of acetonitrite and run in HPLC.

HPLC gave reading (AUC), we compared AUC with plotted standard graph gave unknown concentration of Betamethasone dipropionate in serum sample.

RESULTS

This study was conducted in MGM Medical College and Hospital Kamothe, Navi Mumbai in healthy volunteers This study included 10 healthy volunteers, who were not using corticosteroid treatment for more than one month were included.
Study was carried out after the permission of Institutional Ethics Committee, proper consents were taken from each healthy volunteers.

Ten Healthy volunteers between the age group of 18 to 50 years were included. Before enrolment of healthy volunteers in the study, they were explained each and every information of study including the use of study, effects and side effect of drug, procedure of blood pressure measurement, blood sugar level estimation, drug application and blood sample collection.

Ten healthy volunteers were enrolled in test subgroup 2. Volunteer's age ranging between 23 years and 48 years were included and there were 9 males and 1 female. 1 FTU (fingertip unit) (0.5 gm) betamethasone dipropionate cream 0.05 % w/w (Betamil cream) was applied on 4 cm×4 cm area on medial superior side of forearm. Blood pressure, blood sugar and blood calcium were measured before and four hours after drug application. Three ml blood was collected in plain tube for calcium estimation before drug application. Four hours after drug application two ml blood was collected in EDTA tube for betamethasone dipropionate estimation and three ml blood was collected in plain tube for blood calcium level estimation.

The baseline systolic blood pressure ranged from 112 mm Hg to 128 mm Hg (mean 121.8 mm Hg, SD = 4.83). The diastolic blood pressure was between 76 mm Hg and 84 mm Hg (mean 80.8 mm Hg, SD = 3.56) on first day. Four hours after 1 FTU of betamethasone dipropionate cream application, the systolic blood pressure was raised on an average by 1.2 mm Hg, whereas rise in diastolic

blood pressure was less than 0.2 mm Hg. Both changes were not significant statistically. No volunteers developed frank hypertension. Betamethasone dipropionate concentration in blood after four hours drug application was in range between 01 ng/ml to 11 ng/ml and mean blood concentration was 5.9 ± 4.18. [Table 1]

The baseline blood sugar level ranged from 76 mg/dl to 90 mg/dl (mean 84.20 mg/dl, SD = 4.71) on first day. Four hours after 1 FTU of betamethasone dipropionate cream application, the blood sugar level ranged from 80 mg/dl to 91 mg/dl. (mean 85.30 mg/dl, SD = 3.16) Blood sugar level was raised on an average by 1.1 mg/dl. This change was not significant statistically. No volunteers developed frank hyperglycemia. Betamethasone dipropionate concentration in blood after four hours drug application was in range between 01 ng/ml to 11 ng/ml and mean blood concentration was 5.9 ± 4.18. [Table 2]

The baseline blood calcium level ranged from 7.9 mg/dl to 10.6 mg/dl (mean 8.8 mg/dl, SD = 0.8) on first day. Four hours after 1 FTU of betamethasone dipropionate cream application, the blood calcium level ranged from 7.7 mg/dl to 10.3 mg/dl. (mean 8.8 mg/dl, SD = 0.7) there were minor changes in the blood calcium level. This change was not significant statistically. No volunteers developed frank hypercalcemia or hypocalcemia. Betamethasone dipropionate concentration in blood after four hours drug application was in range between 01 ng/ml to 11 ng/ml and mean blood concentration was 5.9 ± 4.18. [Table 3]

Comparison of systolic blood pressure and diastolic blood pressure before and after betamethasone dipropionate topical application in healthy volunteers. Mean systolic and diastolic blood pressure before the betamethasone dipropionate application was 121.8 ± 4.83 mm Hg and 80.8 ± 2.70 mm Hg and after the betamethasone dipropionate cream application was 123 ± 3.56 mm Hg and 81 ± 2.54 mm Hg respectively. There was no statistically significant difference in systolic blood pressure (P = 0.5363) and in diastolic blood pressure (P = 0.8664) after the betamethasone dipropionate cream application. [Table 4]

Comparison of blood sugar level before and after betamethasone dipropionate topical application in healthy volunteers. Mean blood sugar level before the betamethasone dipropionate application was 84.2 ± 4.71 mg/dl and after the betamethasone dipropionate application was 85.3 ± 3.16 mg/dl. There was no significant difference in blood sugar level (P = 0.5475) after the betamethasone dipropionate application in healthy volunteers [Table 4]

Comparison of blood calcium level before and after betamethasone dipropionate topical application in healthy volunteers. Mean blood calcium level before the betamethasone dipropionate application was 8.8 ± 0.8 mg/dl respectively and after the betamethasone dipropionate application was 8.8 ± 0.7 mg/dl. There was no significant difference in blood calcium level (P = 0.9534) before and after the betamethasone dipropionate application in healthy volunteers. [Table 4]

There is no significant correlation between concentration of betamethasone dipropionate with Blood pressure (systolic and diastolic) and blood sugar in healthy human volunteers. [Table 5]

DISCUSSION

In our study there was no change in systolic and diastolic blood pressure after four hours and Betamethasone dipropionate concentration in blood after four hours was5.9 ng/ml. In 1983 animal experiment conducted by Häusler A and coworkers on spontaneously hypertesive rat and normotensive wister rat, concluded that both rats responded with a significant elevation in average blood pressure after seven weeks of oral betamethasone treatment.[5] Koenen SV and co-workers conducted animal experiment in 2002 on pregnant baboon investigated that fetal blood pressure increased significantly after intramuscular betamethasone treatment.[6] A comparative animal study conducted by Derks J B et al in 1997 concluded that prenatal betamethasone and dexamethasone treatment of late-gestation fetal sheep, in doses similar to those employed clinically, is associated with fetal cardiovascular, endocrine and behavioural effects. Both betamethasone and dexamethasone induce similar increases in fetal blood pressure.[7] In another study by Krishnankutty Sudhir and coworkers in 1989 showed that Oral hydrocortisone increases blood pressure, diastolic blood pressure remained unchanged, systolic blood pressure increased from 119 to 135 mm Hg.[8] A study conducted by

MarinisPirpiris and coworkers in 1992also showed increase mean arterial pressure from 82±3 to 91 ±3 mm Hg by dexamethasone.[9] Another study conducted by Atsuhisa Sato and coworkers in 1995 showed glucocorticoid-induced hypertensionin elderly patients and/or in those with positive family history of essential hypertension.[10] In 1999 Dodic M and coworkers concluded that foetal exposure to maternal dexamethasone during defined developmental stage or 'window' programmes elevated blood pressure, which persists later in life.[11] Case-control study conducted by Marie-Josée Martel and coworkers in 2005 explained that, there was no significance dose-response relation was observed between inhaled corticosteroids and pregnancy induced hypertension or pre-eclampsia. Oral corticosteroids were significantly associated with the risk of pregnancy induced hypertension.[12]

From our results and from literature, it showed that corticosteroids increases blood pressure and change in blood pressure was more after oral administration than topical application. Ultra high potent and high potent corticosteroids increase only systolic blood pressure and there is no effect of moderately potent corticosteroids on blood pressure this may be due to the molecular weight of corticosteroids.

In our study four hour after application of betamethasone dipropiponate has not changed blood sugar level. In 2009 study conducted by Peter Gonzalez MD and coworkersin patients with Diabetes mellitus they showed that Lumbosacral transforaminal and caudal epidural betamethasone injections are associated with statistically significant elevations in blood glucose levels in diabetic subjects.[13] Ramírez-Torres MA and co-workers in 2011 reported that betamethasoneinduced hyperglycemia was greater in insulin treated women with gestational or type 2 diabetes.[14] Study conducted by Jolley JA and coworkers in 2016 on pregnant women of diabetic and without diabetic, administration of betamethasone for threatened preterm delivery they find out that both subjects with and without diabetes demonstrate significant hyperglycemia after receipt of antenatal betamethasone.[15]

Iwamoto T and co-workers in 2004 investigated that Steroid-induced diabetes mellitus was diagnosed if the patient had either a fasting glucose concentration of 126 mg/dl or greater, or a random glucose concentration of 200 mg/dl or greater.[16] In 2006 study conducted by Angela A and co-workers on diabetic patients, reported that blood glucose level increased in diabetic patients who received methylprednisolone injection.[17] Van Raalte DH and co-workersin 2013 investigated that prednisolone-induced impairment of insulin-stimulated capillary recruitment was paralleled by insulin resistance, increased postprandial glucose levels, hypertension and increased circulating resistin concentrations in healthy men.[18] From our results and from literature, it showed that corticosteroids increases blood sugar level and change in blood sugar was more after oral and injectable administration than topical application.

In our study blood calcium levels have not changed after four hours application of betamethasone. Betamethasone absorbed in blood after topical application, but it does not produce any change in blood calcium. Study conducted by C. Gennari, in 1993 revealed that low and high doses of betamethasone and high doses of prednisone induced a significant decrease in intestinal calcium absorption.[19] In 1981 Theodore J. Hahn et al investigatedthe tintestinal calcium absorption reduced by 31 % after prednisone administration.[20] YasuoSuzuki and coworkers in 1983 studied Parathyroid function and calcium metabolism in 44 patients under glucocorticoid therapy conclude that urinary calcium excretion increased in patients under glucocorticoid therapy.[21] Most of the literature explained that corticosteroids decreases calcium absorption and increases excretion of calcium but not a single study correlated blood concentration of corticosteroids with calcium level in the blood.

CONCLUSION

In healthy volunteers four hour after betamethasone dipropionate cream application, does not get adsorbed into blood stream in substantial measurable levels. After single application of betamethasone to skin in normal volunteers have not produced any change in blood pressure, blood sugar and blood calcium levels after four hours.

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Table 1: Blood pressure (mm Hg)

Sr. No.	Age in year	Sex	Baseline BP (mmHg) at 3 p.m.		1st day BP (mm/hg) at 3 p.m.		Concentration of betamethasone dipropionate in ng/ml
			S	D	S	D	
1	25	F	124	80	124	80	10
2	28	M	118	78	122	80	09
3	23	M	126	84	124	84	04
4	39	M	122	80	124	80	02
5	41	M	112	76	116	76	01
6	40	M	128	82	130	82	01
7	33	M	124	84	124	84	09
8	48	M	118	80	122	80	9.5
9	29	M	126	84	124	84	11
10	48	M	120	80	120	80	02
Mean (mm Hg)			121.8±4.83	80.8 ±2.70	123 ±3.56	81 ± 2.54	5.9 ± 4.18

S = Systolic blood pressure; D = Diastolic blood pressure

Table 2: Blood sugar (mg/dl)

Sr. No.	Age in year	Sex	Blood sugar (mg/dl)		Concentration of betamethasone dipropionate in ng/ml
			Before	After	
1	25	F	88	86	10
2	28	M	82	86	09
3	23	M	90	86	04
4	39	M	76	80	02
5	41	M	85	88	01
6	40	M	79	81	01
7	33	M	84	86	09
8	48	M	90	91	9.5
9	29	M	87	85	11
10	48	M	81	84	02
Mean (mg/dl)			84.20 ±4.71	85.30 ±3.16	5.9 ± 4.18

Table 3: Blood calcium (mg/dl)

Sr. No.	Age in year	Sex	Blood calcium (mg/dl)		Concentration of betamethasone dipropionate in ng/ml
			Before	After	
1	25	F	8.3	8.3	10
2	28	M	9.4	9.5	09
3	23	M	8.7	8.8	04
4	39	M	8.6	8.7	02
5	41	M	7.9	7.7	01
6	40	M	9.2	9.2	01
7	33	M	9.0	9.1	09
8	48	M	8.4	8.3	9.5
9	29	M	11.0	10.3	11
10	48	M	8.3	8.3	02
Mean (mg/dl)			8.8 ± 0.8	8.8 ± 0.7	5.9 ± 4.18

Table 4: Comparison of blood pressure, blood sugar and blood calcium before and after betamethasone dipropionate application in healthy volunteers

		Mean	SD
SBP (mm Hg)	Before	121.8	4.83
	After	123	3.56
DBP (mm Hg)	Before	80.8	2.70
	After	81	2.54
Blood Sugar (mg/dl)	Before	84.2	4.71
	After	85.3	3.16
Blood Calcium (mg/dl)	Before	8.8	0.8
	After	8.8	0.7

Significant at $P < 0.05$; Name of test : t-test (paired)

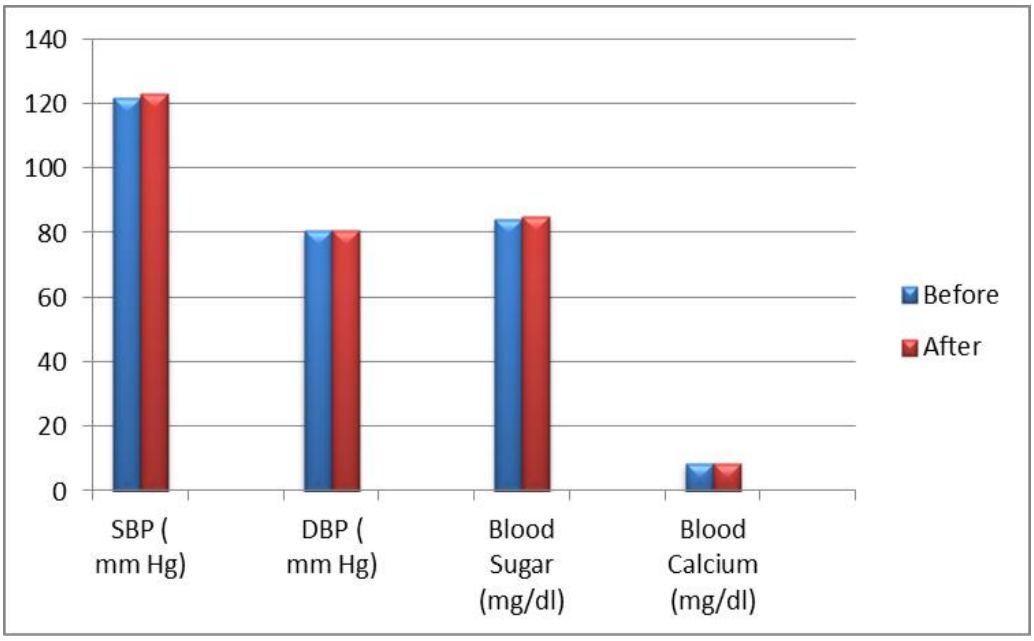

Fig. 1: Comparison of blood pressure, blood sugar and blood calcium before and after betamethasone dipropionate application in healthy volunteers.

Table 5: Correlation between concentration of betamethasone dipropionate with Blood pressure (systolic and diastolic) and blood sugar.

	Blood Concentration (ng/ml) Betamethasone dipropionate
SBP (mm of Hg)	r = -.184
DBP (mm of Hg)	r = -.265
BS (mg/dl)	r = .438

*. Correlation is significant at the 0.05 level. Test used KarlPearson

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