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Levels of Cyclic-AMP, Cyclic-GMP and Betamethasone in the Aqueous Humor Following Topical Administration of Betamethasone in Rabbit Eyes*)

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ABSTRACT

The levels of cyclic-AMP, cyclic-GMP and betamethasone (BM) in the aqueous humor and serum BM following topical treatment with BM were determined using radioimmunoassay (RIA) methods. One drop of a solution containing 0.1%, 0.5% or 1.0% BM was applied to the rabbit eye of 6 rabbits. The left eyes served as controls.

BM was detected in the aqueous humor from right eyes 15 minutes after administration and peaked in 60-90 minutes. BM was also detected in the aqueous humor of the left eyes and in serum.

Basal levels of cAMP and cGMP in the aqueous humor before BM treatment were 61.5 and 4.1 picomole/ml respectively, and changed little after BM treatment.

INTRODUCTION

Neufeld et al.[5)] first reported that topical application of adrenergic agents to rabbit eyes increases the cAMP concentration in aqueous humor and induced ocular hypotension. It has also been reported that cAMP injected directly into the anterior chamber of rabbits increased outflow facility by Neufeld et al.[6)].

On the other hand, it is well known that patients treated with topical or systemic corticosteriods sometimes show increased intraocular pressure which can result in established glaucoma.

Since cAMP lowers intraocular pressure, betamethasone (BM), which causes intraocular hypertension, might affect cAMP levels in the aqueous humor.

In an attempt to demonstrate a relationship between steroid treatment and cyclic nucleotide levels in the aqueous humor we measured the concentrations of BM, cAMP and cGMP in the aqueous humor of albino rabbits after topical treatment with BM.

MATERIALS AND METHODS

Powdered betamethasone (Rinderon® A) was kindly provided by Shionogi Co. (Osaka, Japan). Aqueous solutions containing 0.1, 0.5 and 1.0% BM were prepared by the Department of Pharmacy in this hospital.

Female albino rabbits, weighing 2.8-3.1 kg, were used in this study. One drop of local anesthetic was applied to both eyes about ten seconds before aspiration of aqueous humor. Fifty μl of aqueous humor was obtained from both eyes by inserting a 27 gauge needle attached to a 0.5 ml tuberculin syringe. One ml of blood was also withdrawn from the marginal auricular vein. One drop of BM was then applied to the left eye and the right eye served as a control. Fifteen, 30, 60, 90 and 120 minutes after BM treatment, aqueous humor and blood were collected in the same manner as described

*) 馬場さえ子, 三嶋 弘, 宮地幸隆：Betamethasone 点眼投与後の前房水中および血中 Betamethasone 濃度と，前房水中 cAMP, cGMP 濃度の経時的変化

above.

Each samples of aqueous humor was immediately mixed with 50 μl of 6% EDTA and 50 μl was used for BM assay and 20 μl for the assay of cyclic nucleotides. BM was assayed as previously reported by present authors[4]. Prior to the RIA for cyclic nucleotides, standard solution of cAMP and cGMP and test samples (100 μl) were succinylated with 20 μl of succinylation reagent. The succinylation reagent was prepared by mixing 400 mg of succinic anhydride, 2 ml of acetone and 0.72 ml of triethylamine. Each succinylated sample was then diluted with 50 mM acetate buffer (pH 6.0) to a final volume of one ml. Fifty μl were used for the cAMP assay and 200 μl for the cGMP assay. The RIA incubation mixture contained 100 μl of ^{125}I-cAMP (or ^{125}I-cGMP) and standard solutions or samples in total of 500 μl in 50 mM acetate buffer. After incubation of at 4°C overnight, 50 μl of 1% bovine gamma-globulin and one ml of 20% polyethylene glycol were added and mixed throughly. After centrifugation at 2,500 rpm for 15 minutes supernatants were removed and radioactivity in the precipitate was determined with a gamma-counter (EC-461, Shimazu Co.). The concentrations of cAMP and cGMP present in each sample were determined from standard curves prepared on logit-logarithmic scaled paper.

RESULTS

I) *Betamethasone (BM) levels in the aqueous humor:*

BM levels in the aqueous humor determined after administration of one drop of BM solution to right eyes are shown in Fig. 1. Small amounts of BM were detected in the treated eyes fifteen minutes after treatment with a 0.1% solution of BM. BM concentration reached a peak value of 72.2 ng/ml in 60 minutes. In control eyes, trace amounts of BM were first detected in the aqueous humor in 60 minutes and remained detectable for 120 minutes. After treatment with a 0.5% BM solution, a peak value of 332.5 ng/ml was obtained in the aqueous humor of treated eyes. This is almost 5 times higher than that observed after treatment with a 0.1% solution of BM. On the other hand, BM levels in the aqueous humor of control eyes were very low. After treatment with a 1.0% solution of BM, BM was detectable in 15 minutes in both treated and controled eyes. BM levels in the aqueous humor increased rapidly in treated eyes reaching a peak value of 583.3 ng/ml in 90 minutes. In control eyes BM reached a peak of 39.1 ng/ml at 120 minutes.

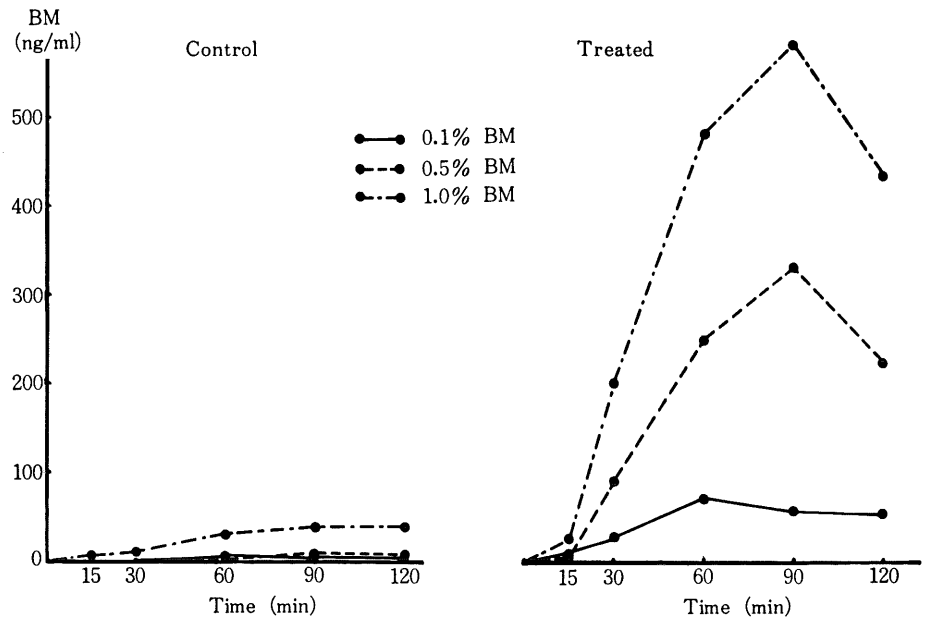

Fig. 1. Betamethasone levels in the aqueous humor following topical betamethasone administration

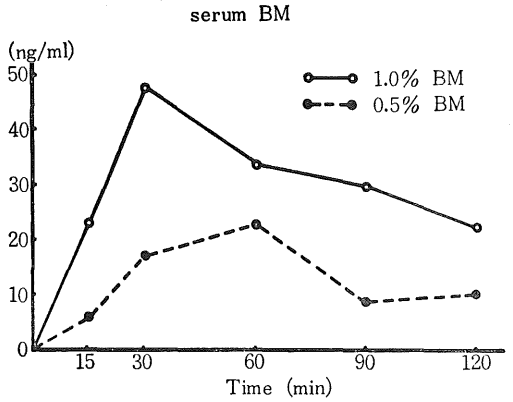

Fig. 2. Serum betamethasone levels after topical betamethasone administration

II) *Serum BM:*

After application of a 0.1% solution of BM, trace amounts of BM could be detected in the serum (less than one ng/ml in 120 minutes). Significant amounts of BM appeared in the blood after treatment with 0.5 and 1.0% solution of BM (Fig. 2) Peak value were 23.0 ng/ml in 60 minutes for 0.5% solution of BM and 48.0 ng/ml in 30 minutes for 1.0% solution of BM.

III) *The levels of cAMP and cGMP in the aqueous humor (Fig. 3, 4 and 5):*

The levels of cAMP and cGMP in the aqueous humor before treatment with BM solution were 61.2 picomole/ml and 4.1 picomole/ml, respectively. Treatment with 0.1% BM did not result in remarkable changes in either cAMP or cGMP levels in the aqueous humor or in significant differences between treated and control eyes (Fig. 3). Treatment with a 0.5% solution (Fig. 4) and as high as a 1.0% solution (Fig. 5) of BM has no effect whatever on cMP or cGMP

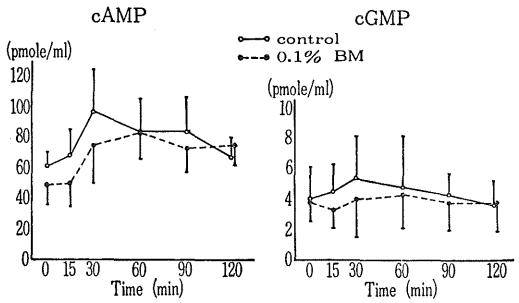

Fig. 3. cAMP and cGMP levels after 0.1% betamethasone administration

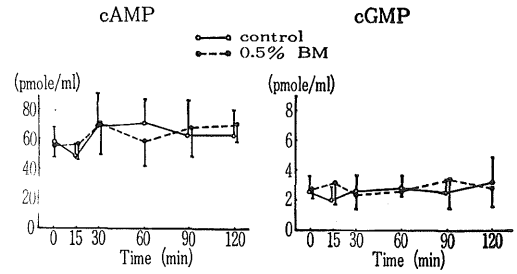

Fig. 4. cAMP and cGMP levels after 0.5% betamethasone administration

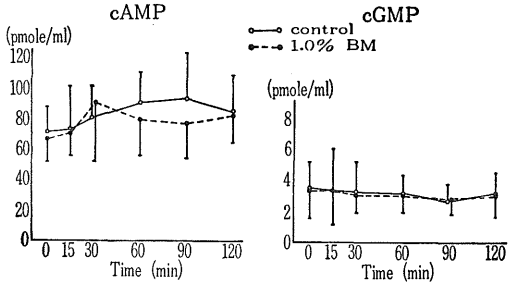

Fig. 5. cAMP and cGMP levels after 1.0% betamethasone administration

levels in the aqueous humor.

DISCUSSION

Yamauchi et al.[9] have reported intraocular penetration of certain synthetic steroids applied to rabbit eyes. They have observed that administered drugs in the aqueous humor peak in 45–60 minutes after topical treatment. The present study showed similar results with a clinical dosage (0.1% BM). After treatment with higher concentration of BM (0.5 and 1.0%), however, BM levels in the aqueous humor reached a maximal value in 90 minutes. It was reported that the contralateral eye often showed an attenuated response to the drug administered[2,3].

Chiang and Thomas also have suggested that the response observed in the untreated eye was due to the drug derived from blood circulation. Our present very sensitive RIA study which could detect minimum value of 20 picomole BM demonstrated the appearance of BM in the control eye as well as in the serum, and supports their hypothesis. Ros et al.[7] have detected radioactivity in the nontreated contrateral eye after topical treatment with radioactive atenolol. They used a 4% solution but the specific radioactivity was diluted to about 1/40 of the original

[^{14}C]-atenolol when cold atenolol was added to make a 0.1% solution. They couldn't detect atenolol in the blood. Since our data indicate that the serum BM levels are less than one ng/ml after treatment of 0.1% BM, it is understandable why they have failed to detect serum atenolol.

Neufeld et al.[5] first demonstrated the effect of epinephrine in enhancing cAMP levels in the rabbit eye. This was followed by a number of reports in which ocular cyclic nucleotides and chemicals, especially sympathomimetic or sympatholytic agents, were studied. Neufeld et al.[6] reported that cyclic AMP injected directly into the anterior chamber causes a decrease of intraocular pressure. We attempted to clarifty whether or not steroid treatment had any effect on cyclic nucleotide levels in the aqueous humor. As shown in Fig. 3, 4 and 5, one drop of BM application failed to affect cAMP levels in the aqueous humor. On the other hand, Tamura et al.[8] have shown that bupranolol hydrochloride which has been shown to have ocular hypotensive effects did not affect cAMP levels in the aqueous humor. Therefore it may be reasonable to conclude that cAMP is not the only transmitter for controlling intraocular pressure although it would play an important role when stimulation of the adrenergic receptor is involved.

Cyclic GMP levels in ocular tissues have been reported by Bonomi et al.[1]. They found no remarkable changes in cGMP levels in the aqueous humor after topical application of a 1.0% atropin. They attributed this result to high concentrations of cGMP found in the retina. Significant changes of the cGMP levels in the aqueous humor could have been obscured by the cGMP derived from the retina by diffusion. Considering the presumed large amounts of cGMP in the retina, it seems important to develop other reliable experimental methods to demonstrate changes in the cGMP levels in the aqueous humor. The present experiments show that BM applied topically to one eye is absorbed into the systemic circulation and enters the other eye. BM had little effect in cAMP and cGMP levels in the aqueous humor. Further investigations are required to determine whether steroid induced ocular hypertension is related to cAMP levels in the aqueous humor.

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