alone for 5 consecutive days significantly prolonged the patency of a thermal sclerostomy in non-glaucomatous albino rabbits.

Topical dexamethasone ointment was not additive to subconjunctival D-penicillamine, although each drug alone caused a significant increase in the duration of filter function. The reason for this lack in additivity is unclear. Perhaps the two drugs would have been more additive if subconjunctival D-penicillamine had been given several days later, at a more beneficial time in the sequence of scar tissue formation. D-penicillamine acts by preventing the cross linkages which are needed to form mature collagen after new tropocollagen has been secreted extracellularly. If some retardation of the surgically induced influx of inflammatory cells can be accomplished with topical dexamethasone, and if subsequent inflammatory-cells-turned-fibroblasts and migrated fibroblasts can be blocked from producing new extracellular matrix collagen by D-penicillamine, perhaps the scarring process can be foiled at two steps rather than one. Clearly, in this study, the optimum method of combining these two drugs was not found.

Key words: glaucoma, D-penicillamine, dexamethasone, sclerostomy, outflow facility, intraocular pressure, filtering surgery

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References


Potentiation of Collagen Synthesis in Explants of the Rabbit Eye by 5 β-Dihydrocortisol

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The biologic effect of 5 β-dihydrocortisol on collagen synthesis was evaluated. The metabolite was found to potentiate subthreshold levels of dexamethasone in increasing ³H-proline incorporation in cells of the outflow region of the rabbit. Digestion of the tissue with highly purified collagenase indicated that the ³H-proline was incorporated into collagen type protein. This study demonstrates another biologic activity of 5 β-dihydrocortisol, a metabolite found to accumulate in cells cultured from trabeculectomy specimens from patients with primary open angle glaucoma. Invest Ophthalmol Vis Sci 27:1757–1760, 1986

Topical ocular administration of 5 β-dihydrocortisol, a cortisol metabolite, has been shown to potentiate the dexamethasone and cortisol induced high-affinity nuclear binding of the glucocorticoid receptor in the rabbit iris-ciliary body, an early and necessary event in steroid hormone action. In addition, this metabolite was found to potentiate the dexamethasone-induced elevation of intraocular pressure (IOP) in young rabbits. The present study utilizes ³H-proline incorporation to demonstrate an additional glucocorticoid potentiating activity of 5 β-dihydrocortisol. Dexamethasone has been previously found to increase the incorporation of ³H-proline in the outflow pathway cells of the rabbit.

Materials and Methods. Eyes from young albino rabbits (<2 Kg) were enucleated and rinsed in sterile phosphate buffered saline. The anterior segment was then removed by cutting the sclera 2 mm posterior of the limbus. The central region of the cornea was removed. The tissue was then sectioned radially into slices 1–2 mm thick. The tissue slices contained the outflow region, adjacent sclera, cornea, and iris-ciliary body. The explants were incubated at 37°C in 1 ml of Dulbecco's modified Eagle medium supplemented with
Fig. 1. Localization of incorporated $^3$H-proline in the outflow pathway cells of the rabbit. Autoradiography of 2 $\mu$m section from an explant incubated with $^3$H-proline. The silver grains were counted over trabecular meshwork cells (TM) surrounding the aqueous plexus (AP). Sclera (S). Exposure time 4 days at 4°C (toluidine blue, x250).

2.5% dialyzed fetal bovine serum, freshly dissolved ascorbic acid (100 $\mu$g/ml), 10 $\mu$Ci/ml $^3$H-proline (Spec. Act 160 Ci/mmol), with and without the indicated steroids. Crystalline dexamethasone (9-$\alpha$-fluoro-16-methyl prednisolone) and 5 $\beta$-dihydrocortisol (11$\beta$,17,21 trihydroxy-$\beta$-pregnane-3,20-dione) were purchased from Steroloids Inc (Wilton, NH) and Research Plus (Bayonne, NJ), respectively. Stock solutions of the steroids ($10^{-2}$ M) were prepared in ethyl alcohol and stored at -20°C. Aliquots were evaporated under nitrogen and dissolved in distilled water to a final concentration of $10^{-5}$ M, filter sterilized, and further diluted aseptically in incubation media. After incubation for 24 hr at 37°C, the explants were fixed in 2.5% glutaraldehyde for 8 hr, washed in phosphate buffer, and embedded in Epon 812. Sections 1–2 $\mu$m thick were placed on slides and dipped in NTB2 nuclear track emulsion (Kodak, Rochester, NY), exposed for 4–5 days at 4°C, developed, and stained with toluidine blue.

Silver grains were counted within 100 $\mu$m$^2$ areas superimposed to individual cells of the outflow pathway region. Two or three explants were incubated in separate tubes under identical conditions in each experiment. At least 50 areas of 100 $\mu$m$^2$ were counted in the outflow region of each explant. The results are expressed as number of silver grains per 100 $\mu$m$^2$ ± standard deviation (SD). A student's t-test was used to determine the significance of differences.

In one experiment, explants were incubated with $^3$H-proline with and without $10^{-7}$ M dexamethasone as described above, and the tissues were fixed in 2.5% glutaraldehyde and embedded in paraffin. Sections 6 $\mu$m thick were digested with 145 units/ml of a highly purified Clostridium collagenase (Collagenase form III, Advance Biofactures Corp., Lynbrook, NY) overnight at 37°C in the presence of N-ethylmaleimide, conditions found to minimize nonspecific proteolysis. Control slides were incubated in the same buffer without enzyme. After digestion, the slides were washed in phosphate buffer, dried and processed for autoradiography.

The handling of the rabbits in this investigation adhered to the ARVO Resolution on the Use of Animals in Research.

Results. Autoradiography of explants incubated with $^3$H-proline for 24 hr show incorporation of the precursor into cells of the outflow pathway region (Fig. 1). Dexamethasone resulted in an increase in $^3$H-proline incorporation in the cells of the outflow pathway (as reported previously). Digestion of the tissue with highly purified collagenase virtually eliminated the incorporated $^3$H-proline in the explants (Fig. 2A, B). This indicated that the $^3$H-proline is incorporated into col...
lagen type protein. Figure 3 shows the effects of several concentrations of dexamethasone or 5 β-dihydrocortisol on the incorporation of 3H-proline in these cells. As can be seen, 4 × 10^{-9} M dexamethasone was fully active in causing an increase in 3H-proline incorporation; larger concentrations of this steroid did not result in any further increase in incorporation. Dexamethasone at 2 × 10^{-9} M was inactive as was 10^{-8}, 10^{-7}, and 10^{-6} M 5 β-dihydrocortisol. Figure 4 shows the effects of various concentrations of 5 β-dihydrocortisol in combination with 2 × 10^{-9} M dexamethasone (a subthreshold dose). As can be seen 10^{-7} M 5 β-dihydrocortisol potentiated the subthreshold dose of dexamethasone in causing an increase in 3H-proline incorporation.

Discussion. The present study shows that 5 β-dihydrocortisol can potentiate subthreshold levels of dexamethasone in increasing collagen synthesis in cells of the outflow region of the rabbit. This potentiation of collagen synthesis may be the mechanism by which the metabolite potentiates the dexamethasone-induced increase in IOP in these animals.

Normal mammalian tissues metabolize cortisol to the tetrahydrocortisols with no significant accumulation of the intermediate dihydrocortisols. The tetrahydrocortisols are then conjugated with glucuronide and excreted in the urine. The dihydrocortisols were previously thought to be inactive cortisol metabolites. A number of studies, however, have indicated these metabolites are biologically active. These studies include potentiation by 5 β-dihydrocortisol of the glucocorticoid effects in the young rabbit: (a) induction of high-affinity nuclear binding of the glucocorticoid receptor in the iris-ciliary body, (b) the increase in IOP, and (c) the present observation of an increase in collagen synthesis. In addition, dihydrocortisol (5 α) has been shown to have weak mineralocorticoid activity and to potentiate the activity of low concentrations of aldosterone in the rat. A recent study demonstrated that dihydrocortisol (isomer unidentified) could inhibit angiogenesis in the presence of heparin or a heparin fragment in the chick embryo choioallantoic membrane.

The importance of the biologic activity of dihydrocortisols to POAG is based on the observation that cells cultured from trabeculectomy specimens from patients with POAG had abnormal cortisol metabolism resulting in accumulation of large quantities of these metabolites. To the extent that these metabolites are produced in vivo and have similar glucocorticoid potentiating activity in man, they may be the basis for the sensitivity of POAG patients to exogenous glucocorticoids and for their ocular hypertension.

Key words: 5 β-dihydrocortisol, potentiation of glucocorticoid activity, collagen synthesis, rabbit outflow tissue


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References


Retinoblastoma: A Primitive Tumor With Multipotential Characteristics

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The histogenesis of retinoblastoma, the most common intraocular tumor of childhood, has been recently attributed to a primitive retinal cell which is capable of differentiation into both neuronal and glial elements.1 By manipulating the culture medium constituents and using differentiating agents, such as dibutyryl-cyclic AMP and butyrate, we have now been able to differentiate a population of Y-79 human retinoblastoma cells into cells of pigment epithelial character, as well as into cells of neuronal or glial nature. These studies additionally implicate the outer layer of the optic vesicle in retinoblastoma etiology, and introduce a new system for studying the biologic properties of primitive neural tumors as well as the embryonic development of the retina and central nervous system. Invest Ophthalmol Vis Sci 27:1760–1764, 1986

Y-79 human retinoblastoma cells in monolayer culture can be induced to partly differentiate morphologically and biochemically into cells which demonstrate neuronal and glial characteristics.1,2 Photoreceptor differentiation of these cells has also been achieved as assessed by their ability, under proper conditions,4 to synthesize and secrete the interphotoreceptor retinoid-binding protein (IRBP), a glycoprotein known to be produced by retinal photoreceptor cells.3,6 More recently, we have been able to achieve firm attachment and morphological differentiation of 20–30% of initially seeded Y-79 cells by plating them in serum-free medium containing 10 μg/ml laminin (LN) for 3 days and, subsequently, replacing laminin with 2 mM butyrate of 4 mM dibutyryl cyclic AMP (db-cAMP).7 We now report that, in this system, butyrate supports the differentiation of a population of Y-79 cells into cells of pigment epithelial character, while dibutyryl cyclic AMP (db-cAMP) supports the development of cells more similar to conventional neurons.

Materials and Methods. Y-79 cells were cultured as previously described.7 Briefly, cells in suspension culture were centrifuged, dissociated by pipetting after addition of 5 ml of culture medium, and diluted to a final density of 4 × 10⁶ cells/ml. The culture medium consisted of Eagle’s Minimum Essential Medium (MEM) with Earle’s salts, supplemented with 2 mM glutamine (Gibco, Grand Island, NY), 5 μg/ml insulin, 5 μg/ml transferrin, 4 μg/ml sodium selenite, 5 ng/ml putrescine, and 5 μg/ml progesterone, as described by Bottenstein.8 Laminin (10 μg/ml) was added when appropriate. Two milliliter portions of the cell suspension were seeded in 35 mm tissue culture plastic dishes (for electron microscopy), or tissue culture plastic chamber slides (Miles Scientific, Naperville, IL) (for immunohistochemistry). Three days after plating, floating cells were removed by replacement of the culture medium with fresh serum-free medium without laminin, containing 2 mM sodium butyrate or 4 mM dibutyryl-cAMP (Sigma, St. Louis, MO). This medium was changed every 3 days thereafter, and the cells were tested on the 12th day of culture.

For transmission electron microscopy, cells were scraped from the dishes, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 hr at 23°C, postfixed in O3O4, and embedded in Maraglas 655 (Ladd Research Industries, Burlington, VT). Sections...