The effects of methylprednisolone acetate on macromolecular synthesis and glucose oxidation in epithelial cells of the ocular surface

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Epithelial cells that re-epithelialized corneas after the normal corneal epithelium was removed limbus to limbus by scraping were found to be metabolically responsive to methylprednisolone acetate. Seventy-two hours after scraping, the re-epithelializing cells from steroid-treated animals were found to have a 26% slower rate of DNA synthesis, a 30% slower rate of glucose oxidation via glycolysis, and a 79% greater rate of RNA synthesis with no apparent change in gross protein synthesis as compared with vehicle-treated controls. The DNA content of the epithelial layer isolated from steroid-treated corneas was reduced by 31% as compared with that of vehicle-treated controls. (INVEST OPHTHALMOL VIS SCI 23:501-506, 1982.)

Key words: epithelium, methylprednisolone acetate, DNA, RNA, protein, glucose

Glucocorticoids are potent metabolic effectors. The nature of the effects observed when cells are treated with steroids is dependent on numerous factors, including cell type, conditions of treatment, and concentration of steroid.1 There are metabolic functions that steroids can either inhibit or stimulate such as transport, macromolecular synthesis, and gene regulation.2 The mechanisms by which steroids affect cellular processes are unknown; however, all steroid-responsive cells appear to have specific cytoplasmic receptors for steroid binding, the ability to activate the receptor/steroid complex, and the ability to translocate the complex to the nucleus.3 The presence of steroid receptors does not mean that the cell will respond to steroid treatment, however. Cells in this latter category are usually transformed by viruses or chemicals.1

Corneal and conjunctival epithelial cells, as well as cells of the corneal stroma and endothelium, all appear to be able to specifically bind steroids and translocate them to the nucleus.4,5 The data indicate that wound healing by the stromal kerocytes and the corneal endothelium is inhibited by steroid therapy.6,7 Topical prednisolone acetate as well as other steroids have been shown to inhibit epithelial wound closure of epithelial wounds made by scraping limbus to limbus.8 Wound closure rates of 6 mm central corneal epithelial wounds were not affected by steroids. The mitotic rate of corneal epithelial cells has been observed to be dramatically
utilized to maximize the rate of molecular synthesis as well as glucose oxidation were studied. The effects studied of methylprednisolone acetate on DNA, RNA, and protein synthesis were totally denuded by limbus-to-limbus scraping. The effects studied of methylprednisolone acetate on DNA, RNA, and protein synthesis as well as glucose oxidation were investigated. Re-epithelializing corneas were utilized to maximize the rate of molecular synthesis.

Materials and methods

Rabbit and tissue preparation. The corneal epithelium was removed from limbus to limbus from rabbit corneas by scraping with a Gill knife and the aid of a dissecting microscope after the rabbit had been anesthetized with 10 mg/kg ketamine hydrochloride (Ketaset) given intravenously, and two drops of 0.4% Dorsacaine were dropped onto the cornea. Both eyes of each animal were treated identically and the epithelium was always removed between 9 and 10 a.m. to avoid diurnal variation. The rabbits, weighing 2 to 3 kg each, were assigned randomly to one of three groups: (1) no treatment, (2) vehicle, (3) methylprednisolone acetate (Depo-Medrol). The steroid and vehicle were given subconjunctivally immediately after removal of the corneal epithelium (total dose of 18 mg/eye).

After removal of the corneal epithelium and treatment, the rabbits were returned to their cages for 72 hr. The rabbits were then sacrificed, the eyes were enucleated, and the corneas were excised with a scleral rim. The excised corneas were placed in bicarbonate Ringer’s solution containing 0.3 mm reduced glutathione (GBR), 100 U/ml penicillin, and 1.00 mg/ml streptomycin and were incubated for 1 hr at 34°C. All animals were sacrificed between 9 and 10 a.m. on the third day after scraping to avoid diurnal variation.

Thymidine incorporation. The rate of DNA synthesis was measured by incubating the excised corneal tissue in GBR containing 0.1 mM 3H-thymidine (1.0 mCi/μmole). As described above for the measurement of DNA synthesis, the tissue was pulse labeled for 3 hr, washed, and fractionated in perchloric acid by the Schmidt-Thannhauser procedure. The amount of 3H-thymidine incorporated into the DNA fraction during a 3 hr pulse labeling was taken as a measure of the rate of DNA synthesis. All data are expressed as picomoles of 3H-thymidine incorporated per microgram of DNA per unit of time ± the standard error of the mean. Corneas were fractionated and analyzed individually; corneas were not pooled for analysis.

Uridine incorporation. The rate of RNA synthesis was measured by incubating the excised corneal tissue in GBR containing 0.1 mM 3H-uridine (1.0 mCi/μmole). As described above for the measurement of DNA synthesis, the tissue was pulse labeled for 3 hr, washed, and fractionated in perchloric acid by the Schmidt-Thannhauser procedure. The amount of 3H-uridine incorporated into the KOH-soluble but cold perchloric acid-insoluble fraction was a measure of RNA synthesis. Incorporation of 3H-uridine into other fractions such as the DNA and protein fractions served as a control to monitor the fractionation procedure. All data were expressed as the picomoles of 3H-uridine incorporated per microgram of DNA per unit of time ± the standard error of the mean. Corneas were not pooled for analysis.

Leucine incorporation. The rate of protein synthesis was measured by the incubation of excised corneas as described above in GBR containing 2.24 μM L-[4,5-3H(N)]-leucine (45 mCi/μmole). The amount of leucine in the cold soluble fractions was a measure of leucine uptake, while the amount of tritium in the hot acid-soluble fraction, which remained precipitable upon chilling, was a measure of the amount of protein synthesis.

Glucose oxidation. Corneas were excised from the eyes of animals in each of the treatment groups 72 hr after scraping of the epithelium. Either whole corneas or epithelial stripings were then incubated in bicarbonate Ringer’s containing 5.0 mM [14C-6]-glucose (0.015 μCi/μmole). The individual tissues were placed in Erlenmeyer flasks fitted with rubber caps and polyethylene center...
wells and were incubated for 2 hr at 34°C. At the end of the 2 hr incubation, the medium was acidified with 0.3 ml 6N H2SO4 and 0.3 ml of hydroxide of hyamine was added to the center well to trap the 14CO2 produced. After 2 hr, the 14CO2 trapped was quantitated by liquid scintillation counting in Complete Counting Cocktail Neutralizer (Research Products International, Elk Grove Village, Ill.).

**Histology.** Eyes were enucleated from animals in each treatment group and fixed in 4% buffered formalin for 24 hr, after which the corneas were excised with a scleral rim and fixed in 4% formalin for an additional 24 hr. The tissues were then embedded in paraffin, sectioned, mounted, and stained with hematoxylin and eosin.

**Analytical methods.** Scintillation counting, unless otherwise stated, was performed in a Searle Isocap 300 with the external standard method of quench correction. Samples were counted in Insta-Gel by Packard unless otherwise stated.

All nucleosides and the cold acid-soluble nucleotide pools were chromatographed in two thin-layer chromatographic systems: (1) MN-cellulose developed in n-butanol-glacial-acetic acid: H2O 2:1:1 (v/v), (2) silica gel G developed in chloroform:methanol 4:1 (v/v). The thin layer in the area of internal standards was visualized under short-wave ultraviolet light and removed by scraping. The amount of radioactivity in the thin layer was quantitated by scintillation counting after elution of the isotope from the thin layer into 1 ml of water.

**Materials.** All isotopes were purchased from New England Nuclear, Boston, Mass.: L-[4,5-3H(N)]-leucine, [5,6-3H]-uridine, [methyl-3H]-thymidine, and [6-14C]-glucose. Methylprednisolone acetate (Depo-Medrol) and its vehicle were provided by the Upjohn Co., Kalamazoo, Mich. The nonradioactive nucleoside and nucleotide standards were purchased from P-L biochemicals, Milwaukee, Wis.

**Results**

**DNA content and histology.** The DNA content of epithelial stripings obtained from corneas 72 hr after scraping and initiation of treatment with steroids was 28.4 ± 1.3 μg/cornea (n = 20), while corneas from the control groups contained 41.1 ± 4.0 μg/cornea (n = 12). The difference in DNA content between these groups was significant at the p < 0.01 level as determined by the Student's t test. The mean area of the corneas used in this study was 1.2 ± 0.1 cm². The fact that the DNA content of the corneal epithelium was reduced in the steroid-treated corneas suggested the presence of fewer cells.

![Fig. 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933335/)
in the epithelial layers of these corneas. Histologic sections obtained from control and steroid-treated groups after 72 hr of healing showed that one thin layer of epithelial cells covered the corneas of steroid-treated animals, while two or three layers of cells covered the control corneas (Fig. 1).

**Rate of incorporation of 3H-thymidine.**
The rate of DNA synthesis as measured by a 3 hr pulse labeling showed that 3H-thymidine was incorporated into the DNA-containing fraction of control cells at 4.3 ± 0.8 pmoles/μg DNA (n = 4), while steroid-treated corneas incorporated thymidine at 2.9 ± 0.2 pmoles/μg DNA (n = 4). Despite the decreased rate of DNA synthesis, the rate of incorporation of 3H-thymidine into the acid-soluble pool was 13.1 ± 1.1 and 15.9 ± 1.0 pmoles/μg DNA for control and steroid-treated epithelial cells, respectively. Preliminary chromatography of the acid-soluble fractions indicated that less thymidine 5′-triphosphate was formed from the 3H-thymidine taken up in the presence of steroids.

**Rate of incorporation of 3H-leucine.**
The rate of RNA synthesis as measured by a 3 hr pulse of 3H-uridine into the RNA fraction of epithelial cells was 6.8 ± 1.2 pmoles/μg DNA (n = 6) in control corneas and 12.2 ± 0.8 pmoles/μg DNA (n = 6) in steroid-treated corneas. The difference was significant at p < 0.01. Analysis of the acid-soluble fractions indicated that the rate of incorporation of uridine into the nucleotide pool was not altered in the steroid-treated epithelial cells.

**Rate of incorporation of 3H-leucine.**
The acid-precipitable protein obtained from control corneas after removal of the DNA and RNA showed that leucine was incorporated at a rate of 1.6 ± 0.6 pmoles/μg DNA (n = 8). Leucine was incorporated into the protein of steroid-treated corneas at 1.8 ± 0.6 pmoles/μg DNA (n = 8) during a 3 hr pulse. Although there was no significant difference in the rate of protein synthesis, the rate of incorporation of leucine into the acid-soluble fraction of steroid-treated epithelial cells was twice as great as in control corneas (i.e., 14.4 ± 0.9 vs. 28.8 ± 5.1 pmoles/μg DNA, n = 8 for each).

**Glucose oxidation.** The oxidation of glucose to CO₂ via the Emden Meyheroff and tricarboxylic acid pathways of glucose oxidation was significantly reduced in steroid-treated epithelial tissues. A significantly lower rate of 14CO₂ production from [14C-6]-glucose was observed when either whole corneas or epithelial stripings from the corneas of steroid-treated rabbits were used. During 2 hr incubations, the rates were 0.16 ± 0.01 pmoles/μg DNA (n = 8) for control epithelial tissues and 0.10 ± 0.02 pmoles/μg DNA (n = 8). The difference in glucose oxidation rate was significant at the level of p < 0.02.

**Discussion**
The responses of cells to glucocorticoids have confused investigators for years because of the diversity of biologic effects observed. The conditions of steroid treatment and the time at which the biologic parameters are measured can dictate the result. We selected to utilize tissue re-epithelialization in situ for our study because the rapidly proliferating cells gave us measurable parameters and allowed epithelial/mesodermal interactions, which appear to be important in the response of epithelial cells to factors that influence their growth.

The response of epithelial cells to topical therapy is typified by the mouse dermis. DNA synthesis was suppressed for 5 days after a single dose of dexamethasone, while RNA synthesis was stimulated shortly after therapy. On the tenth day after therapy, DNA synthesis was actually increased above normal levels and RNA synthesis returned to normal levels. In studies on the corneal epithelium of a rat, the mitotic rate was suppressed for at least 24 hr by a single intraperitoneal dose of dexamethasone. After the suppression, a burst of mitotic activity was observed during the next 24 hr period. The burst of mitotic activity was inhibited by a second dose of steroid. The mechanism by which mitosis was regulated was not reported.

Our data clearly indicate that corticosteroid therapy inhibited DNA synthesis or altered the circadian rhythm of DNA synthesis in re-epithelializing rabbit corneas.
stimulation of RNA synthesis and leucine uptake as well as the inhibition of glucose oxidation concurrently with inhibition of DNA synthesis have been reported in other epithelial cell systems. Studies by Srinivasan and Kulkarni have shown that the rate of re-epithelialization, as measured by wound closure rates, was significantly reduced in animals treated with prednisolone acetate.

The mechanism by which steroids exert the reported metabolic effects remains unclear. The epithelial cells of the cornea and conjunctiva appear to have specific steroid receptors and the ability to translocate the receptor/steroid complex to the nucleus; therefore, one may hypothesize that the mechanisms are similar to those of other steroid-responsive cells. The data reported here suggest that nucleoside transport was not inhibited because the total amount of uridine and thymidine incorporated into cytoplasmic pools was not reduced, although a slight reduction in the conversion of thymidine to thymidine-5'-triphosphate was observed. Our data cannot be used to determine whether glucose transport is truly reduced because we measured the evolution of 14CO2. Studies with a nonmetabolizable substrate such as 3-O-methylglucose would be required to evaluate glucose transport. Our leucine incorporation data were similar to those reported for other epithelial tissues in that the total amount of leucine uptake increased during the time frame of steroid therapy.

We have not measured the specific activity of any key metabolic enzymes to determine whether steroids induce the synthesis of enzymes important to metabolic regulation. The fact that RNA synthesis is increased in these tissues suggests that steroids may regulate the expression of specific genes, as hypothesized by others.

These data indicate that the metabolic interaction of corticosteroids with corneal and conjunctival cells requires further investigation. The lack of epithelial response to steroids observed by others may have been due to the insensitivity of the parameters measured or observed and the time frame under which the parameters were observed.

REFERENCES


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