The Use of Mucoadhesive Polymers to Enhance the Hypotensive Effect of a Melatonin Analogue, 5-MCA-NAT, in Rabbit Eyes

Vanessa Andrés-Guerrero, Irene T. Molina-Martínez, Assumpta Peral, Beatriz de las Heras, Jesús Pintor, and Rocío Herrero-Vanrell

PURPOSE. 5-Methoxy-carbonylamino-N-acetyltryptamine (5-MCA-NAT, a melatonin receptor agonist) produces a clear intraocular pressure (IOP) reduction in New Zealand White rabbits and glaucomatous monkeys. The goal of this study was to evaluate whether the hypotensive effect of 5-MCA-NAT was enhanced by the presence of cellulose derivatives, some of them with bioadhesive properties, as well as to determine whether these formulations were well tolerated by the ocular surface.

METHODS. Formulations were prepared with propylene glycol (0.275%), carboxymethyl cellulose (CMC, 0.5% and 1.0%) of low and medium viscosity and hydroxypropylmethyl cellulose (0.3%). Quantification of 5-MCA-NAT (100 µM) was assessed by HPLC. In vitro tolerance was evaluated by the MTT method in human corneal-limbal epithelial cells and normal human conjunctival cells. In vivo tolerance was analyzed by biomicroscopy and specular microscopy in rabbit eyes. The ocular hypotensive effect was evaluated measuring IOP for 8 hours in rabbit eyes.

RESULTS. All the formulations demonstrated good in vitro and in vivo tolerance. 5-MCA-NAT in CMC medium viscosity 0.5% was the most effective at reducing IOP (maximum IOP reduction, 30.27%), and its effect lasted approximately 7 hours.

CONCLUSIONS. The hypotensive effect of 5-MCA-NAT was increased by using bioadhesive polymers in formulations that are suitable for the ocular surface and also protective of the eye in long-term therapies. The use of 5-MCA-NAT combined with bioadhesive polymers is a good strategy in the treatment of ocular hypertension and glaucoma. (Invest Ophthalmol Vis Sci. 2011;52:1507–1515) DOI:10.1167/iovs.10-6099
As in the pineal gland, retinal melatonin content significantly changes during the 24-hour cycle, with a peak concentration occurring at night.\textsuperscript{38,39} Studies in humans revealed a diurnal rhythm of IOP with the highest level occurring during daytime and the lowest at night,\textsuperscript{41–44} when melatonin levels increase. In view of this relation, it has been suggested that melatonin influences IOP rhythm.\textsuperscript{38}

5-Methoxy-carbonylamo-Y-acetyltryptamine (5-MCA-NAT) is described as an MT<sub>1</sub> melatonin receptor agonist, although there is still some controversy about the identification of its binding site.\textsuperscript{45–48} 5-MCA-NAT has been demonstrated to reduce IOP in glaucomatous monkeys\textsuperscript{49} and in New Zealand White rabbits.\textsuperscript{50} Consequently, it has been regarded as a potential substance for the treatment of ocular hypertension, which is associated with glaucoma. In this work, we prepared several formulations containing 5-MCA-NAT and bioadhesive polymers. The polymers used as vehicles were carboxymethyl cellulose low viscosity (0.5% and 1.0%), carboxymethyl cellulose medium viscosity (0.5% and 1.0%), and hydroxypropylmethyl cellulose (0.3%). The active substance was first dissolved in propylene glycol, which is recognized by the U.S. Food and Drug Administration (FDA) as an ophthalmic demulcent.\textsuperscript{51} We evaluated the improvement of the hypertensive effect of 5-MCA-NAT by these polymers after instillation in rabbit eyes.

**Materials and Methods**

**Compounds**

5-MCA-NAT was provided by Tocris Bioscience (Ellisville, MO) and 1,2-propylene glycol (PG) by Guinama (Valencia, Spain). Carboxymethyl cellulose low viscosity (CMC1; 25–50 cps, 2% solution at 20°C), carboxymethyl cellulose medium viscosity (CMC2; 400–800 cps, 2% solution at 20°C), and hydroxypropylmethyl cellulose (HPMC; 1390 cps, 2% solution at 20°C) were purchased from Abaran Materias Primas SL (Madrid, Spain). Phosphate-buffered saline (PBS) was purchased from Invitrogen-Gibco. The mixture was centrifuged (3000 rpm, for 4 minutes at room temperature) and finally, contained 10% calf serum and 2% penicillin-streptomycin. Then it was sterilized first by filtration (0.20-µm filter pore) and then by autoclaving (121°C, 20 minutes). All the procedures were performed under aseptic conditions.

The dose of 5-MCA-NAT in formulations was quantified by HPLC under the conditions described earlier.

**Characterization of Formulations: Surface Tension and pH**

The surface tension of each formulation was determined by the plate method (PL21; Krüss, Hamburg, Germany), with a digital tensiometer (model K11; Krüss, Hamburg, Germany) in combination with the integrated software (Laboratory Desktop software, ver. 3.11; Krüss) at 33 ± 0.2°C (corneal surface temperature).\textsuperscript{52–55} The plate was immersed in the solution (speed of 8 mm/min) to a depth of 2.0 mm. Before each measurement, the tensiometer was calibrated with double-distilled water (72 ± 0.5 mN/m). The time required for equilibration of the formulations was set to 3 minutes. The formulations were assayed in triplicate, and the measurements were repeated three times for every formulation.

pH measurements of formulations were performed at room temperature (25°C) with a pH meter (model GLP 21; Crison, Barcelona, Spain) equipped with a combined Ag/AgCl glass electrode (model 52/02; Crison).

**Viability Assays**

The viability assays were performed with immortalized human corneal-limbal epithelial cells (HCLE cells; Schepens Eye Research Institute, Harvard Medical School, Boston, MA) and normal human conjunctival cells (IOBA-NHC; Instituto de Oftalmobiologia Aplicada, Valladolid University, Valladolid, Spain).

Cytotoxicity studies were assessed by the mitochondrial-dependent reduction of the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan (MTT method).\textsuperscript{54,55}

HCLE cell cultures were plated in 15-mL culture flasks in a keratinocyte serum-free medium (SEM), a medium nutritionally optimized for proliferation of keratinocytes, supplemented with 0.5 mL CaCl<sub>2</sub> 0.3 M, 1.25 mL bovine pituitary extract and 40 µL epidermal growth factor (EGF). Cultures were grown at 37°C in a 5% carbon dioxide atmosphere. To inactivate trypsin, this mixture was neutralized with the same volume of Dulbecco’s modified Eagle’s medium: nutrient mixture F-12 (DMEM/F12), which contained 10% calf serum and 2% penicillin-streptomycin. All reagents were purchased from Invitrogen-Gibco (Barcelona, Spain). The mixture was centrifuged (3000 rpm, for 4 minutes at room temperature), and finally, the cells were resuspended in SEM.

IOBA-NHC cells were plated at 15-mL culture flasks in DMEM/F-12 medium supplemented with 10% calf serum, 2% penicillin-streptomycin, 2.5 µg/mL amphotericin B, 1 µg/mL bovine pancreas insulin, 0.5 µg/mL hydrocortisone, 0.1 µg/mL cholaer toxin, and 0.2 ng/mL EGF. Cultures were grown at 37°C in a 5% carbon dioxide atmosphere. The mixture was neutralized with the same volume of Dulbecco’s modified Eagle’s medium: nutrient mixture F-12, which contained 10% calf serum and 2% penicillin-streptomycin. Then it was centrifuged (1000 rpm, for 5 minutes at room temperature) and finally, the cells were resuspended in culture medium. All reagents were purchased from Invitrogen-Gibco.

For the cytotoxicity studies, cells were seeded into 96-well culture plates (50,000 cells/well). After adhering to plates (37°C for 24 hours in an atmosphere of 5% CO<sub>2</sub>/95% air), the medium was removed and the testing formulation was added.\textsuperscript{57}

Cell lines were exposed to (1) formulations with 5-MCA-NAT (100 µM) and the corresponding vehicle (CMC1 0.5%, CMC1 1.0%, CMC2 0.5%, CMC2 1.0%, HPMC 0.3%, or PBS) and (2) vehicles without 5-MCA-NAT. All the formulations (with and without 5-MCA-NAT) contained PG (0.275%).

The exposure time of cells to formulations was set at 15 minutes, 1 hour, and 4 hours to simulate short- and long-term treatments. After that, the media were carefully removed, and the MTT solution (5 mg/mL in PBS) was added to the plates, which were then incubated for 5 hours at 37°C. After careful aspiration of the MTT solution, the cells were solubilized in DMSO (100 µL/well). MTT and DMSO were purchased from Sigma-Aldrich (Madrid, Spain). The extent of the reduc-
Table 1. Experimental Concentrations Obtained for Formulations with 5-MCA-NAT

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Experimental Results (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC1</td>
<td>0.5% 27.59 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>1%   27.80 ± 0.19</td>
</tr>
<tr>
<td>CMC2</td>
<td>0.5% 27.25 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>1%   27.93 ± 0.21</td>
</tr>
<tr>
<td>HPMC</td>
<td>0.3% 28.02 ± 0.41</td>
</tr>
<tr>
<td>PBS</td>
<td>27.50 ± 0.15</td>
</tr>
</tbody>
</table>

n = 3. Data are expressed as micrograms per milliliter. Initial solutions of 5-MCA-NAT in PG (10 mg/mL) were always used to prepare the formulations. All formulations contained PG 0.275%. The theoretical concentration of 5-MCA-NAT was 27.53 µg/mL.

Statistical Analysis

Data are expressed as the mean ± SEM (n > 3, indicated in each case). Significant differences between two mean values were evaluated by two-tailed Student’s t-test. If necessary, an analysis of variance (ANOVA) was used. Results were taken as significantly different at P < 0.05.

The plotting and fitting of dose–response curves was performed with commercial software (OriginPro 8 SR2; Originlab, Northampton, MA).

Results

Determination of the Dose of 5-MCA-NAT in Formulations

The HPLC method of quantifying 5-MCA-NAT was validated with respect to linearity, accuracy, and reliability in the range of concentrations between 5 and 50 µg/mL. The retention time of 5-MCA-NAT was 10.66 ± 0.54 minutes.

The chromatographic method separated CMC1, CMC2, and HPMC from 5-MCA-NAT. In all cases, the retention time of the vehicles was found to be smaller (±2.5 minutes) than the one obtained for 5-MCA-NAT. Results are summarized in Table 1.

In all cases, nonsignificant differences (P > 0.05) were observed between the theoretical concentration of 5-MCA-NAT and the experimental values obtained for the formulations. Taking into account that the instillation volume of the formulations in rabbit eyes was 25 µL, the administered dose of 5-MCA-NAT was 0.693 ± 0.01 µg.

Characterization of Formulations

The resulting data for surface tension and pH are shown in Table 2. All the formulations were nearly neutral, but they showed pH significantly lower than the corresponding value of PBS (P < 0.001 in all cases).

Surface tension of PBS (solvent of the formulations) was measured and used as a reference. No significant differences were found between the surface tension achieved with PBS (71.13 ± 0.5 mN/m) and that obtained with ultrapure water (72 ± 0.5 mN/m; P = 0.095). On the other hand, the surface tension for the rest of the formulations (with 5-MCA-NAT, PG, and the corresponding vehicles) were significantly lower than the reference in all cases (P < 0.001). The result obtained for 5-MCA-NAT/PBS (64.67 ± 1.6 mN/m) was found to be the closest to PBS, whereas the formulation 5-MCA-NAT/CMC2 1.00% achieved the lowest value (46.51 ± 1.64 mN/m).

Table 2. Physicochemical Parameters of the Formulations of 5-MCA-NAT 100 µM, PG 0.275%, and the Vehicles Dissolved in Isotonic PBS

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Concentration</th>
<th>pH (SD)</th>
<th>Surface Tension mN/SD (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC1</td>
<td>0.50%</td>
<td>7.07 (0.03)</td>
<td>60.26 (0.380)</td>
</tr>
<tr>
<td></td>
<td>1.00%</td>
<td>7.02 (0.02)</td>
<td>53.89 (2.474)</td>
</tr>
<tr>
<td>CMC2</td>
<td>0.50%</td>
<td>7.05 (0.01)</td>
<td>49.87 (0.652)</td>
</tr>
<tr>
<td></td>
<td>1.00%</td>
<td>7.07 (0.03)</td>
<td>46.51 (1.641)</td>
</tr>
<tr>
<td>HPMC</td>
<td>0.30%</td>
<td>7.09 (0.01)</td>
<td>49.42 (3.769)</td>
</tr>
<tr>
<td>PBS</td>
<td>7.06 (&lt;0.01)</td>
<td>64.67 (1.614)</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of three determinations. PBS pH 7.2.
the contrary, no significant differences were found when the formulations are compared with CMC1 1.0% \( (P = 0.29) \), CMC2 1.0% \( (P = 0.21) \), and HPMC 0.3% \( (P = 0.15) \).

Data regarding the area under the \( \Delta \text{IOP} \)–time curve from 0 to 8 hours (AUC) are shown in Table 5. The AUC of the reference formulation (5-MCA-NAT/PBS) was significantly lower than the values obtained for formulations with CMC1 0.5% and with CMC2 0.5% \( (P = 0.02 \) and \( P = 0.0003 \), respectively). However, there were no significant differences in the AUCs for the formulations with CMC1 1.0% \( (P = 0.98) \), CMC2 1.0% \( (P = 0.19) \), and HPMC 0.3% \( (P = 0.18) \).

The mean time effect, which is a term used to define the average duration of the hypotensive effect, was considered to be the period in which the IOP value was at or below half of the maximum value achieved in the experiment. The formulation composed of 5-MCA-NAT and PBS (reference formulation) produced an effect that lasted approximately 6 hours. This effect was exceeded only by the formulation that contained the bioadhesive agent CMC2 0.5%, which lasted approximately 7 hours (Table 5).

**Short-Term Tolerance Evaluation**

The images taken with the specular microscope, both before and after instillation, showed a uniform distribution of the corneal endothelial cells in size and shape for all the formulations. The cellular distribution was within the expected range for rabbit corneas (2000–2500 cells/mm²).

The study performed by means of the slit lamp showed a slight redness in the free margins of the eyelids just before instillation in all cases, as a natural state of the rabbit eyes (Table 7).

None of the vehicles produced irritation or discomfort in the rabbit eyes up to 6 hours after administration. Tearing, redness, and blepharospasm were not visible with any formulation.

**Discussion**

The present experimental work describes the ocular hypotensive effect of six formulations containing 5-MCA-NAT, a melatonin receptor agonist and different cellulose derivatives used as vehicles (carboxymethyl- and hydroxypropylmethyl cellulose). We obtained formulations that were demonstrated to be well tolerated by the ocular surface. In addition, the polymers present in some of the formulations increased the hypotensive effect of 5-MCA-NAT.

Glaucoma is a chronic disease that commonly leads to loss of visual acuity and visual field. Most forms of glaucoma are associated with high IOPs; in these cases, chronic therapies are frequently associated with some adverse effects, such as a solution of benzalkonium chloride (BAK, 0.005%) was used as the positive control.
as allergic reactions, allergic contact dermatitis, or punctate corneal staining. Moreover, several studies have demonstrated that dry eye syndrome or damage of the corneal epithelium are usual after the long-term administration of topical antihypertensive agents.

The ocular toxicity of topical treatments is frequently associated with the preservative used in the formulation. Most of preservatives have been described to increase macrophages, lymphocytes, and fibroblasts in the conjunctiva and Tenon capsule. Furthermore, they can also destabilize the tear film indirectly by decreasing the density of goblet cells in the conjunctival epithelium compromising the ability of the tear film to provide protection and trophic factors to the cornea.

### Table 5. Eight-Hour Time Course of the Ocular Hypotensive Effect of Six Formulations with 5-MCA-NAT, PG (0.275%), and the Vehicles or PBS

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>CMC1 0.5%</th>
<th>CMC1 1.0%</th>
<th>CMC2 0.5%</th>
<th>CMC2 1.0%</th>
<th>HPMC 0.3%</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>1</td>
<td>94.60 ± 7.60</td>
<td>85.17 ± 7.51</td>
<td>77.52 ± 7.02</td>
<td>88.38 ± 7.57</td>
<td>91.34 ± 8.52</td>
<td>86.16 ± 6.75</td>
</tr>
<tr>
<td>2</td>
<td>88.99 ± 3.13</td>
<td>82.00 ± 6.39</td>
<td>76.95 ± 3.04</td>
<td>85.89 ± 6.30</td>
<td>89.03 ± 5.51</td>
<td>86.98 ± 5.47</td>
</tr>
<tr>
<td>3</td>
<td>87.10 ± 4.37</td>
<td>86.50 ± 5.41</td>
<td>77.09 ± 6.86</td>
<td>86.52 ± 5.78</td>
<td>87.38 ± 4.43</td>
<td>85.59 ± 5.71</td>
</tr>
<tr>
<td>4</td>
<td>88.05 ± 2.35</td>
<td>87.82 ± 3.99</td>
<td>80.55 ± 3.10</td>
<td>89.14 ± 5.37</td>
<td>93.04 ± 6.80</td>
<td>87.03 ± 4.25</td>
</tr>
<tr>
<td>5</td>
<td>95.85 ± 4.92</td>
<td>88.59 ± 5.51</td>
<td>80.96 ± 8.85</td>
<td>90.80 ± 8.10</td>
<td>93.20 ± 4.72</td>
<td>84.51 ± 6.10</td>
</tr>
<tr>
<td>6</td>
<td>91.96 ± 6.19</td>
<td>90.48 ± 7.37</td>
<td>85.21 ± 5.64</td>
<td>94.88 ± 6.27</td>
<td>89.22 ± 6.50</td>
<td>91.05 ± 6.10</td>
</tr>
<tr>
<td>7</td>
<td>100.66 ± 7.35</td>
<td>97.51 ± 4.44</td>
<td>87.20 ± 6.04</td>
<td>95.72 ± 6.73</td>
<td>91.45 ± 5.74</td>
<td>93.98 ± 5.29</td>
</tr>
<tr>
<td>8</td>
<td>99.66 ± 6.57</td>
<td>97.51 ± 4.45</td>
<td>91.81 ± 7.58</td>
<td>99.72 ± 6.55</td>
<td>91.45 ± 6.65</td>
<td>98.72 ± 7.20</td>
</tr>
</tbody>
</table>

Data are represented as mean percentage ± SD of 10 determinations. Bold data represent the mean time (hours) in which the duration of the effect is maintained (mean time effect).

### Figure 1. Ocular hypotensive effect of 5-MCA-NAT 100 μM in six different vehicles: (a) PBS, (b) CMC1 0.5%, (c) CMC1 1.0%, (d) CMC2 0.5%, (e) CMC2 1.0%, and (f) HPMC 0.3%. The corresponding vehicles without 5-MCA-NAT are considered as controls in each case. Data are represented as the mean ± SEM of 10 determinations.
chloride, damage to the corneal epithelial cells and inhibition of the growth of trabecular meshwork cells have been reported.10

In most cases, ocular surface modifications directly impact the effectiveness of long-term therapies. Fechtner et al.70 demonstrated that ocular surface disease symptoms in patients with glaucoma using topical IOP-lowering therapy correlates positively with the number of IOP-lowering medications used. From the clinical point of view, the management of patients with glaucoma or ocular hypertension who also have dry eye syndrome must reduce eye drop-induced toxicity as well as develop a specific treatment that is well tolerated by the ocular surface. Extending the limits of tolerability, by improving the tolerability of medications, should become one of the key objectives of glaucoma therapy. Attending to these facts, the employment of polymers that combine good properties for the ocular surface with the ability of increasing the ocular contact time of the formulation is a good strategy for the treatment of glaucoma. Furthermore, the increase in ocular contact time may add to the bioavailability of the drug, that so the drug dosage and the number of instillations could be reduced.

In the present study, IOP-lowering formulations with no preservative in their composition were prepared. Each ingredient of the ophthalmic formulations developed in this work was selected from FDA-approved ophthalmic products.75

Before elaborating the formulations, we prepared stock solutions of 5-MCA-NAT in PG. PG is recognized by the FDA as an ophthalmic demulcent that protects and lubricates mucous membrane surfaces and relieves dryness and irritation. Therefore, PG is widely used as a solvent, humectant, and preservative substance in commercial ophthalmic preparations at concentrations ranging between 0.3% and 1%. In a previous work,22 we conducted solubility assays to demonstrate that PG dissolved the drug completely in a concentration of 10 mg/mL. For the final formulations, stock solutions of 5-MCA-NAT in PG were diluted with the vehicle (carboxymethyl cellulose or hydroxypropylmethyl cellulose) to obtain a final PG concentration of 0.275% and the corresponding percentage of the polymer.

All cellulose ethers impart viscosity to ophthalmic formulations, have wetting properties, and increase the contact time of solutions by virtue of film-forming properties.30,71–75 Some of them (e.g., hydroxypropylmethyl cellulose) exhibit surface active properties and interact with components of the tear film, increasing its stability.76 Carboxymethyl cellulose also possesses mucoadhesive properties.75 It is believed that mucadhesion takes place when the acidic groups of the bioadhesive polymers link with the sialic moieties present in the mucins of the eye although, at the moment, no theory has successfully explained this mechanism.77

The effects of the formulations of 5-MCA-NAT described herein were compared by using the maximum hypotensive effect of the active compound (percentage) the AUC of the $\Delta$IOP (percentage) versus time (hours) from 0 to 8 hours, the mean time (hours) in which the duration of the hypotensive effect is maintained, mean time effect, and the lower number of toxic/secondary effects of the vehicles.

The formulation 5-MCA-NAT 100 µM/PBS produced a maximum IOP reduction of 18.11%, and it was taken as the reference. The hypotensive effect of the active compound increased only when CMC1 1.0% or CMC2 0.5% was added to the formulation, but it was statistically significantly only for CMC2 0.5% (30.27%, $P < 0.0001$). The AUC of 5-MCA-NAT 100 µM/PBS was significantly lower than the values obtained with CMC1 0.5% or CMC2 0.5% ($P = 0.02$ and 0.0003, respectively). Concerning the mean time effect, the reference formulation produced an effect that lasted approximately 6 hours. This effect was exceeded only by the formulation that contained CMC2 0.5%, whose effect lasted approximately 7 hours.

The film-forming and bioadhesive properties of polymers depend on the concentration, and that may be the reason why there were differences among the formulations that we tested. It is evident that there is a range of concentrations for each polymer to provide an improvement of the drug activity. Within the polymers and concentrations that we tested, the formulation containing the bioadhesive polymer CMC2 0.5% was the best at lowering IOP in rabbit eyes.

In vitro cytotoxicity studies in human corneal-limbal epithelial cells and normal human conjunctival cells were performed to determine the tolerance of formulations. Taking into account that a conventional topical ophthalmic formulation is eliminated in only 5 minutes from the ocular surface, short contact times (15 minutes) are enough to determine tolerance. Nevertheless, in chronic therapies, such as hypotensive therapies, cells are exposed several times to topical formulations. Moreover, bioadhesive polymers are able to increase the ocular contact time of the formulation in the ocular surface.75 Thus, to simulate longer exposures, contact times of 1 and 4 hours were used for the assay. In all cases, formulations did not prove cytotoxic to cells showing cell viability values higher than 80%. Good in vivo short-term tolerance was also demonstrated, as no irritation or discomfort was present in the rabbit eyes with any of the formulations.

In conclusion, we used several formulations containing different bioadhesive polymers to study the hypotensive effect of the melatonin analogue 5-MCA-NAT in rabbit eyes.

### Table 6. Maximum IOP Reduction Induced by 5-MCA-NAT 100 µM in the Vehicles and PBS

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Maximal IOP Reduction</th>
<th>AUC (0–8 h)</th>
<th>Mean Time Effect (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC1</td>
<td>10.0%</td>
<td>13.80 ± 1.40</td>
<td>86.29 ± 8.94</td>
</tr>
<tr>
<td></td>
<td>1.00%</td>
<td>20.73 ± 1.74</td>
<td>58.99 ± 5.84</td>
</tr>
<tr>
<td>CMC2</td>
<td>0.50%</td>
<td>30.27 ± 1.49</td>
<td>138.61 ± 6.20</td>
</tr>
<tr>
<td></td>
<td>1.00%</td>
<td>15.76 ± 2.13</td>
<td>69.61 ± 8.65</td>
</tr>
<tr>
<td>HPMC</td>
<td>0.30%</td>
<td>15.68 ± 1.20</td>
<td>83.17 ± 4.23</td>
</tr>
<tr>
<td>PBS</td>
<td>18.11 ± 1.07</td>
<td>70.81 ± 11.86</td>
<td>6</td>
</tr>
</tbody>
</table>

$n = 10$. Data are the mean percentage ± SEM.
The polymers that we used (cellulose derivatives) are suitable for use on the ocular surface, are protective of the eye in long-term therapy, and are currently widely used in artificial tear solutions in the treatment of dry eye. Furthermore, we found that the hypotensive effect of 5-MCA-NAT was enhanced with the use of CMC1 1.0% and CMC2 0.5%, the latter being the most effective at reducing IOP (30.27%). We have developed an optimal formulation with a bioadhesive polymer (CMC) able to increase the hypotensive effect of the active substance while being, at the same time, gentle with the ocular surface. Finally, from the IOP values obtained after the instillation of 5-MCA-NAT in rabbit eyes, we can also conclude that the use of 5-MCA-NAT combined with bioadhesive polymers may be possible in the treatment of ocular hypertension and glaucoma.

**References**


17. Mundorf T, Noecker RJ, Earl M. Ocular hypotensive efficacy of brimonidine 0.15% as adjunctive therapy with latanoprost 0.005% in patients with open-angle glaucoma or ocular hypertension. *Adv Ther.* 2007;24:302–309.


