4-9-2019

Fluorometholone Modulates Gene Expression of Ocular Surface Mucins

Jonathan Taniguchi  
Chapman University

Ajay Sharma  
Chapman University, sharma@chapman.edu

Follow this and additional works at: https://digitalcommons.chapman.edu/pharmacy_articles

Part of the Genetic Processes Commons, Medical Biochemistry Commons, Medicinal and Pharmaceutical Chemistry Commons, Musculoskeletal, Neural, and Ocular Physiology Commons, Other Pharmacy and Pharmaceutical Sciences Commons, and the Sense Organs Commons

Recommended Citation


This Article is brought to you for free and open access by the School of Pharmacy at Chapman University Digital Commons. It has been accepted for inclusion in Pharmacy Faculty Articles and Research by an authorized administrator of Chapman University Digital Commons. For more information, please contact laughtin@chapman.edu.
Fluorometholone Modulates Gene Expression of Ocular Surface Mucins

Comments
This is the accepted version of the following article:


which has been published in final form at https://doi.org/10.1111/aos.14113. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Copyright
Acta Ophthalmologica Scandinavica Foundation

This article is available at Chapman University Digital Commons: https://digitalcommons.chapman.edu/pharmacy_articles/652
Fluorometholone modulates gene expression of ocular surface mucins

Jonathan Taniguchi, Ajay Sharma*
Department of Biomedical and Pharmaceutical Sciences
Chapman University School of Pharmacy
Chapman University
Irvine, California 92618

*Corresponding Author
email: sharma@chapman.edu
Phone: 001-714-516-5498
Fax: 001-714-516-5481
Abstract:

Purpose: Mucins are vital to keep the ocular surface hydrated. Genes encoding for mucins contain a glucocorticoid response element. The purpose of this study is to evaluate the effect of fluorometholone, a glucocorticoid receptor agonist used in the management of dry eye, on the gene expression of conjunctival and corneal epithelial cell mucins.

Methods: Stratified cultures of human conjunctival and corneal epithelial cells were exposed to 25, 50 & 100 nM of fluorometholone alone or in presence of mifepristone, a glucocorticoid receptor antagonist. The mRNA was isolated from the cells and reverse transcribed to cDNA. The cDNA was used for quantification of gene expression of mucin (MUC) 1, 4, 16 &19 using real time PCR.

Results: Fluorometholone caused a dose and time dependent increase in the gene expression of MUC1, MUC4, MUC16 and MUC19 in the conjunctival as well as corneal epithelial cells. Mifepristone, a glucocorticoid receptor antagonist, inhibited fluorometholone-mediated increase in the gene expression of conjunctival and corneal mucins. At the tested concentration, neither fluorometholone nor mifepristone caused any notable changes in the cellular phenotype or viability of conjunctival and corneal epithelial cells.

Conclusion: Fluorometholone increases the gene expression of MUC1, MUC4, MUC16 and MUC 19 in the conjunctival and corneal epithelial cells through activation of glucocorticoid receptors. The increased expression of mucins can be an additional possible mechanism contributing to the beneficial effects of fluorometholone in dry eye in addition to its well-known anti-inflammatory effects.
Introduction

Mucins are high molecular weight glycoproteins that are expressed on the wet epithelial surface of many organs including respiratory tract, gastrointestinal tract, genitourinary tract, and the ocular surface (Corfield et al., 2001; Gipson 2001; Rose & Voynow, 2006; Govindarajan & Gipson, 2010). Structurally, mucins consist of extensive glycan N-acetyl galactosamine side chains attached to the amino acids of a protein core. The heavy glycosylation imparts the mucins with hydrophilicity and a high negative charge. These structural features account their two key physiological functions of repelling pathogens and keeping the organ surface hydrated. Mucins can be tethered to the cell membrane of epithelial cells or can be secreted. The ocular surface expresses 3 membrane-bound mucins (MUC): MUC1, MUC4, MUC16, one secreted mucin MUC 19, and one gel-forming secreted mucin, MUC5AC (Gipson & Argüeso, 2003; Gipson, 2004; Ablamowicz & Nichols, 2016). The membrane-bound mucins are expressed on the apical surface of corneal and conjunctival epithelial cells and the large gel-forming mucin is primarily secreted by the goblet cells present in the conjunctiva (Gipson & Argüeso, 2003; Gipson, 2016). Besides ocular surface epithelial cells, lacrimal gland also releases two secretory mucins into the tear film, gel-forming MUC5B and soluble MUC7. The ocular surface mucins have been shown to play a critical role in keeping the eye surface hydrated and lubricated. The ocular mucins have also been shown to perform janitorial functions of removing allergens and pathogens from the eye surface (Gipson & Argüeso, 2003; Gipson, 2004; Govindarajan & Gipson, 2010; Ablamowicz & Nichols, 2016). Given their role in keeping the eye surface hydrated, multiple studies have shown altered levels or degree of glycosylation of various mucins in the patients suffering from dry eye
Dry eye is one of the most frequently encountered conditions in ophthalmic clinics. Current clinical management of dry eye includes the use of artificial tears, punctual plugs, and anti-inflammatory drugs (Pflugfelder, 2004; Dogru & Tsubota, 2011). Topical cyclosporine and short-term corticosteroids are frequently prescribed anti-inflammatory drugs in the management of moderate to the severe dry eye. Multiple clinical studies have shown the beneficial effect of corticosteroids on the signs and symptoms of dry eye disease (Avunduk et al., 2003; Pflugfelder et al., 2004; Pinto-Fraga et al., 2016; Cutolo et al., 2017). Suppression of inflammation, a decrease in the proinflammatory cytokines and chemokines, a reduction in the matrix metalloprotease activity and an increase in the goblet cell density have been shown as the possible mechanisms for the ameliorative effect of corticosteroids on dry eye.

Corticosteroids modulate transcription of a wide variety of genes via activation of glucocorticoid receptors. Corticosteroids such as dexamethasone and budesonide have been shown to modulate the gene expression of mucins in airway epithelial cells and cancer cell lines (Imai et al., 2004; Bai et al., 2007; Milara et al., 2017). Corneal and conjunctival epithelial cells express glucocorticoid receptors raising the possibility that topical corticosteroids can modulate the gene expression of ocular mucins by activating glucocorticoid receptors (Cavet et al., 2013; Kadmiel et al., 2016). Fluorometholone is a fluorinated corticosteroid that has been shown to be efficacious in relieving the symptoms of dry eye in non-Sjogren and Sjogren’s patients (Jee et al., 2015; Lin & Gong 2015, Pinto-Fraga et al., 2016). Fluorometholone is a preferred and frequently prescribed
topical ophthalmic corticosteroid because of its lower propensity to cause an increase in intraocular pressure. Compared to other corticosteroids, fluorometholone is a less potent glucocorticoid receptor agonist, and its effect on modulation of mucins gene expression has not been evaluated. Therefore, the present study is designed to examine the effect of fluorometholone on the gene expression of mucins in conjunctival and corneal epithelial cells.

**Materials and Methods**

**Conjunctival and corneal cell culture**

Telomerase-transformed human conjunctival and human corneal cells were used for the experiments. Human conjunctival epithelial cells were plated at the density of 2X10^4/cm^2 in keratinocyte serum-free medium supplemented with 25 μg/ml bovine pituitary extract, 0.2 ng/mL epidermal growth factor, and 0.4 mM CaCl_2. Once the cultures achieved 50% confluence, they were switched to a 1:1 mixture of supplemented keratinocyte serum-free medium: low-calcium DMEM/F12. The cells were then allowed to reach 100% confluence. The cells were then switched to DMEM/F12 medium supplemented with 1mM CaCl_2, 10% calf serum and 10 ng/mL EGF for about 5 to 7 days to induce stratification (Gipson et al., 2003).

The human corneal epithelial cells were grown and propagated in keratinocyte growth medium supplemented with bovine pituitary extract, human epidermal growth factor, insulin, hydrocortisone, epinephrine, transferrin and 0.15 mM calcium chloride. For inducing stratifications, the cells were grown on collagen-coated 3.0μm PTFE transwell membrane inserts in the supplemented keratinocyte growth medium containing 1.15 mM
CaCl$_2$. Once the cells reached 100% confluence, the growth medium was added to the bottom wells only, and the membrane inserts with the growing cells were exposed to air. Creating this air-liquid interface resulted in stratification within one week (Robertson et al., 2005).

**Isolation of RNA and preparation of cDNA**

The cells were harvested, and mRNA was isolated using QIAGEN’s FastLane Cell cDNA kit. According to the kit protocol, the cells were washed with a washing buffer to remove any remaining cell culture medium and extracellular material. The cells were then lysed with a hypotonic buffer that causes the release of the intracellular contents. The lysate was then mixed with a gDNA wipeout buffer to remove the genomic DNA. At this point, the lysate was mixed with a master mix containing reverse transcriptase, a buffer, dNTPs, and a reverse transcription primer mix to create cDNA.

**Real time PCR for quantification of mucins gene expression**

The relative quantification of mucins 1, 4, 16 & 19 mRNA was performed using the real-time PCR system (Biorad CFX thermocycler, Bio-Rad Laboratories). A 20 µl reaction mixture containing 2 µl of cDNA, 2 µl of forward primer (200 nM), 2 µl of reverse primer (200 nM), and 10 µl of 2X SYBR green super mix was run at a universal cycle (95°C for 10 min, 40 cycles at 95°C for 15 s, and 55°C for 60 s) as described in our previous papers [19-21], using the primers listed in Table 1. β-actin was used as housekeeping genes. The relative change in gene expression was calculated using ΔΔCt method.
Experimental Design:

To test the effect of fluorometholone on human conjunctival and corneal epithelial cell mucins gene expression, the cells were exposed to fluorometholone 25nM, 50nM, and 100nM for 12 hours and 24 hours. These doses were selected based on the previously published reports demonstrating that fluorometholone activates glucocorticoid receptors to bring about biological effects at these concentrations in the in vitro experiments (Hos et al., 2011; Huynh et al., 2015; Yan et al., 2017). To test whether the effect of fluorometholone on mucins gene expression was glucocorticoid receptor-mediated, human conjunctival and corneal epithelial cells were exposed to fluorometholone (100nM) alone and in the presence of a glucocorticoid receptor antagonist, mifepristone (10µM), for 24 hours. The mifepristone (10µM) alone treatment group was also included. Pyridine (1µl/ml) was used to dissolve both fluorometholone and mifepristone. Therefore, pyridine 1µl/ml was used to serve as the vehicle control. Each experiment was conducted in quadruplicate.

Statistical Analysis: The data is presented as mean ± standard error. The data was checked for normal distribution prior to using one-way ANOVA for calculating the statistical significance. The statistical analysis for comparing the effect of various doses of fluorometholone to the vehicle control was performed using one-way ANOVA and Dunnett’s test (Fig. 1 & Fig. 2). The statistical analysis for comparing the effect of mifepristone to vehicle control and fluorometholone was performed using one-way ANOVA and Tukey’s range test (Fig. 3 & Fig. 4). A p value of <0.05 was considered to be statistically significant.
Results

Dose and time dependent effect of fluorometholone on human conjunctival epithelial cell mucins gene expression

To test the dose- and time-dependent effect of fluorometholone on mucins gene expression, we exposed the human conjunctival epithelial cells to three different doses (25, 50 & 100 nM) of fluorometholone for 12 and 24 hours. Stratified conjunctival cells are reported to express membrane-tethered MUC1, MUC4, MUC16 and secreted MUC19. Fluorometholone caused a significant increase in the gene expression of all the four mucins in the conjunctival epithelial cells. Moreover, the effect of fluorometholone on the mucins gene expression was time-dependent and a notably greater increase in the gene expression of all the four mucins was observed at 24 hours compared to 12 hours (Fig. 1). Fluorometholone-mediated increase in MUC1 gene expression at 24 hours was dose dependent. The 25 nM dose did not cause any notable increase in MUC1 gene expression whereas 50nM and 100nM dose caused a 1.5-fold and 2.5-fold increase respectively (Fig. 1A). For MUC4, no increase in gene expression was observed at 25 nM dose whereas 1.5 folds increase was observed at both 50nM and 100nM dose (Fig. 1B). For MUC 16 and MUC19, a 1.5- fold and 2-fold increase respectively, was noted with all the three tested doses suggesting that effect of fluorometholone on these mucins may have already reached a ceiling effect at 25 nM dose (Fig. 1C &D).

Effect of Flurometholone on Human Corneal Epithelial Cell Mucins Gene Expression

Based on the data obtained from conjunctival epithelial cells, fluorometholone-mediated increase in mucins expression was significantly higher at 24-hour time point
compared to the 12-hours. Therefore, the effect of fluorometholone on corneal epithelial cell mucins gene expression was tested only at 24-hour time point. The effect of fluorometholone on corneal epithelial cell mucin gene expression was dose-dependent, with a significantly higher increase in gene expression observed at 100 nM dose compared to the 50 nM dose. A statistically significant 1.4-fold, 1.8-fold and 2.5-fold increase in the gene expression of MUC 1 & 4, MUC16 and 19 respectively, was observed in the corneal epithelial cells treated with 100 nM fluorometholone (Fig. 2).

**Effect of mifepristone on fluorometholone-mediated mucins gene expression**

To test whether the fluorometholone-mediated increase in conjunctival and corneal epithelial cell mucins gene expression was mediated through activation of glucocorticoid receptors, we exposed human conjunctival and corneal epithelial cells to fluorometholone in presence of mifepristone, a glucocorticoid receptor antagonist. As is evident from Fig. 3, mifepristone prevented fluorometholone-dependent increase in the gene expression of mucins 1, 4, 16, and 19 in conjunctival epithelial cells suggesting that the fluorometholone-mediated increase in mucins gene expression was indeed mediated through activation of glucocorticoid receptors. Additionally, mifepristone treatment alone did not affect the gene expression of conjunctival epithelial cell mucins (Fig. 3).

Mifepristone also markedly attenuated fluorometholone-mediated increase in mucins 1, 4, 16, and 19 gene expression in the corneal epithelial cells (Fig. 4). It is worthwhile to note that mifepristone treatment alone also caused a significant decrease (0.5 to 0.75 fold) in the gene expression of MUC1 & 4 compared to control cells. *In vitro* cultures of human corneal epithelial cells require hydrocortisone, a glucocorticoid receptor agonist, for their growth and survival. It is likely that the hydrocortisone may have caused an
increase in the baseline mucins 1& 4 gene expression in control corneal epithelial cells, which is antagonized by mifepristone.

**Effect of Fluorometholone and Mifepristone on Human Conjunctival and Corneal Epithelial Cell Morphology and Viability**

To examine the possibility of cellular toxicity or phenotypic changes by the tested doses of the pharmacologic agents, we exposed the human conjunctival and corneal epithelial cells to fluorometholone 50nM and 100nM, and mifepristone 10µM for 24 hours. No visible decrease in cell density was observed with the tested dose of fluorometholone or mifepristone. Additionally, no apparent change in the morphology of the conjunctival (Fig. 5) or corneal (Fig. 6) epithelial cells was noted with either fluorometholone or mifepristone compared to the control cells. Cellular viability was determined using trypan blue and neither fluorometholone nor mifepristone caused any decrease in cell viability of the stratified human conjunctival and corneal epithelial cells (data not shown).

**Discussion**

Artificial tears are the first-line therapy for the management of dry eye. However, a large proportion of patients with moderate to severe dry eye do not respond adequately to tear supplementation, thus necessitating the use of alternative therapeutic interventions including the use of topical corticosteroids. Clinical studies have consistently shown the beneficial effect of short-term topical corticosteroids on the signs and symptoms of moderate to severe dry eye (Avunduk et al., 2003; Pflugfelder et al., 2004; Pinto-Fraga et al., 2016 Cutolo et al., 2017). Corticosteroids are potent anti-inflammatory agents. Inflammation is known to play a significant role in the pathogenesis of dry eye disease.
Therefore, the therapeutic effect of corticosteroids on dry eye is largely attributed to their anti-inflammatory properties. Results of the present study demonstrate that fluorometholone increased the gene expression of mucins in cultured human conjunctival and corneal epithelial cells. Mucins are vital for ocular surface hydration and lubrication. Theoretically, an increase in ocular surface mucins will retard the evaporation of tear film, thus alleviating the symptoms of dry eye. Therefore, our results suggest that an increase in ocular surface mucins can be an additional possible mechanism contributing to the therapeutic effects of corticosteroids in dry eye. To our knowledge, this is the first report demonstrating that fluorometholone, a weak glucocorticoid receptor agonist, can cause an increase in the gene expression of mucins in the conjunctival and corneal epithelial cells. Our data is further supported by a recent case study of two patients suffering from dry eye who upon topical fluorometholone instillation showed an increase in ocular gene expression of MUC16 (Machida et al., 2016). Previous studies have tested the effect of corticosteroids on mucins gene expression in the airway epithelial cells and cancer cells (Imai et al., 2004; Bai et al., 2007; Milara et al., 2017). The corticosteroid-mediated changes in mucin gene expression have been shown to differ from one cell type to another. The modulation has also been shown to be differential for various mucins. For example, corticosteroids have been shown to cause an increase in MUC1 expression in human prostate cancer cells, but an opposite effect is observed in the gastric mucosa (Okazaki et al., 1998; Imai et al., 2004). Further, corticosteroid treatment caused an increase in expression of membrane-tethered mucins but decreased the expression of secreted mucins in nasal polyp epithelium (Martínez-Antón et al., 2008). In the present
study, an increase in all the ocular surface mucins was observed with fluorometholone in both the corneal as well as conjunctival epithelial cells.

Glucocorticoids exert their effects by binding to glucocorticoid receptors that are ubiquitously present in most mammalian cells, including corneal and conjunctival epithelial cells. The glucocorticoid receptors exist as a complex regulated by chaperones such as heat shock proteins 90 and 70. Activation of the glucocorticoid receptor by ligand initiates a conformational change, releasing the chaperone molecules and creating a glucocorticoid-glucocorticoid receptor (GC-GCR) complex. The GC-GCR complex translocates to the nucleus and can bring about gene expression changes by directly binding to glucocorticoid response elements located on the promoter regions of corticosteroid-regulated genes. Mucins genes have been shown to contain a glucocorticoid response element in their promoter (Chen et al., 2006; Chen et al., 2012). Our results demonstrate that the fluorometholone-mediated modulation of ocular mucins is glucocorticoid receptor mediated because the effects are completely blocked by mifepristone, a glucocorticoid receptor antagonist. Our results are further supported by the earlier reports demonstrating that fluorometholone activates glucocorticoid receptors to mediate its biological effects at the concentrations that were used for this study (Hos et al., 2011; Huynh et al., 2015; Yan et al., 2017). Fluorometholone for ophthalmic use is available as 0.1% suspension which is equivalent to 2600 μM concentration. No published data is available regarding the concentration of fluorometholone that is achieved in the tear film of patients after the topical instillation of 0.1% (or 2600μM) suspension. Just like any other eye drops, fluorometholone ocular suspension will undergo dilution and rapid drainage upon instillation. However, it can be contemplated that despite dilution or
drainage, a tear film concentration of ≥ 100 nM that was used in this study, is very likely to be present in the tear film upon instillation of 2600 μM fluorometholone suspension. Therefore, it may be suggested that ocular instillation of fluorometholone will achieve enough tear film concentration to cause modulation of ocular surface mucins in patients as was observed in the cultures of conjunctival and corneal epithelial cells exposed to 100 nM dose of fluorometholone used in this study. Our assumption is directly supported by a recent report showing that ocular instillation of 0.1 % fluorometholone caused an increase in ocular gene expression of MUC16 in the dry eye patients (Machida et al., 2016).

In conclusion, our data shows that fluorometholone increases the gene expression of MUC1, MUC4, MUC16 and MUC 19 in the conjunctival and corneal epithelial cells through activation of glucocorticoid receptors. The increased expression of mucins can be an additional possible mechanism contributing to the beneficial effects of fluorometholone in dry eye in addition to its well-known anti-inflammatory effects.

Acknowledgements: Authors thank Dr. Ilene Gipson, Professor of Ophthalmology, Harvard Medical School, Boston, USA, for providing human conjunctival epithelial cells and Dr. James V Jester, Professor, Ophthalmology, School of Medicine, University of California, Irvine, USA, for providing human corneal epithelial cells.

References:


Fig. 1. Real-time PCR quantification of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) gene expression in human conjunctival epithelial cells treated with fluorometholone (25, 50, and 100nM) for 12 hours and 24 hours.

* p < 0.05 compared to control

Fig. 2. Real-time PCR quantification of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) gene expression in human corneal epithelial cells treated with fluorometholone (50 and 100nM) for 24 hours.

* p < 0.05 compared to control

Fig. 3. Effect of mifepristone (10µM), a glucocorticoid receptor antagonist, on fluorometholone (100nM)-mediated changes in the gene expression of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) in human conjunctival epithelial cells quantified using real-time PCR at 24 hours after the treatment.

* p < 0.05 compared to control; θ p < 0.05 compared to fluorometholone

Fig. 4. Effect of mifepristone (10µM), a glucocorticoid receptor antagonist, on fluorometholone (100nM)-mediated changes in the gene expression of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) in human corneal epithelial cells quantified using real-time PCR at 24 hours after the treatment.

* p < 0.05 compared to control; θ p < 0.05 compared to fluorometholone
Fig. 5. Representative phase contrast microscopy images of human conjunctival epithelial cells treated with mifepristone (10µM) or fluorometholone (50nM and 100nM) for 24 hours.

Fig. 6. Representative phase contrast microscopy images of human corneal epithelial cells treated with mifepristone (10µM) or fluorometholone (50nM and 100nM) for 24 hours.

Table 1: Sequence and accession number of primers used for the gene expression quantification of human mucins using real time PCR
Fig. 1. Real-time PCR quantification of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) gene expression in human conjunctival epithelial cells treated with fluorometholone (25, 50, and 100nM) for 12 hours and 24 hours.

* p < 0.05 compared to control

199x191mm (300 x 300 DPI)
Fig. 2. Real-time PCR quantification of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) gene expression in human corneal epithelial cells treated with fluorometholone (50 and 100 nM) for 24 hours.

* p < 0.05 compared to control
Fig. 3. Effect of mifepristone (10µM), a glucocorticoid receptor antagonist, on fluorometholone (100nM)-mediated changes in the gene expression of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) in human conjunctival epithelial cells quantified using real-time PCR at 24 hours after the treatment.

* p < 0.05 compared to control; θ p < 0.05 compared to fluorometholone.
Fig. 4. Effect of mifepristone (10µM), a glucocorticoid receptor antagonist, on fluorometholone (100nM)-mediated changes in the gene expression of MUC1 (A), MUC4 (B), MUC16 (C) and MUC19 (D) in human corneal epithelial cells quantified using real-time PCR at 24 hours after the treatment.

* p < 0.05 compared to control; θ p < 0.05 compared to fluorometholone
Fig. 5. Representative phase contrast microscopy images of human conjunctival epithelial cells treated with mifepristone (10µM) or fluorometholone (50nM and 100nM) for 24 hours.
Fig. 6. Representative phase contrast microscopy images of human corneal epithelial cells treated with mifepristone (10µM) or fluorometholone (50nM and 100nM) for 24 hours.
<table>
<thead>
<tr>
<th>Mucin</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>5’AGCACCAGACTACTACAAGA3’</td>
<td>5’CAGATCTGGCCTGAACTTAAT3’</td>
<td>NM_002456.5</td>
</tr>
<tr>
<td>MUC4</td>
<td>5’GTGACCTCAGCAGTCTCAATAA3’</td>
<td>5’AGAAGCTGATGTCTTTGATAATAG3’</td>
<td>NM_018406</td>
</tr>
<tr>
<td>MUC16</td>
<td>5’GAAGCCCTCCAGAGATACAAGAG3’</td>
<td>5’TATGGCCTGGGATAGGAGATAA3’</td>
<td>NM_024690.2</td>
</tr>
<tr>
<td>MUC19</td>
<td>5’CCTCTCTGGATGTGGGAATTG3’</td>
<td>5’CAGATCCACTGCCTCTAGTTTC3’</td>
<td>HM801842.1</td>
</tr>
<tr>
<td>β -actin</td>
<td>5’GGACCTGACTGACTTCTCAT3’</td>
<td>5’CGTAGCACAGGTTCTCATTAT3’</td>
<td>NM_001101.3</td>
</tr>
</tbody>
</table>