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"In vitro" hyperosmolar design in human corneal epithelial cells and inflammation in macrophages: avoiding the use of animals in the screening of ocular surface therapies

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1. Introduction

Keratoconjunctivitis sicca (SS-KCS) is a pathology affecting the ocular surface and tear film stability [1]. When a hypertonic environment occurs, specific biochemical pathways are activated leading to apoptosis [2]. Substances protecting cells against hyperosmolarity by stabilization of cell´s membrane through their accumulation are commonly described as osmoprotectants. There are substances employed for the treatment of SS-KCS with anti-inflammatory properties. L-Carnitine, Taurine or Betaine are known to possess osmoprotective properties [2, 3] and the mucoadhesive polymer hyaluronic acid (HA) has demonstrated to have anti-inflammatory effect [1]. Commonly, the use of animals is considered for ocular drug screening, however following the principle of replacement, reduction, and refinement (3Rs), alternative in vitro models should be developed. The aim of the present study is to simulate in vitro hyperosmolar and inflammatory conditions for fast screening of ocular therapies avoiding the use of animals following the 3Rs principle.

2. Materials and methods

2.1. In vitro tolerance evaluation of osmoprotectants

Cell viability of different substances (Betaine, L-Carnitine and Taurine, HA, and hydroxy propyl methyl cellulose; HPMC) was assessed at 8 h in Human corneal cells (HCECs) and evaluated by MTT.

2.2. Simulation of hypertonic environment in human corneal epithelial cells

2.2.1. In vitro tolerance under hypertonic stress

Initially, HTERT-HCECs were exposed to different hyperosmolar concentrations (350, 400, 450, 460, 470, 480, 490 and 500 mOsm/L) for 16 h and the 470 mOsm/L was selected. Then, HCECs were exposed to the osmoprotectants and anti-inflammatory substances (8 h) and incubated with 470 mOsm/L overnight. Finally, cell viability was determined as mentioned above.

2.2.2. Flow cytometry apoptosis and cell size studies

Hypertonic environment was simulated and osmoprotective activity assessed. Cells were isolated, stained with 7-AAD, YO-PRO™-1 Iodide, taken to the flow cytometer (FC500) and apoptosis was evaluated. Besides, after hyperosmotic stress simulation, variations in relative cell size (RCS) were evaluated. The in vitro efficacy of osmoprotectants and HA was studied.

2.3. TNF-α determination in an inflammation model of macrophages

Inflammatory conditions were recreated in macrophages (J774A.1) by addition of LPS and TNF-α detection by Enzyme-linked immunosorbent assay (ELISA).

3. Results and Discussion

Cells exposed to hyperosmolar concentrations showed a threshold at 450 mOsm/L where viability decrease dramatically from 66.66 % ± 13.54 up to 12.77 % ± 5.57 at 500 mOsm/L. Pre-incubation with osmoprotectants and polymers which were well tolerated increased survival compared with hypertonic environment in absence of protective compounds (Fig. 1). Particularly, Betaine (200 mM) and Taurine (80 mM) were the most effective substances in protecting cells against hyperosmolarity (66.01 % ± 3.65 and 52.08 % ± 3.36 respectively). Among polymers, HA 0.8 % showed the best results (60.24 % ± 6.29).

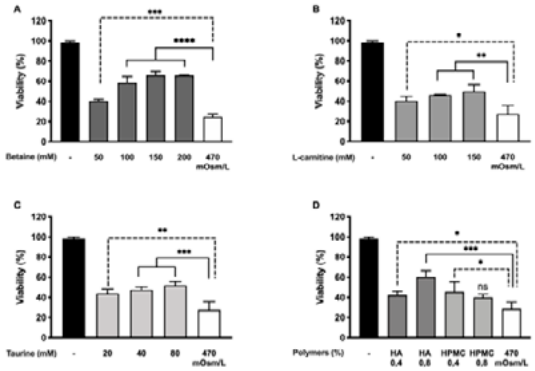

Fig. 1. Osmoprotection of osmoprotectants and polymers in response to 470 mOsm/L.

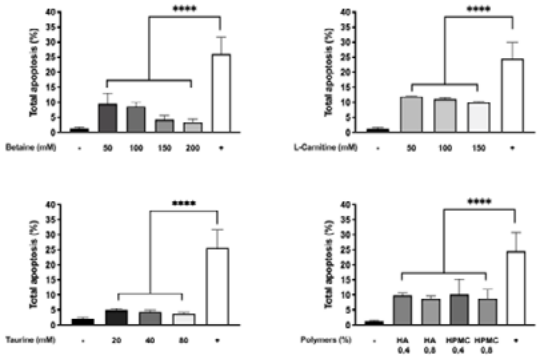

Fig. 2. Total apoptosis of cells pre incubated with the different substances and polymers.

It has been previously reported that an increment in osmolarity causes cell apoptosis. We confirmed the activity of osmoprotectants and polymers to prevent cell apoptosis [3]. Particularly, taurine and betaine at high concentrations were the most effective. It was also assessed that HA and HPMC can protect cells against apoptosis caused by hypertonic stress (Fig.2).

Some have studied that cells under hyperosmolarity develop a change in volume [3]. We showed that osmoprotectants balanced the RCS (≈ 100 %) under hyperosmolarity but polymers, drastically increased RCS (HA 0.8 % increased RCS up to 113.8 % ± 4.11) (Fig.3).

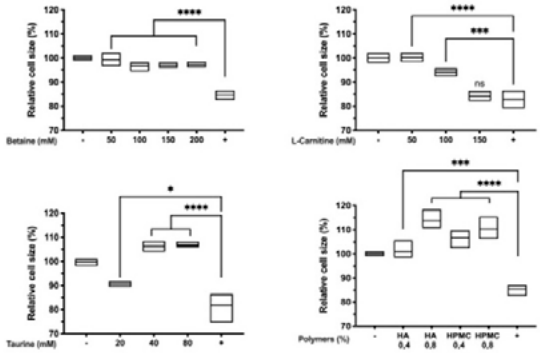

Fig. 3. Modification in cell size under hypertonic stress (470 mOsm/L).

Finally, we assessed the anti-inflammatory activity of the osmoprotectants and polymers. L-Carnitine, taurine and HA were the most anti-inflammatory ones (≈ 60 %) as hypothesized by some authors.

4. Conclusions

In vitro hyperosmolar models in HCECs and inflammation in macrophages allow to screen potential therapies to prevent cell damage when chronic hypertonic stress and inflammation have been established such as occur in SS-KCS. This tool could be useful before the use of animal models to follow the 3Rs rules.

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References

1. Jones L, Downie LE, Korb D, Benitez-del-Castillo JM, Dana R, Deng SX, et al. TFOS DEWS II Management and Therapy Report. Ocul Surf [Internet]. 2017;15(3):575–628. Available from: http://dx.doi.org/10.1016/j.jtos.2017.05.006

2. Khajavia N, Reinach PS, Skrzypski M, Lude A, Mergler S. L-Carnitine Reduces in Human Conjunctival Epithelial Cells Hypertonic- Induced Shrinkage through Interacting with TRPV1 Channels. 2014;49(030):790–803.

3. Garrett Q, Khandekar N, Shih S, Flanagan JL, Simmons P, Vehige J, et al. Betaine stabilizes cell volume and protects against apoptosis in human corneal epithelial cells under hyperosmotic stress. Exp Eye Res. 2013;108:33–41.

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