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Original article

Effects of tryptophan, kynurenine and kynurenic acid exerted on human reconstructed corneal epithelium *in vitro*

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ABSTRACT

Background: Tryptophan metabolites formed along kynurenine pathway may affect cell proliferation and tissue function. This pathway presents potential sites for drug discovery. Tryptophan and its metabolites kynurenine and kynurenic acid may be involved in the physiology and pathology of the ocular surface. The purpose of this work is to investigate the effect exerted by tryptophan, kynurenine and kynurenic acid upon corneal epithelium.
Methods: A SkinEthic™ HCE human reconstructed corneal epithelium model was used. WST-1 test was used to examine cell proliferation and viability, and the Griess reaction for nitric oxide determination. The levels of IL-6 and IL-10 were measured by means of ELISA assay. All analyses were carried out after the cells were exposed to tryptophan, kynurenine and kynurenic acid at concentrations of 5, 50 or 100 μM for 0–24 h and 24–48 h.
Results: Tryptophan (100 μM), kynurenine (100 μM) and kynurenic acid (5–100 μM) slightly increased the viability and proliferation of corneal epithelium. All of the tested compounds decreased cellular NO release. Kynurenine (50–100 μM) and tryptophan (50–100 μM) decreased while tryptophan (5 μM) and kynurenic acid (100 μM) increased the release of IL-6. All of the tested substances increased the level of IL-10 and decreased the IL-6 to IL-10 ratio.
Conclusions: Tryptophan, kynurenine and kynurenic acid affect physiological processes in corneal epithelium and therefore may play a significant role in the physiology and pathology of the ocular surface. Our results indicate that the use of these compounds may be considered in the treatment of ocular surface diseases.

Introduction

Human corneal epithelium (HCE) plays an important role in corneal homeostasis and is essential for the maintenance of normal visual acuity [1,2]. HCE covers the cornea and comprises about 10 percent of its thickness. It consists of two to three cell layers of flattened superficial cells, two to three cell layers of wing cells, and a single layer of columnar basal cells [3]. HCE has a rich sensory nerve supply. The reduction of the sensory nerve plexus may lead to a loss of corneal sensitivity and serious complications [3,4]. HCE absorbs oxygen and cell nutrients from tear fluid for the rest of the cornea. Moreover, HCE forms a barrier to pathogens, dust, foreign matter and changes in temperature [1–3].

The homeostasis of corneal epithelium is maintained by an integrated process of cell proliferation, migration, differentiation, stratification, and desquamation. An impairment of these processes may result in ocular surface diseases such as superficial punctate keratitis, persistent epithelial defects and other debilitating disorders [5,6]. Since the cornea is not a static system but it relies

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on dynamic cellular and molecular processes, it is widely accepted that not only unfavorable external conditions but also changes in intrinsic metabolism may affect normal vision [7].

In ophthalmology, novel drugs are being developed to improve corneal metabolism and epithelial wound healing [8]. Tryptophan (TRP) and its selected metabolites: kynurenine (KYN) and kynurenic acid (KYNA), hold a promise. TRP, an essential amino acid, is used in the biosynthesis of proteins. TRP plays a rate-limiting role during protein synthesis because it has the lowest overall concentration of all amino acids in the human body [9,10]. TRP is present in tear fluid, aqueous humor and serum at the following concentrations: $7.1 \pm 0.7\ \mu M$; $0.9 \pm 0.1\ \mu M$; $52.9 \pm 1.8\ \mu M$, respectively [11]. It was reported that TRP deficiency caused corneal neovascularisation and cataracts in rats [12]. As TRP possesses strong antioxidant properties [10,13], its use as a therapeutic antioxidant was considered in certain disorders [9,14]. TRP may also improve cutaneous wound healing [15].

TRP is a precursor of serotonin, a neurotransmitter and the other biologically active compounds collectively known as kynurenines. Almost 99% of available TRP is catabolized in the kynurenine pathway [16]. The first enzyme of the kynurenine pathway which converts TRP into N-formylkynurenine is tryptophan-2,3-dioxygenase (TDO) and indole-2,3-dioxygenase (IDO) [17,18]. TDO is present mainly in the liver and is active under physiological conditions. IDO is present in many extrahepatic tissues and its activity is accelerated by cytokines released during immune activation. The intermediate, N-formylkynurenine is metabolized to KYN by kynurenine formamidase. Kynurenine aminotransferases (KATs) I–IV exhibit a transamination activity towards KYN leading to the formation of KYNA [19].

KYN, a metabolic intermediate, is a constant component of body fluids. Its concentration in serum is $1.78 \pm 0.42\ \mu M$ [20]. Recently we have communicated the presence of KYN in tears from healthy donors [21]. KYN protects corneal endothelium and keratocytes against oxidative stress and in consequence apoptosis induced by UV-B light [22]. KYN is thought to be involved in the immune privilege of the cornea and the anterior chamber. For example, adequate levels of endothelium-derived KYN might contribute to the preservation of corneal allogenic cells after corneal transplantation [23,24]. Similarly, the IDO activity in stroma is considered to be a factor of ocular immune privilege in corneal keratocytes [25]. It was noticed that corneal transplantation increased IDO activity [23,24].

KYNA is widely distributed throughout the mammalian body with serum concentration of 4.4–28.4 nM [26]. Recently we communicated the presence of KYNA in human tears [21]. Moreover, the presence of KATs I, II and III in human corneas, especially pronounced in endothelium and epithelium was documented immunohistochemically [27]. This finding may suggest that KYNA is synthesized in the cornea. This compound exhibits anti-inflammatory, antioxidative, analgesic, neuroprotective and antiproliferative properties [11,19,28–32]. It seems that KYNA may regulate peripheral cellular responses by both antagonism on N-methyl-D-aspartate (NMDA) receptors and the activation of G-protein-coupled receptor 35 (GPR35) which is predominantly detected on immune cells [29,33]. KYNA has been considered in therapy of certain neurobiological diseases [34] and as an antifibrogenic agent to improve healing outcomes in subjects threatened with hypertrophic scarring [32]. It could be speculated that KYNA may exert a beneficial effect on corneal wound healing and protect the corneal nerves, thereby contributing to the transparency of the cornea.

Overall, TRP, KYN and KYNA may affect several processes in corneal cells and as such play a significant role in the physiology and pathology of the ocular surface. To further analyze the effects exerted by these compounds on corneal epithelium we used a commercially available three-dimensional (3D), multilayer, standardized SkinEthic™ model of HCE, which is morphologically and functionally similar to corneal epithelium [35–37]. Cell viability and proliferation, the release of mainly the pro-inflammatory cytokine interleukin 6 (IL-6) and the anti-inflammatory cytokine interleukin 10 (IL-10) and nitric oxide (NO) level were investigated.

Materials and methods

Cell culture

A commercially available, HCE three-dimensional model (SkinEthic™ from Episkin, Lyon, France) was used. This reconstructed HCE model consists of immortalized human corneal epithelium cultured on an inert, permeable polycarbonate filter of $0.5\ cm^2$ for 5 days at the air–liquid interface in a supplemented chemically defined medium (modified MCDB 153) [36]. The resulting construct is a multilayered, stratified epithelium similar to that of the normal human corneal epithelium. This model represents 5–7 viable cell layers (columnar basal cells, transitional wing cells and flattened superficial cells) without significant histological abnormalities and has been characterized for different relevant markers [36,37].

The tissues were shipped in a room temperature in an agarose semi-solid culture medium. After delivery cells were immediately removed and placed in a 24-well culture plate with 1 ml of chemically defined maintenance medium supplied by SkinEthic which was changed every 24 h [36,37]. The precise composition of medium is not specified due to producer's policy.

Substances, products, control and treatments

Tryptophan (TRP), kynurenine sulphate (KYN) and kynurenic acid (KYNA) were purchased from Sigma (St. Louis, MO, USA). All of the tested substances were used at final concentrations of 5, 50 and 100 μM.

Experimental procedure

The tested substances at selected concentrations, diluted in culture medium, were applied on the HCE. Medium in a volume of 150 μl was directly applied to the insert and gently spread over the whole epithelium surface while plate wells were filled with 600 μl of culture medium (Fig. 1). Incubation of cells was performed at 37 °C, 5% CO_2 in a humidified incubator. HCE placed in a standard conditions without tested substances serves as a negative control.

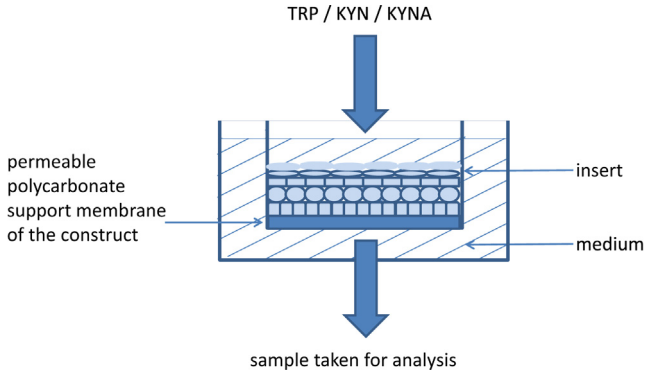

Fig. 1. Scheme of the experiment. Insert containing corneal epithelium is placed inside a well which is filled with culture medium. Medium containing investigated drug was applied directly into the insert with corneal cells. After the incubation time the medium from the compartment outside insert was collected for further analyses.