Assessment of Toxicities of Moxifloxacin, Cefuroxime, and Levofloxacin on Corneal Endothelial Cells *in Vitro*

Matsuura K [1], Haruki T [1] , Miyazaki D [1] , Terasaka Y [1] , Noguchi Y [1] , Inoue Y [1]

[1]Tottori University

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Background

Although intracameral moxifloxacin and cefuroxime are being used for perioperative prophylaxis, there is still have limited information on which is the optimal treatment regimen.

For example, the currently used concentrations were empirically determined, and appear not to be completely tested for their purpose or safety.

In addition, there has been some concern on their toxicity on corneal endothelial cells when used intracamerally.

Purpose

- Aim of this study is to evaluate and compare the toxic effects of moxifloxacin, cefuroxime, and levofloxacin on human corneal endothelial cells *in vitro.*

- In addition, to determine the safe intracameral concentrations for these antibiotics.

Methods

- Human corneal endothelial cells (HCEn) in culture were exposed to moxifloxacin, cefuroxime, and levofloxacin at concentrations up to 2000 µg/ml.
- Evaluation of membrane damage was determined by ethidium homodimer-1 (EthD-1) uptake, and cell viability by intrinsic esterase activity.

Assay of cell viability and membrane damage

EthD-1 uptake was measured with a fluorescent microplate reader (Tecan, Männedorf, Switzerland) with excitation by 495 nm and emission at 635 nm. Dead cells were used as positive control.

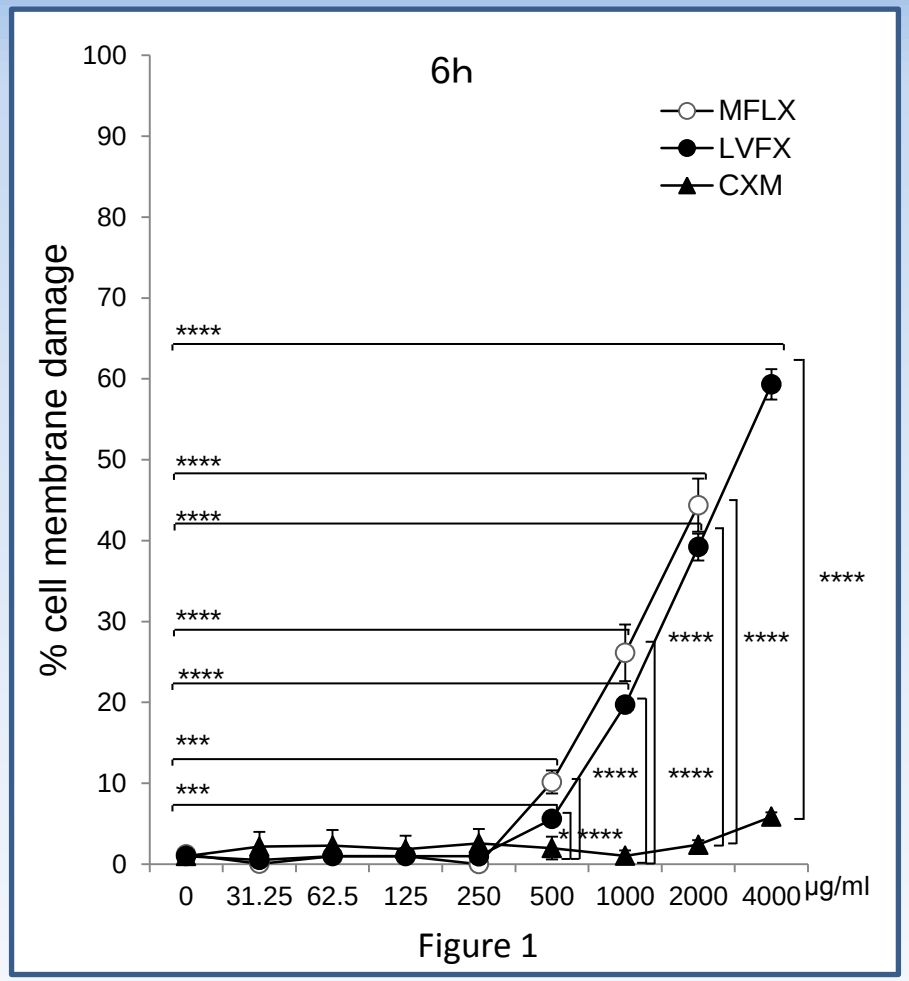
For measurement of esterase activity, HCEn cells were added with non-fluorescent calcein AM,which is converted to fluorescent calcein by the esterase activity of the cells. The optical density of the fluorescence due to the esterase activity was measured by a fluorescent microplate reader with excitation at 495 nm and emission at 530 nm.

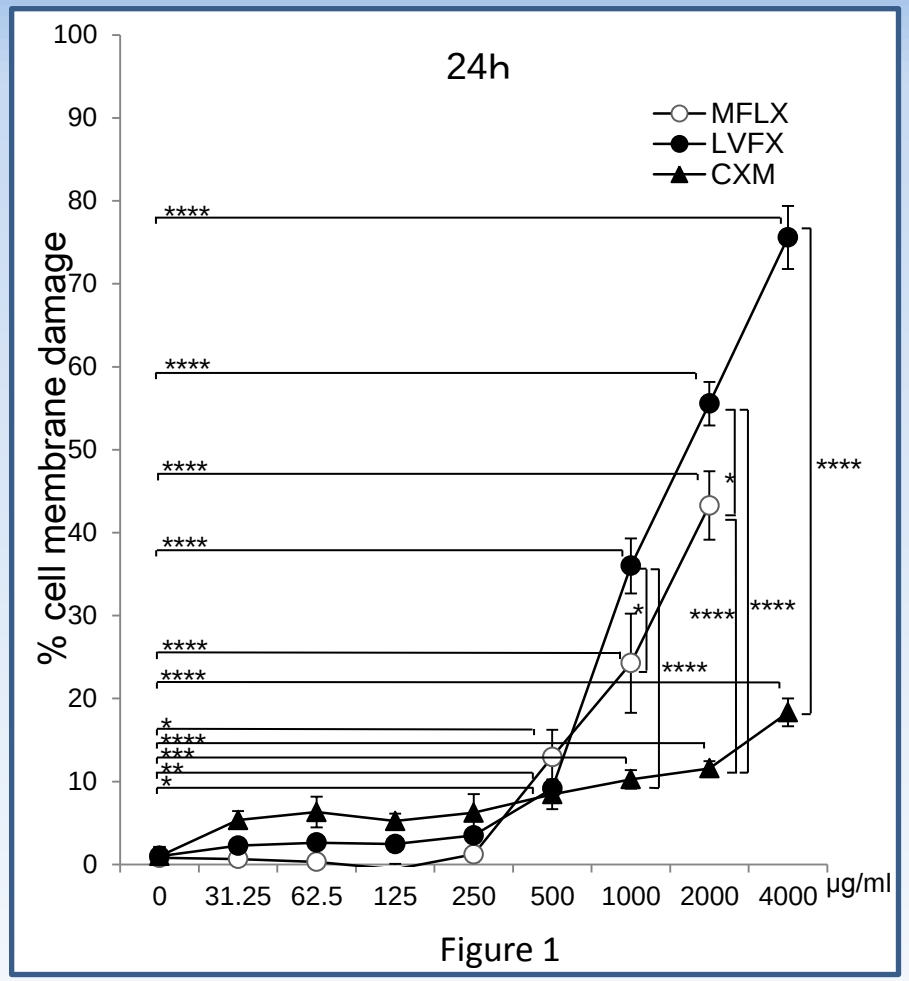
Methods2

- The inhibitory effects of the three antibiotics on the constitutive secretion of interleukin-6 (IL-6) by HCEn cells was determined by ELISA.

Enzyme-linked immunosorbent assay (*ELISA*)
The supernatants of the HCEn cells were collected 72 h after antibiotic exposure, and the level of IL-6 was measured with an ELISA kit (PeproTech, New Jersey). The supernatants were diluted 20-fold with the diluent of the kit according to the manufacturers' instructions. The supernatant was incubated on the captured antibody-coated plates overnight at 4º C and processed for ELISA measurements.

Results1





*: *P* <0.05, **: *P* <0.005, ***: *P* <0.001, N=6

Comparative toxicity (EthD-1 uptake).

Moxifloxacin and levofloxacin had a dose-dependent increase of cell membrane damage at 6 h. Cell membrane damage by cefuroxime was detected at 24 h

Results2

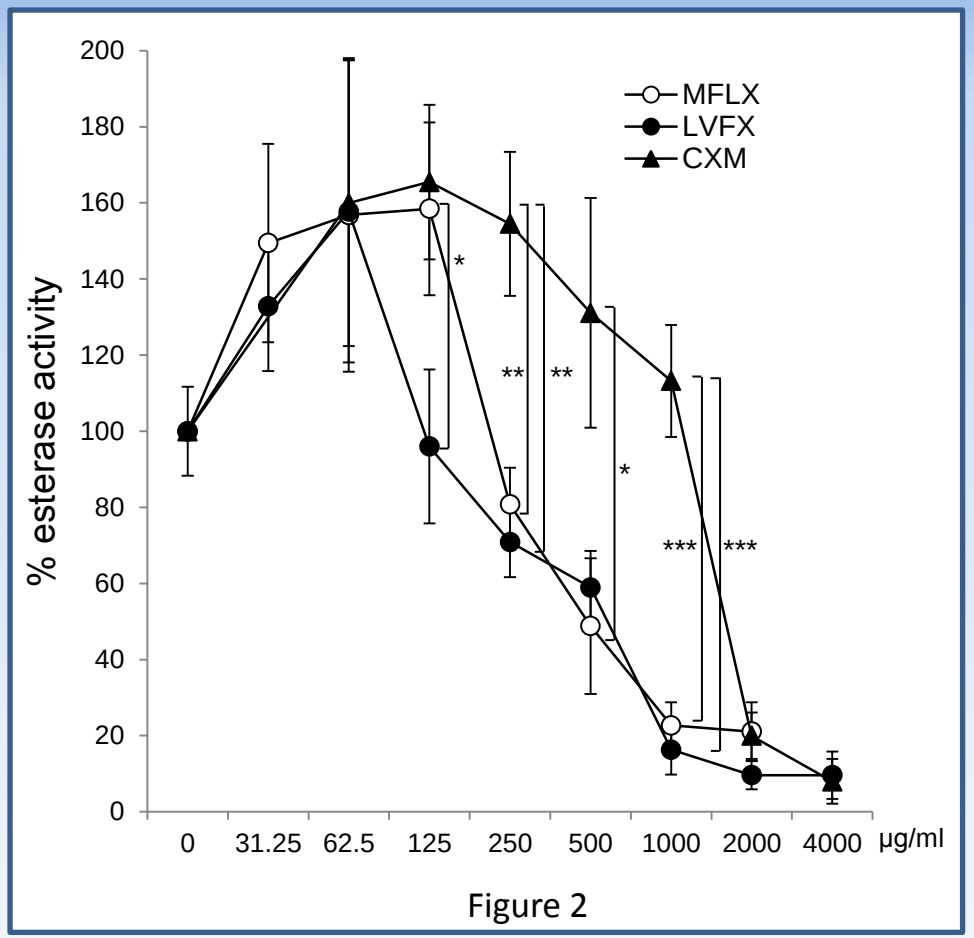


Figure 2

*: *P* <0.05, **: *P* <0.005, ***: *P* <0.001, N=6

Comparative toxicity (cell intrinsic esterase activity)

Cell viability was significantly decreased with moxifloxacin and levofloxacin treatment at ≥1000 µg/ml. A significant reduction of cell viability by cefuroxime was observed at ≥2000 µg/ml.

Membrane damage

The acute membrane damage effect was not detectable with <500 µg/ml of moxifloxacin and levofloxacin, however damage was greatly increased when higher than 500 µg/ml of moxifloxacin and ofloxacin were used (Figure 1).

The concentrations of antibiotics that inhibited cell growth by 50% (IC_{50}) was determined. After 24 h exposure, the IC_{50} of moxifloxacin, levofloxacin, and cefuroxime was 487 µg/ml, 578 µg/ml, and 1600 µg/ml, respectively, indicating that cefuroxime was less toxic when used at the same concentration.

Results3

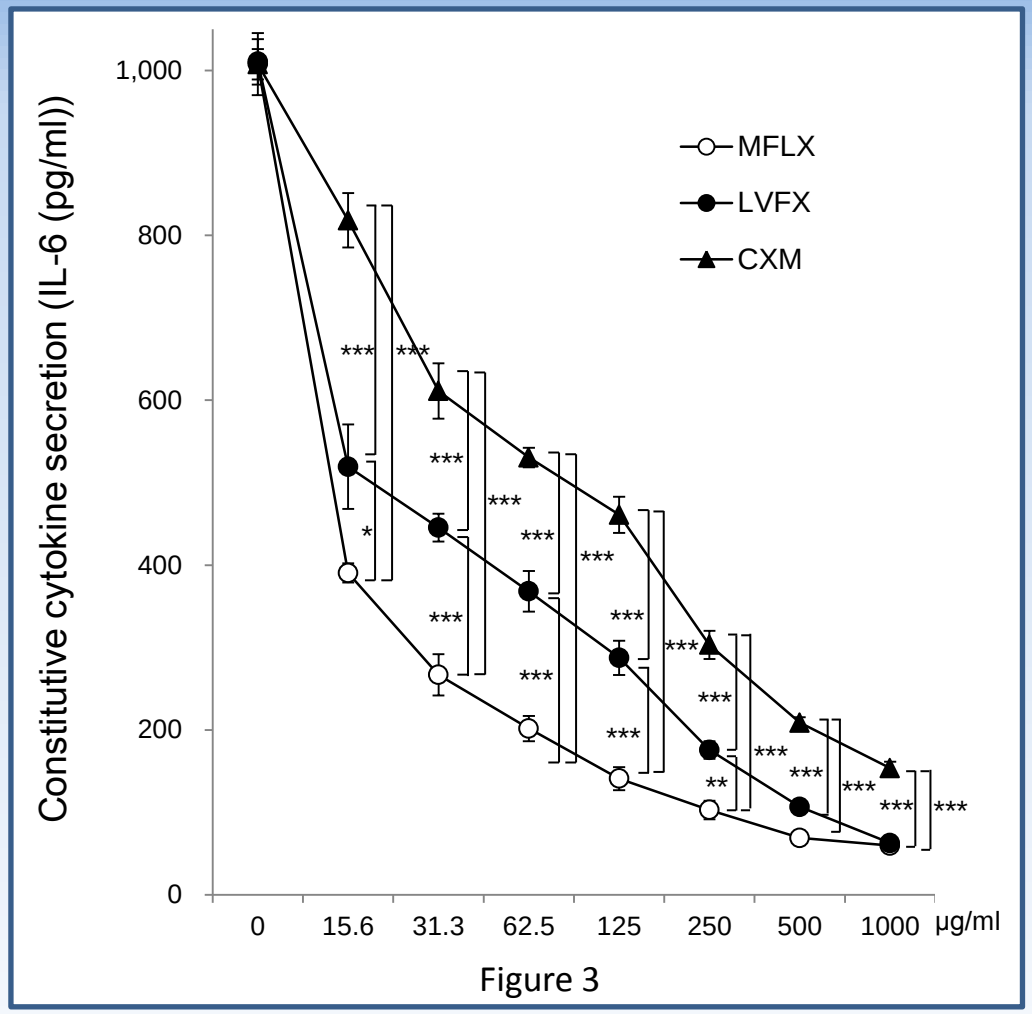


Figure 3

*: *P* <0.05, **: *P* <0.005, ***: *P* <0.001, N=6

Effect of antibiotics on cytokine secretion (IL-6).

The supernatant was measured for secreted IL-6 by ELISA. All three antibiotics significantly impaired IL-6 secretion at ≥15.6 μg/ml (*P* <0.005), and the decrease was dose-dependent.

Inhibitory effects

After microbial infections, IL-6 is abundantly produced by corneal endothelial cells, and the cells also induce numerous anti-microbial proteins and cytokines. Because IL-6 is constitutively produced by corneal endothelial cells, we assessed whether exposure to the three antibiotics affected the constitutive IL-6 synthesis (Figure 3). Interestingly, all three of these antibiotics significantly impaired IL-6 secretion at concentrations as low as 15.6 μg/ml ($P<0.005$).

No endothelial function loss has been reported, and this impairment appears reversible and not lead to permanent reduction of cell viability. However, we need to be aware that the protective arm mediated by IL-6 can be impaired by intracameral antibiotics. This disarmament might cause adverse effects when intracameral antibiotics are used especially for resistant strains.

Why moxifloxacin?

- ***Commercial MFLX (Vigamox®) is preservative free and can be diluted and used for intracameral administration.***

- ***MFLX is concentration dependent and requires approximately 2 hours to be effective.***

- ***Antibacterial spectrum of MFLX is wide, MFLX is effective against Enterococcus faecalis, whereas cefuroxime is not effective.***

Discussion & Conclusion

Considering the half-life of intracameral moxifloxacin as approximately 1 h, 500 μg/ml of moxifloxacin would achieve a concentration four times higher than the MIC_{90} of highly resistant pathogen (32~64 μg/ml) for the first 2 hours.

500 μg/ml of moxifloxacin would be effective for the refractory strains without noticeable endothelial damage.

Thus, we propose a dose of 500 μg/ml for moxifloxacin which should be safe for general prophylactic use.