

Rose Bengal score in eyes irrigated with tap water and PSS with chlorine was significantly increased compared with before irrigation and eyes receiving irrigation with PSS only ($P < 0.05$; Fig. 1B). The volunteers did not complain of any subjective symptoms the next day after instillation of the fluids.

Changes in Barrier Function of the Corneal Epithelium

Fluorescein uptake evaluated by anterior fluorometry was not significantly changed in the eyes irrigated with PSS, tap water, and DW. Eyes irrigated with PSS with chlorine showed a significant increase of fluorescein uptake compared with all other groups (Fig. 2).

Changes in Morphology of the Corneal Epithelial Cells

Confocal microscopy was performed before and after irrigation with PSS with or without chlorine. The central cornea before irrigation revealed a healthy appearance.⁵ The superficial epithelial layers revealed light cell boundaries with bright visible nuclei (Fig. 3, top left). The basal epithelial layers also showed light cell boundaries, but the cells were smaller (Fig. 3, top right). Both superficial and basal epithelial cells showed healthy structure. In the stroma, keratocytes were visible as light cell bodies against the dark background. No stromal collagen fibers were visible. Confocal scans also showed corneal nerve fibers as bright lines. The corneal endothelial cells appeared as regularly arranged hexagonal light cells with dark borders.

Eyes after irrigation with PSS confocal scans showed an identical appearance in superficial (Fig. 3, middle left) and basal (Fig. 3, middle right) epithelium, stroma, and endothelium compared with scores before irrigation. In contrast, superficial epithelial cells after irrigation with PSS with chlorine showed extremely high reflectivity with granules and

deposits (Fig. 3, bottom left). The epithelial basal layer showed increased reflectivity in the cell boundaries (Fig. 3, bottom right). The corneal stroma and endothelial cells did not show remarkable changes compared with scores before irrigation.

DISCUSSION

This study showed that eye irrigation with PSS with chlorine was associated with an increase in vital staining scores of the corneal epithelium. Confocal microscopy showed impairment of cell-to-cell junction and cytosolic edema of superficial and basal epithelial cells after irrigation with PSS with chlorine. Moreover, PSS with chlorine caused damage to the corneal barrier function, as shown by the anterior fluorometer. These results indicate that PSS with chlorine adjusted to the same concentration of pool water has deleterious effects on the ocular surface, although this solution is isotonic.

By Japanese law, the concentration of free residual chlorine in pool water in Japanese schools should be 0.4–1.0 mg/L, and this concentration in tap water should be >0.1 mg/L. Chlorine in the water creates free residual chlorine, which has a strong disinfectant property while also having an unstable existence. Free residual chlorine reacts with nitrogenous compounds in the water (eg, saliva, urine and sweat) to form chloramines (monochloramine, dichloramine, trichloramine). Compared with free residual chlorine, these chloramines exist stably but have less effectiveness for disinfection. Thus, the disinfectant property of chlorine attenuates with time. For this reason, the concentration of free residual chlorine in school pools is being verified before classes and every hour during classes, and chlorine disinfectant is added to adjust the concentration to the required 0.4–1.0 mg/L. Because we dissolved a tablet of chlorine disinfectant just before the eye irrigation, most of the residual chlorine should be free in this solution. This free residual chlorine could explain the increased damage to the corneal epithelium caused by PSS with chlorine compared with that by tap water.

Although Rose Bengal scores of eyes irrigated with tap water were increased compared with eyes irrigated with PSS only, irrigation with tap water did not disrupt the corneal barrier, as shown by the results of anterior fluorometry. Irrigation with DW also did not result in an increase of fluorescein uptake. These results suggest that hypotonicity of the solution does not have a major harmful effect on the corneal epithelium. Increased staining with Rose Bengal reflected the damage of both the cornea and conjunctival epithelium. Irrigation with tap water showed increases of Rose Bengal scores, but not in fluorescein scores, which indicated that tap water caused damage mostly to the conjunctival epithelium. Increased scores of Rose Bengal after eye irrigation with tap water and PSS with chlorine suggest alteration or washout of mucin coating.⁶ There are several kinds of mucins in the cornea and conjunctiva,⁷ and their presence is thought to be necessary for the stability of tear film on the ocular surface. Rinsing eyes with tap water also causes damage to the ocular surface. Swimming without goggles in the pool and rinsing eyes with tap water after swimming have harmful effects on the cornea, conjunctiva, and tear film.

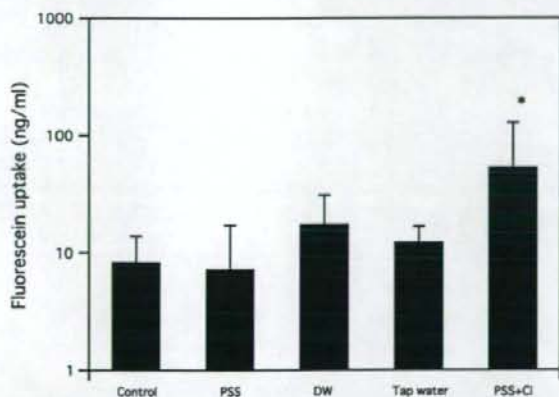


FIGURE 2. Fluorescein uptake measured by anterior fluorometry. Fluorescein uptake is significantly increased in eyes irrigated with PSS with chlorine compared with all other groups ($*P < 0.05$).

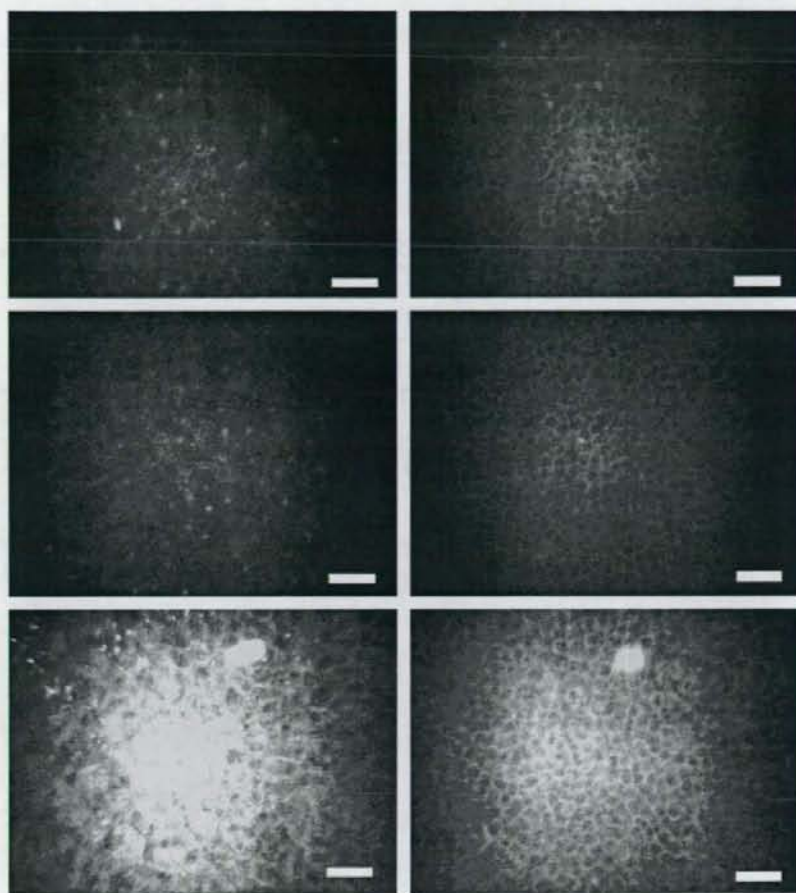


FIGURE 3. Confocal microscopic photographs of the central cornea before and after irrigation with PSS with chlorine. Superficial epithelial layer before irrigation (top left) shows flat regular epithelium with regular cells. Basal epithelial layer before irrigation (top right) appears as smaller cells with light cell boundaries. Superficial (middle left) and basal (middle right) epithelial layer after irrigation with PSS shows identical figures as before irrigation. Bottom left, Superficial epithelial layer after irrigation with PSS with chlorine shows highly reflective cells with granule and deposits. Bottom right, Basal epithelial layer after irrigation with PSS with chlorine shows increased reflectivity in cell boundaries (Bar = 50 μ m).

The effect of any mechanical factors such as irrigation with 250 mL of fluid may not have completely simulated the condition in swimming pools. Also, we did not perform experiments to show the threshold of exposure in time that prompts corneal epithelial toxicity. Other factors not investigated in this study should be considered in relation to ocular surface epithelial damage (ie, the number of hours actually spent in the swimming pool, the frequency of opening the eyes in the water, and the kind of activity done in the swimming pool).

In conclusion, we showed in this study that chlorine, as a disinfectant, and solution hypotonicity can be potentially harmful to the ocular surface health. Although this harmful effect is temporary because the volunteers in our study did not complain of any subjective symptoms the next day after instillation of the fluids, we believe that it is important to

educate people to wear swimming goggles and to not rinse their eyes with tap water after swimming.

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