

## 論文内容要旨

報告番号	甲 先 第 <b>328</b> 号	氏 名	山本 将司
学位論文題目	In Vitro Studies on the Biocompatibility of Bis-Quaternary Ammonium Compounds (ビス型第四アンモニウム化合物の生体適合性に関するインビトロ研究)		
<p>内容要旨</p> <p>Antiseptic agents are extensively used for hand hygiene by HCWs and for skin antiseptics of patients to control and prevent the spread of HAI. Antiseptic agents, such as CHG, PHMB, BAC, and OCT, are used widely as active ingredients in antiseptics. However, there are several reports on their cytotoxicity in human cells and adverse effects, such as irritant contact dermatitis or allergic contact dermatitis, caused by these antiseptic agents. Therefore, the development of new antimicrobial substances that can be used as a topical skin antiseptic is expected to create more options for skin antiseptics, not only in terms of increased antimicrobial activity, but also in terms of reducing adverse effects such as contact dermatitis.</p> <p>In addition, most studies on the conventional toxicity evaluation of antiseptic agents evaluated only cytotoxicity; there are few studies focusing on changes in the transcription and translation of inflammation-related genes induced by the antiseptic agents. Therefore, in order to predict the skin irritation potential of antiseptic agents, not only a cytotoxicity assay of cell death was used, but a more accurate evaluation was performed through the combination of multiple evaluation methods.</p> <p>To meet these objectives, in the first chapter of this study, we examined the synthesis method of various bis-QACs, in order to obtain the molecular structure of a novel antimicrobial substance with greater biocompatibility than OCT, which is known to have excellent bactericidal activity. Three series of novel bis-QACs were obtained by linking two molecules of pyridinium salt with a spacer structure such as pentaerythritol or hydroquinone, followed by N-alkylation with alkyl halides. The antimicrobial activity of the newly synthesized bis-QACs, 4TOSU-n, 3PHBO-n and 3HHDMP-n, and the existing antiseptic agents was compared through the examination of the MIC and MBC. Our results indicated that 4TOSU-10, 12, 3PHBO-10, 12, and 3HHDMP-10, 12 exerted potent bacteriostatic activity against gram-negative bacteria, gram-positive bacteria, and fungi, which demonstrated their broad antibacterial spectra. Furthermore, the measurement of MBC to evaluate the bactericidal activity of the novel bis-QACs revealed excellent MBC values as the alkyl chain length increased from 8 to 12. In particular, as the bactericidal activity of bis-QACs with an alkyl chain length of C12 was comparable with that of OCT, which showed the best bactericidal activity among the existing antiseptic agents, it was determined that these compounds had potent bactericidal activity.</p> <p>To compare the cytotoxicity of the novel bis-QACs with existing antiseptic agents, an MTT assay was performed in NHEK (NB) cells. The cytotoxic effect of several bis-QACs was lower</p>			

than that of existing QACs and was comparable with that of biguanide-based compounds. The comparison of BIs revealed that novel bis-QACs, such as 4TOSU-12, 3PHBO-12 and 3HHDMP-12, had equal or greater biocompatibility than the existing antiseptic agents tested. Thus, the biocompatibility of 4TOSU-12, 3PHBO-12, and 3HHDMP-12 was equal to or greater than that of existing antiseptic agents; therefore, these bis-QACs were expected to be useful as topical skin antiseptics.

In Chapter 2, to predict the potential skin irritation of novel bis-QACs more accurately, we focused on the inflammatory cytokines induced by antiseptics and compared their expression at both the mRNA and protein level. In this study, novel bis-QACs such as 3PHBO-12, 3HHDMP-12, which showed particularly excellent BI values as reported in the first chapter, were used for the test. The cytotoxicity of 3PHBO-12 and 3HHDMP-12 in NHEK (AD), NHDF (AD), THP-1 and HL-60 cells was evaluated by using a WST assay. The cytotoxicity of these bis-QACs was lower than that of the existing QACs, but higher than that of the biguanide-based compounds. Subsequently, each antimicrobial substance at a concentration lower than the  $IC_{50}$  obtained from the cytotoxicity assay was applied to NHEK (AD) cells for 24 to 72 h to compare the mRNA expression levels of inflammatory cytokines. Our results showed that BAC or OCT induced an increase in inflammatory cytokine expression over time compared with the novel bis-QACs. In particular, 3HHDMP-12 did not induce the mRNA expression of inflammatory cytokines, which suggested that skin irritation may be milder than existing antiseptic agents.

Therefore, we evaluated the potential irritancy of the novel bis-QACs in more detail by using a reconstructed human skin model, LabCyte EPI-MODEL which is known to exhibit a good correlation with in vivo skin irritation. 3PHBO-12 and 3HHDMP-12 were applied to the LabCyte cultures at the actual use concentration and their cytotoxicity was compared. Cytotoxicity was observed only when cultures were treated with OCT and BAC, and not when treated with novel bis-QACs. In addition, the expression of inflammatory cytokines in the LabCyte cultures treated with each antimicrobial substance was compared at both the mRNA and protein level. As seen in the results of the cytotoxicity assay, inflammatory cytokine gene expression and IL-1 $\alpha$  secretion was significantly increased in OCT or BAC-treated LabCyte cultures. In contrast, even at actual use concentrations, the novel bis-QACs did not alter inflammatory cytokine mRNA expression or IL-1 $\alpha$  secretion. From the results of experiments using LabCyte cultures, it was suspected that the skin irritancy of 3PHBO-12 and 3HHDMP-12 was equal to or less than that of existing antiseptic agents.

In summary, this paper is a comparative study of the efficacy and safety of newly synthesized bis-QACs and existing antiseptic agents and has clarified the antimicrobial properties, cytotoxicity, biocompatibility, and potential skin irritancy of these bis-QACs. Unlike antibiotics, which are intended to have selective toxicity to microorganisms, skin antiseptics are toxic not only to microorganism cells, but also to human skin cells. This is an essential property and there are therefore no antiseptic agents that completely overcome the problems of both efficacy and safety. However, the development of new antimicrobial substances is expected to create more options for skin antiseptics, not only through increased antimicrobial activity, but also through a reduction in adverse effects such as contact dermatitis. In particular, the biocompatibility and potential skin irritation of 3PHBO-12 and 3HHDMP-12 were equal to or less than those of existing antiseptic agents. Therefore, were

considered that these novel bis-QACs were applicable antiseptics to human skin, which prevent many HAI, including drug-resistant bacteria.