광화학반응으로 polyallyamine막이 표면에 고정화된 리팜피신-함유 폴리우레탄으로부터 유리되는 리팜피신의 항균 활성에 관한 연구

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Antimicrobial activity of rifampicin released from the rifampicin-containing-polyurethane immobilized on the surface with the polyallyamine membrane using photochemical reaction

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ABSTRACT

A new method for the prevention of foreign body-associated infections by controlled release of antibiotic was developed. The polyurethane (PU) matrix containing rifampicin was immobilized with hydrophilic photoreactive polyallylamine (PPA) containing azidophenyl groups. The rifampicin release characteristics and the long-lasting antimicrobial activities of the new material was compared with rifampicin-containing PU matrix without PPA membrane. The release rate of antibiotic from rifampicin-containing PU with PPA membrane significantly decreased as the thickness of PPA membrane was increased. The PPA-immobilized rifampicin-containing PU discs immersed in the PBS for 47 days had an efficient antimicrobial activity against both *S. aureus* and *S. epidermidis*.

INTRODUCTION

Infection remains one of the most serious complications of artificial organs, but there are few systems which are able to prevent effectively from the foreign body-associated infections. Sheretz *et al* reported that an antibiotic coating to PU vascular catheters resulted in an *in vitro* half-life of up to 12 hr to 24 hr with the antimicrobial activity studied by subcutaneous mouse model.[1] In 1991, Golomb and Shpigelman developed an antibiotic material containing paraben in PU matrix and slowly releasing the bateriostatic preservative.[2] In order to maintain the sustained antimicrobial activity continuous release of effective doses of antibiotic, immobilization of hydrophilic rate-controlling membrane using the PPA on the surface of antibiotic-containing PU was tried. Rifampicin was selected as an antibiotic of choice because it exhibits broad specrum of antimicrobial activity and is bactericidal even at a very low concentration against staphylococci.[3] *Staphylococcus aureus*(ATCC 27735) and *Staphylococcus epidermidis* RP12(ATCC 35984) were selected for the study of bacterial inhibition due to its presence in the majority of prosthetic infections.[4]

MATERIALS AND METHODS

Preparation of PU discs containing rifampicin (RP)

Rifampicin was dissolved in THF (terahydrofuran). After complete dissolution of rifampicin in the solvent, PU(Pellethane 2363 80AE) was added to make the final concentration of rifampicin to polymer 10% to 30% (w/w). PU discs of 10 mm in diameter were prepared for the test of the antimicrobial activities and for the release kinetics studies.

PPA membrane immobilization on the surface of rifampicin-containing PU discs

In order to study the availability of polyallylamine(PPA) membrane in the control of rifampicin release from rifampicin-containing PU discs, the sample discs were prepared by using photochemical immobilization of polyallylamine (Fig 1).

Measurement of antibiotic release kinetics of the antibiotic-containing PU discs with and without PPA membrane

To observe the antibiotic release kinetics, every two rifampicin-containing PU discs of three different concentrations (10, 20, and 30%) were eluted in 20 ml of phosphate buffered saline(PBS, pH 7.4). The elution buffers were periodically replaced with a fresh PBS at 37'C for 47 days. Rifampicin concentration was measured using spectrophotometer at 334nm. The release rate of rifampicin from discs with and without PPA membrane were compared.

Observation of antimicrobial activity of rifampicin-containing PU

The evaluation of antimicrobial activity of PU with various rifampicin loading was carried by disc diffusion method.[5] *S. epidermidis* and *S. aureus* were cultured in TSB(tryptic soy broth) for 5 hrs to obtain the log phase cultures. Each of bacterial suspensions were diluted to 3.60 X 10^6 CFU/ml and 5.43 X 10^6 CFU/ml with PBS repectively. The RP discs of 10%, 20%, and 30% loading were placed on the surface of nutrient agar plates inoculated with 0.1 ml of bacterial suspension. After 24 hrs, the diameter of inhibitory zone on the culture plate was measured. The sample discs were transferred everyday to a new culture plate and the same procedures were repeated everyday until 12 days. Zones of inhibition were evaluated by measuring the diameter perpendicular to the long axis of the inhibitory zone by Sherertz *et al.*[5] The antimicrobial activity of PU discs with various antibiotic loading was compared.

Observation of long-term antimicrobial activity of rifampicin-containing PU discs with and without PPA membrane

The 10%, 20%, and 30% RP discs without PPA membrane were exposed in 0.01 M PBS for 47 days to evaluate the long-term antimicrobial activity. The discs were gently washed in PBS (3 times) and then it were placed on agar plates inoculated with 0.1 ml of *S. epidermidis* (9.5 X 10^6 CFU/ml). After incubation for 24 hrs, the zones of inhibition were evaluated by measuring the diameter of the narrowist clear zone.

To evaluate the antimicrobial activity of RP discs with PPA membrane, different methods were tried against each of bacterial species. The sample discs (20% and 30%) for the antimicrobial activity test against *S. epidermidis* were prepared by elution in PBS for 47 days. The discs were placed on agar plates with *S. epidermidis* (4 X 10^5 CFU) and incubated for 24 hrs. The 20% RP discs for the antimicrobial activity test against *S. aureus* were prepared by periodical sampling after elution for 1, 2, 3, 4, 5 weeks in 10 ml of PBS. The release buffer was exchanged every week. The evaluation of antimicrobial activity was performed by placing of sample discs on agar plates inoculated with *S. aureus* (2.87 x 10^5 CFU)

RESULTS AND DISCUSSION

Control of release rate was achieved by the hudrophilic polyallyamine membrane immobilized photochemically on the surface of PU containing rifampicin(Fig 1). *In vitro* studies have demonstrated an efficient, long-lasting prevention of bacterial growth on and around the PU discs prepared by this method.

Figure 2 shows the release curves of rifampicin from the PU discs with different antibiotic loading. The initial release rate decreases as the loading of RP increases from 10% to 20%. However, the initial release rate increases again as the loading of RP increase from 20% to 30%.

The marked decrease of release rate was found in the discs with PPA membrane. The release rates of rifampicin from 20% sample discs decreased as the concentration of PPA solution for the treatment of PU discs increased (Fig 3, a). The RP discs coated with 0.5%, 1.0% and 2.0% PPA solution exhibited a zero-order release pattern which is very ideal for the long-term prevention of microorganisms. The 30% rifampicin-containing PU discs treated with 0.1 and 0.5% PPA still showed so-called 'initial burst' as is the case of PU discs without PPA membrane(Fig 3, b). These results suggest that PPA membrane is a suitable rate-limiting membrane or rate-control membrane of rifampicin release.

The use of PPA membrane, results in a long-lasting antimicrobial activity as well as control of antibitic release rate. Figure 4 shows the antimicrobial activities of the PU discs with different antibitic loading. The sample discs maintained the inhibitory zones with diameters larger than 30 mm for at least 13 days against both *S. aureus* and *S. epidermidis*. Significant differences were not found among the three RP discs in the inhibition of bacterial growth until 13 days and the antimicrobial activities according to experimental period were slightly decreased for both *S. aureus* (Fig 4, a) and *S. epidermidis* (Fig 4, b). In this experiment, we found a resistant strain at 3rd days of incubation time (data not shown). In general, it is known that the frequency of developing rifampicin resistance is about 10^{-6} to 10^{-8}. [6] The emergence of resistant strain during the use of RP may be one of the potential problem in the clinical use of

rifampicin-containing artificial organs. Raad *et al* had showed that the combination of minocycline and rifampicin is unique and highly effective in preventing the colonoization of catheters with slime-producing staphylococci and gram-negative bacteria. [7] Therefore, the use of combination of rifampicin with minocycine or other antibiotics is seriously being considered to avoid the appearance of resistant strain to rifampicin in our system.

The weight and thickness of the RP discs without and with PPA membrane was measured prior to the evaluation of antimicrobial activities. The thickness of sample discs increased as the concentraion of rifampicin and PPA solution was increased (Table I). The 10% and 30% RP did not inhibit bacterial growth. But, 20% RP had weak antimicrobial activity against *S. epidermidis* (Fig. 5) after 30 days of elution. It is thought that the maintenance of an antimicrobial activity in 20% RP is to the relatively slow release rate.

Figure 6 illustrates the long-lasting antimicrobial activity of the RP discs treated with various PPA solutions after elution for 47 days. The inhibitory zone of 20% sample disc treated with PPA solutions greater than 0.5% was larger than that of discs treated with 0.1% PPA solution(Fig. 6, a). The antimicrobial activity of 30% sample discs had a linear correlation with the concentration of PPA solutions.

Figre 6, b shows the changes of inhibitory zone according to the release period of rifampicin in PBS. The antimicrobial activities of the 20% RP discs treated with different PPA solutions against *S. aureus* were observed for upto 5 weeks. The 20% RP discs coated with PPA solutions greater than 0.5% maintained a high level (zone sizes, > 35 mm) of inhibitory activity against *S. aureus*. This activity was significantly better than that without PPA membrane.

From the results, the use of PPA membrane in the RP discs for the sustained and controlled release of rifampicin is found to be useful for the prevention of artificial organ-associated infections.

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Fig. 1. Scheme for photochemical immobilization of membrane

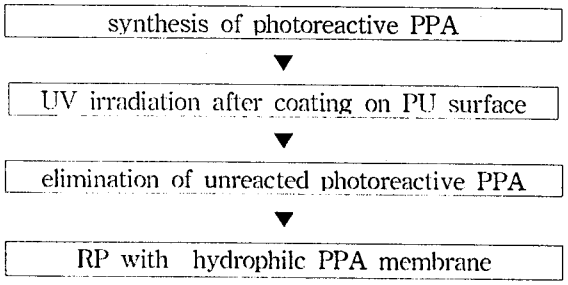

Table I. Characteristics of PU discs with various rifampicin load concentration with or without PPA used in bacterial inhibition study.

Rifampin in PU % (w/w)	Concentration of PPA % (w/w)	Disc weight Mean(+/-SD) (g)	Disc thickness (㎛)
10	0	0.0041(0.0004)	51.67(2.89)
20	0	0.0047(0.0002)	53.33(2.89)
30	0	0.0041(0.0001)	73.33(5.77)
20	0.1	0.0040(0.0002)	55.67(4.93)
	0.5	0.0052(0.0003)	66.00(1.73)
	1.0	0.0053(0.0001)	78.33(4.93)
	2.0	0.0058(0.0002)	80.67(1.15)
30	0.1	0.0035(0.0003)	76.00(3.61)
	0.5	0.0041(0.0002)	82.33(2.52)
	1.0	0.0044(0.0001)	87.33(2.08)
	2.0	0.0051(0.0005)	93.67(3.21)

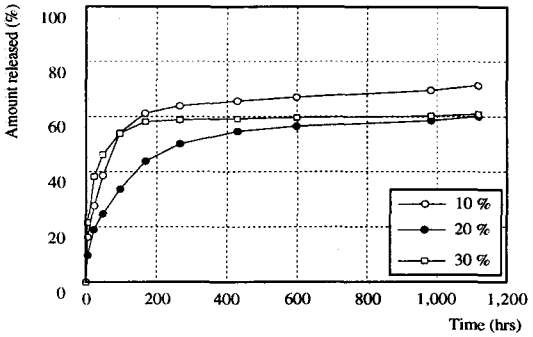

Fig 2. The release profile of rifampin polyurethane discs with various antibiotic load concentration

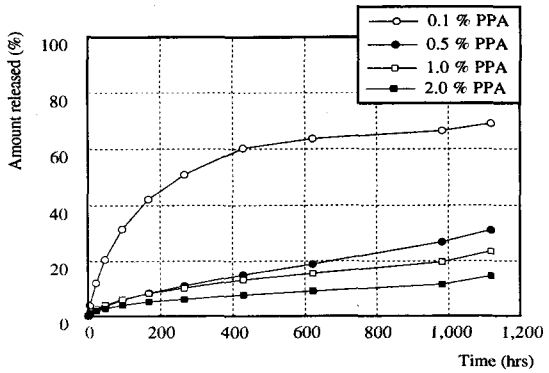

Fig 3 (a). Effect of rate-limiting membrane on the kinetics of rifampin from 20 % rifampin-containing PU discs coated with PPA

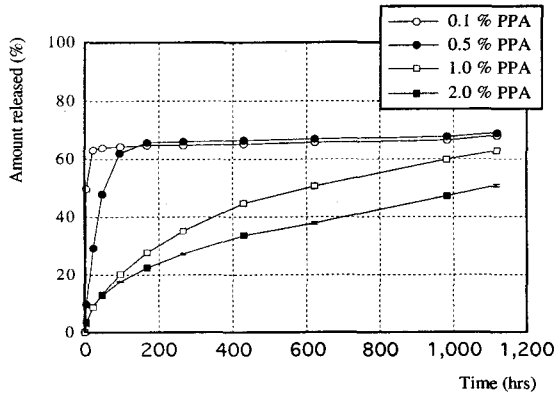

Fig 3. Effect of rate-limiting membrane on the kinetics of rifampin from 30% rifampin-containing polyurethane discs coated with PPA

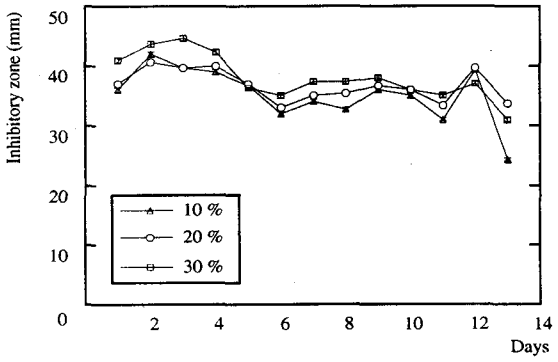

Fig 4 (a). The antimicrobial activity against S. aureus to the PU discs containing with diferrent rifampin load concentrations

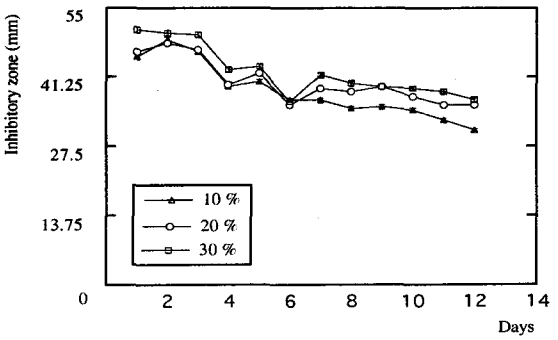

Fig 4 (b). The antimicrobial activities against S. epidermidis to the PU discs containing with different rifampin load concentrations

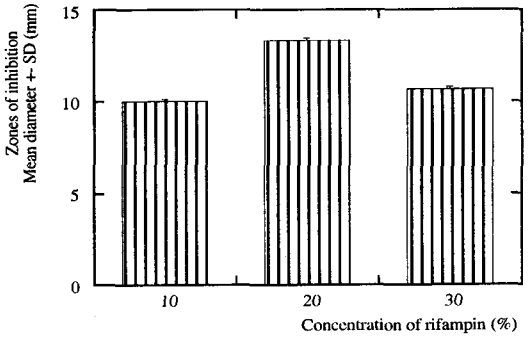

Fig 5. Zones of inhibition of polyurethane containing rifampin against S. epidermidis after release test

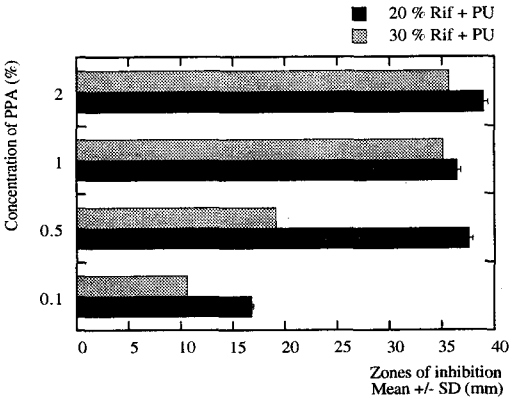

Fig 6 (a). Microbial inhibition of rifampin-containing PU coated with PPA against S. epidermidis after release test

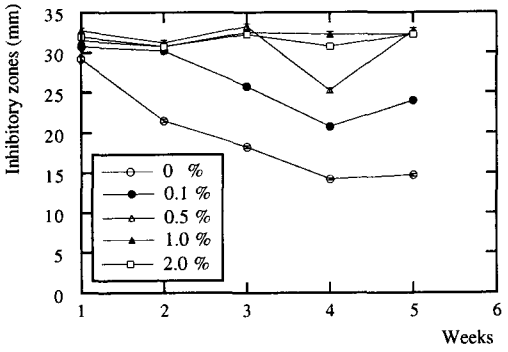

Fig 6 (b). Changes of the inhibitory zone against S.aureus according to the rifampin release period