Journal of Hospital Infection (2003) **53**: 58–63
doi:10.1053/jhin.2002.1332

Combination therapy with polymyxin B-immobilized fibre haemoperfusion and teicoplanin for sepsis due to methicillin-resistant *Staphylococcus aureus*

T. Nakamura*, C. Ushiyama*, Y. Suzuki†, T. Inoue‡, H. Shoji§, N. Shimada¶ and H. Koide¶

*Departments of *Medicine and †Surgery, Misato Junshin Hospital, Saitama; ‡Department of Cardiology, Koshigaya Hospital, Dokkyo University School of Medicine, Saitama; §Artificial Organ Department, Toray Medical Co., Ltd, Tokyo and ¶Department of Medicine, Koto Hospital, Tokyo, Japan*

Summary: The aim of the present study was to determine whether treatment with polymyxin B-immobilized fibre (PMX-F) haemoperfusion, teicoplanin, or both in combination is effective in patients with methicillin-resistant *Staphylococcus aureus* (MRSA) sepsis. Sixty patients with MRSA sepsis were randomly assigned to one of four treatments: (A) PMX-F treatment ($N=15$), (B) teicoplanin treatment ($N=15$), (C) PMX-F and teicoplanin in combination ($N=20$) and (D) conventional therapy ($N=10$). PMX-F treatment was repeated twice. Teicoplanin was administered by intravenous injection. Plasma endotoxin levels were determined by endospecy test. Plasma endotoxin levels were reduced in groups A and C ($P<0.05$). Survival rates were 53, 47, 90, and 20% in groups A, B, C and D, respectively (group C versus group A, $P<0.05$; group C versus group B, $P<0.01$; group C versus group D, $P<0.001$). The mean duration of stay was 44, 42, 28 and 56 days in groups A, B, C and D, respectively. Our data suggest that combination therapy with PMX-F and teicoplanin is effective for sepsis caused by MRSA.

Keywords: Endotoxin; PMX-F; MRSA; Teicoplanin.

Introduction

Despite major advances in monitoring and treatment of sepsis, the mortalities in cases of septic shock remain high.[1] It has been generally assumed that the pathogenesis of sepsis from Gram-positive organisms is similar enough to that of endotoxin shock from Gram-negative bacteria that the same basic mechanisms are involved.[2] It is now understood, however, that Gram-positive sepsis differs from Gram-negative sepsis in that the Gram-positive organisms often arise from skin, wounds, and soft-tissue structures rather than from enteric or genitourinary sources.[3] The polymyxin B-immobilized fibre (PMX-F) cartridge has been used in the management of sepsis patients in Japan since 1989 without critical adverse effects.[4,5] We reported previously that PMX-F treatment effectively removes endotoxin in patients with sepsis caused by

Received 19 February 2002; revised manuscript accepted 30 September 2002.
Author for correspondence: Hikaru Koide, Department of medicine, Koto Hospital, 6-8-5 Ojima, Koto-ku, Tokyo 136-0072, Japan. Tel.: +81-3-3685-2166; Fax: +81-3-3685-7400; E-mail: hkoide@koto-hospital.or.jp

0195–6701/03/010058+06 $35.00/0

Gram-negative bacteria.[6,7] Kodama *et al.*[4] reported that PMX-F treatment is effective in patients with methicillin-resistant *Staphylococcus aureus* (MRSA), suggesting that improved status may be due to the removal of a small amount of endotoxin in Gram-positive or mixed infection, and that such reduction may prove effective in patients with toxic symptoms caused by endotoxin. The precise mechanisms involved are however still unclear.

Many investigators have examined the clinical efficacy of teicoplanin given daily for treatment of MRSA infections.[8,9] Teicoplanin has low potential for causing oto- and nephrotoxicity and the red-man syndrome, and it has a longer half-life than does vancomycin, thus enabling once-daily administration.[10] In the present study, we evaluated the efficacy of PMX-F and teicoplanin in septic patients with MRSA.

Patients and methods

Sixty patients with MRSA sepsis (40 men and 20 women; mean age, 55.5 years) treated in our intensive care units were enrolled in the study during the first 24 h after the onset of sepsis. Patients were considered to have sepsis if they fulfilled the criteria proposed by the American College of Chest Physicians (ACCP)/Society of Critical Care Medicine (SCCM) Consensus Conference Committee.[11] According to ACCP/SCCM definition, sepsis is manifested by two or more of the following features resulting from infection: (1) temperature >38 or $<36^{\circ}$C, (2) heart rate >90 beats/min, (3) respiratory rate >20 breaths/min or $PaCO_2$ <32 torr, and (4) white blood cell count $>12\,000/mm^3$ or $>10\%$ immature forms. The Acute Physiology and Chronic Health Evaluation (APACHE) II score was determined for each patient.[12]

Bacteraemia was diagnosed in 36 of the 60 patients by fever, chills or hypotension, and isolation of MRSA from at least two pretreatment blood cultures; catheter-associated infection was diagnosed in 18 patients based on fever, documented bacteraemia, and use of an intravascular access catheter in the absence of any other apparent source of infection. Diagnosis of soft tissue (18 patients) or bone infection (nine patients) required compatible radiographic studies and isolation of MRSA from a sample obtained by direct closed-space needle aspiration of the infected tissue; pneumonia (nine patients) was defined by the presence of new infiltrates on a chest X-ray and isolation of MRSA from sputum. Peritonitis (six patients) required a purulent exudate culture-positive for MRSA. Exclusion criteria were: treatment with steroids, immunosuppressive agents or nonsteroidal anti-inflammatory agents.

This study was pre-approved by our human research ethics committees. Informed consent was obtained from each participant or a responsible family member.

Standard therapy included: administration of antibiotics (phosphomycin sodium, β-lactams including cefmetazole and arbekacin sulphate; these were used in combination), immunoglobulin infusions, vasoactive drugs, mechanical ventilatory support, corrective measures for metabolic abormalities and any other supportive therapy deemed necessary by the primary physician.

The 60 patients were randomly assigned to one of four treatments (A) PMX-F treatment ($N=15$), (B) teicoplanin treatment ($N=15$), (C) PMX-F and teicoplanin treatment ($N=20$), or (D) conventional treatment ($N=10$). Assignment to study groups was by means of sealed envelopes. There was blinding of allocation, treatment and outcome assessment.

PMX-F and the haemoperfusion column have been described previously.[4–7] To reduce the toxic effect of polymyxin B and derive benefit from the lipopolysaccharide (LPS) blocking property, polymyxin B was immobilized on to insoluble fibres creating a haemoperfusion column to use as an extracorporeal endotoxin removal device for the treatment of patients with sepsis.[4,5] PMX-F therapy was administered twice within a 24 h period (groups A and C). Access to the circulation for direct haemoperfusion with PMX-F was obtained by double-lumen catheter (Arrow International Inc., Reading, PA, USA) inserted into the femoral vein by Seldinger's method. Direct haemoperfusion was performed for 2 h at a flow rate of 80–100 mL/min. Blood endotoxin levels were determined by the Endospecy test (Seikagakukogyo, Tokyo, Japan) after pretreatment by the new perchloric acid method reported previously.[4] Teicoplanin (6 mg/kg) was administered by intravenous injection once daily for three days and then every other day for 14 days (groups B and C).

Data were expressed as mean $\pm$ SD. Results were analysed by one-way analysis of variance (ANOVA) and relative risk and absolute risk reduction were calculated. $P<0.05$ was accepted as statistically significant.

Results

Patient characteristics are shown for each treatment group in Table I. No significant differences were found with respect to age, sex, APACHE II score, systolic blood pressure, heart rate, temperature, white blood cell count, or C-reactive protein. MRSA was detected in all study patients. The sources of MRSA are shown in Table II. Blood endotoxin levels in groups A and C were significantly decreased after treatment (group A, from 16.2 ± 4.8 to 4.6 ± 2.4 pg/mL, $P < 0.05$; group C, from 14.6 ± 5.4 to 3.6 ± 1.8 pg/mL, $P < 0.05$). Levels in groups B and D, however, showed little difference after treatment (group B, from 15.0 ± 4.2 pg/mL to 13.2 ± 3.8 pg/mL and group D, from 14.6 ± 3.9 pg/mL to 13.8 ± 4.0 pg/mL). The survival rates were 53, 47, 90 and 20% in groups A, B, C and D, respectively. Statistical analysis is shown in Table III. Surviving patients were discharged within 60 days after treatment. The mean duration of stay was 44, 42, 28 and 56 days in groups A, B, C and D, respectively. Adverse events including hypersensitivity (erythema) were recognized in two patients (one patient in group B and one patient in group C). These were not seen in group A and group D.

Table I *Patient characteristics*

	Group A ($N=15$)	Group B ($N=15$)	Group C ($N=20$)	Group D ($N=10$)
Mean age (years)	58.5	55.0	54.2	54.4
Sex (M/F)	10/5	10/5	14/6	6/4
APACHE II	23.8 (4 0)	23.5 (3.8)	24.5 (4.5)	22.0 (3.8)
SBP (mmHg)	98 (18)	96 (18)	92 (16)	102 (22)
HR (beats/min)	112 (14)	114 (18)	120 (22)	112 (14)
Temperature (°C)	38.5 (1.2)	38.5 (1.0)	38.8 (1.0)	38.7 (0.7)
WBC ($\times 10^9$/L)	14.6 (1.7)	14.0 (1.6)	15.8 (1.9)	13.8 (1.7)
CRP (mg/dL)	32.2 (4.6)	32.0 (6.2)	37.0 (6.2)	32.4 (4.2)

Numbers in parentheses are standard derivation unless otherwise specified. APACHE, Acute Physiology and Chronic Health Evaluation; SBP, systolic blood pressure; HR, heart rate; WBC, white blood cell; CRP, C-reactive protein; PMX-F, polymyxin B-immobilized fibre. Group A: PMX-F treatment, group B: teicoplanin treatment, group C: PMX-F + teicoplanin treatment, group D: conventional treatment.

Table II *Sources of methicillin-resistant* Staphylococcus aureus

	Group A ($N=15$)	Group B ($N=15$)	Group C ($N=20$)	Group D ($N=10$)
Blood	10	10	12	4
Catheter	4	4	8	2
Soft tissue	4	5	6	3
Bone	4	2	2	1
Sputum	2	2	3	2
Ascites	1	2	1	2

Group A: PMX-F treatment, group B: teicoplanin treatment, group C: PMX-F + teicoplanin treatment, group D: conventional treatment.

Discussion

Sepsis is a major cause of death in critical care units worldwide, including Japan, and it consumes considerable healthcare resources.[2] The role of Gram-positive bacterial pathogens including MRSA in the occurrence of septic shock has received minimal attention because these organisms were thought to be quantitatively less important than Gram-negative bacteria as a cause of septic shock.[2] The prevalence of Gram-positive bacterial pathogens is on the increase because: (1) empiric antimicrobial regimens designed primarily against Gram-negative pathogens select for resistant Gram-positive pathogens, (2) long-term use of intravascular catheters is increasing, (3) the use of surgically implanted foreign materials is increasing, and (4) antibiotic resistance is increasing in Gram-positive organisms.[2]

Table III *Comparisons of survival, relative risk of death and absolute risk reduction of death*

		Risk of death		Absolute risk reduction of death	
Comparison	Survival	RR	95% CI	ARR	95% CI
Group A versus group B	Not significant	0.88	0.43–1.80	0.07	−0.29–0.42
Group A versus group C	$P < 0.05$	0.21	0.05–0.89	0.47	0.15–0.79
Group A versus group D	Not significant	0.58	0.31–1.09	0.33	−0.02–0.69
Group B versus group C	$P < 0.01$	0.19	0.05–0.76	0.52	0.22–0.82
Group B versus group D	Not significant	0.67	0.38–1.17	0.28	−0.09–0.64
Group C versus group D	$P < 0.001$	0.13	0.03–0.48	0.70	0.42–0.98

RR = relative risk, CI = confidence intervals, ARR = absolute risk reduction.

Endotoxin is absent in Gram-positive bacteria, including MRSA. The cell wall contains a thick layer of peptidoglycan, which lies directly over the plasma membrane. The structural components of Gram-positive cell walls are able to mimic some of the properties of endotoxin in their ability to induce proinflammatory cytokine production in mononuclear cells.[13] Endotoxin is the principal component of Gram-negative bacteria responsible for initiating the process of sepsis. Endotoxaemia can sometimes occur in patients with Gram-positive bacteraemia, presumably as a consequence of damage to the integrity of the mucosal barrier in the gastrointestinal tract.[14]

Despite conventional intensive care therapies, the prognosis in septic patients is still poor. Apheresis therapies, which use more selective adsorption techniques, can lower the extent of toxins and cytokines in blood.[15] Various apheresis techniques have been suggested for extracorporeal endotoxin removal. The PMX cartridge has been safely used without adverse effects. We reported previously that PMX-F was effective in patients with Gram-negative bacterial sepsis.[6,7] In the present study, endotoxaemia occurred in septic patients with MRSA, and PMX-F treatment reduced plasma endotoxin levels in these patients. The survival rate with PMX-F treatment was higher than that with conventional treatment. In vitro, polymyxin-B shows antimicrobial activity against MRSA.[16] Jaber *et al.*[17] reported the ability of polymyxin-B to inhibit the biological activity of *S. aureus* lipoteichoic acids, and the ability of a PMX-20R column to remove cytokine-inducing bacterial products other than lipopolysaccharide from 10% human plasma solution containing *S. aureus*. Iwama *et al.*[18] reported PMX-F to be effective in a patient with sepsis due to Gram-positive infection. Small amounts of endotoxin play a key role in the presence of other inflammatory cytokines and mediators in the development of the symptoms of sepsis. Similar findings have been observed with endotoxins and toxic shock syndrome toxin (TSST)-1. The toxicity of TSST-1 in staphylococcal infection increases by 50 000 times in the presence of endotoxin.[19] Miwa *et al.*[20] have developed a sensitive and specific ELISA that enables quantitation of TSST-1. In preliminary studies of sepsis patients with MRSA infection, we recognized that PMX-F reduced TSST-1 concentrations, in part due to the reduction by PMX-F of endotoxin observed in the present study (data not shown).

Teicoplanin is a glycopeptide antibiotic available in Europe and Japan that is active against MRSA.[8,9] Some investigators reported that teicoplanin is effective in vascular access-associated sepsis, soft-tissue infections, osteomyelitis, and pneumonia caused by MRSA.[8,10] Recently, Otsuka *et al.*[21] reported that the antibacterial activity of teicoplanin was attenuated by combination with flomoxef, panipenem or cefmetazole. Several clinical trials have assessed the efficacy of teicoplanin given once daily at doses of 6 mg/kg.[8,22] Bantar *et al.*[8] reported the efficacy and pharmacodynamics of teicoplanin, given daily at 6 mg/kg during the first three days and then on alternate days, for treatment of MRSA. We used this protocol in the present study. Vancomycin is considered the agent of choice for Gram-positive infections, and its use has increased substantially worldwide. Rolston *et al.*[10] reported that teicoplanin and vancomycin show equivalent efficacy and tolerance in septicaemia caused by Gram-positive pathogens. Teicoplanin offers some potential advantages over vancomycin, however, including a favourable toxicity profile and a long half-life.[23,24] In preliminary studies, we compared the survival rate and adverse events between teicoplanin and vancomycin in 20 MRSA-septic patients ($N = 10$ in each group). The survival rate was 50% in teicoplanin-treated patients, and 30% in vancomycin-treated patients. Adverse events including hypersensitivity, hepatotoxicity and nephrotoxicity in vancomycin-treated patients were more than those in the teicoplanin-treated group. Therefore, we selected teicoplanin instead of vancomycin in the present study.

Knapp *et al.*[25] reported that only soluble ELAM-1 is a useful early parameter in predicting outcome of patients with Gram-positive sepsis and that soluble VCAM-1 could not be used as an early prognostic parameter. Staphylococcal enterotoxins are described as 'superantigens' that stimulate T-cell activation and release of T-cell-derived cytokines including interleukins, tumour necrosis factor-alpha (TNF-alpha) as well as interferon-gamma.[26] We are now studying the effect of PMX-F + teicoplanin treatment on these factors. In addition, Yoh *et al.*[26] reported that T cells activated by MRSA-derived enterotoxins and subsequent production of cytokines may play an important role in the pathogenesis of MRSA-associated glomerulonephritis. In the present study, eight out of 20 septic patients in group C developed glomerulonephritis in association with MRSA infection. Combination therapy

with PMX-F and teicoplanin reduced urinary protein excretion from 3.2 ± 1.6 to 0.6 ± 0.3 g/day (data not shown). Therefore, this combination therapy may be effective in MRSA-related glomerulonephritis. Further studies of MRSA-related glomerulonephritis are now in progress.

Our results show that PMX-F and teicoplanin in combination is more effective in patients with sepsis caused by MRSA than is treatment with PMX-F or teicoplanin alone.

Acknowledgements

We thank Ms Yukiko Suzuki, Ms Mie Hirayama, and Mr Hiroyuki Nukui, Misato Junshin Hospital, Saitama, Japan, for their technical assistance. We also thank Mr Noboru Araki, Toray Medical Co., Ltd, Tokyo, Japan for his helpful suggestions.

References

1. Martin C, Viviand X, Leone M, Thirion X. Effect of norepinephrine on the outcome of septic shock. *Crit Care Med* 2000; **28**: 2758–2765.
2. Opal SM, Cohen J. Clinical Gram-positive sepsis: does it fundamentally differ from Gram-negative bacterial sepsis? *Crit Care Med* 1999; **27**: 1608–1616.
3. Bone RC. Gram-positive organisms and sepsis. *Arch Intern Med* 1994; **154**: 26–34.
4. Kodama M, Tani T, Hanasawa K *et al*. The PMX Clinical Study Group. Treatment of sepsis by plasma endotoxin removal: hemoperfusion using a polymyxin-B immobilized column. *J Endotoxin Res* 1997; **4**: 293–300.
5. Shoji H, Tani T, Hanasawa K, Kodama M. Extracorporeal endotoxin removal by polymyxin B immobilized fiber cartridge. Designing and antiendotoxin efficacy in the clinical application. *Therapeut Apheresis* 1998; **2**: 3–12.
6. Nakamura T, Ebihara I, Shimada N *et al*. Effects of haemoperfusion with polymyxin B-immobilized fibre on serum neopterine and soluble interleukin-2 receptor concentrations in patients with septic shock. *J Infect* 1998; **37**: 241–247.
7. Nakamura T, Ebihara I, Shimada N, Koide H. Changes in plasma erythropoietin and interleukin-6 concentrations in patients with septic shock after hemoperfusion with polymyxin B-immobilized fiber. *Int Care Med* 1998; **24**: 1272–1276.
8. Bantar C, Durlach R, Nicola F *et al*. Efficacy and pharmacodynamics of teicoplanin given daily during the first 3 days and then on alternate days for methicillin-resistant *Staphlococcus aureus* infections. *J Antimicrob Chemother* 1999; **43**: 737–740.
9. De Lalla F, Viola R, Pellizzer G, Lazzarini L, Tramarin A, Fabris P. Regional prophylaxis with teicoplanin in monolateral or bilateral total knee replacement: an open study. *Antimicrob Agents Chemother* 2000; **44**: 316–319.
10. Rolston KVI, Chow AW, Bodey GP. Prospective, double-blind, randomized trial of teicoplanin versus vancomycin for the therapy of vascular access-associated bacteremia caused by Gram-positive pathogens. *J Infect Chemother* 1999; **5**: 108–112.
11. Bone RC, Balk RA, Cerra FB *et al*. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644–1655.
12. Knaus WA, Draper EA, Wagner EP, Zimmermann GJ. APACHE II: A severity of disease classification system. *Crit Care Med* 1985; **13**: 818–829.
13. Timmerman CP, Mattsson E, Martinez-Martinez L *et al*. Induction of release of tumor necrosis factor from human monocytes by staphylococci and staphylococcal peptidoglycans. *Infect Immun* 1993; **61**: 4167–4172.
14. Hurley JC. Concordance of endotoxemia with Gram-negative bacteremia in patients with Gram-negative sepsis: a meta-analysis. *J Clin Microbiol* 1994; **32**: 2120–2127.
15. Stegmayr BG. Apheresis as therapy for patients with severe sepsis and multiorgan dysfunction syndrome. *Therapeut Apheresis* 2001; **5**: 123–127.
16. Yoshida T, Hiramatsu K. Potent *in vitro* bactericidal activity of polymyxin B against methicillin-resistant *Staphylococcus aureus*. *Microbial Immunol* 1993; **37**: 853–859.
17. Jaber BL, Barrett TW, Cendoroglo Neto M, Sundaram S, King AJ, Pereira BJ. Removal of cytokine inducing substances by polymyxin-B immobilized polystyrene-derivative fibers during in vitro hemoperfusion of 10% human plasma containing *Staphylococcus aureus* challenge. *ASAIO J* 1998; **44**: 48–53.
18. Iwama H, Komatsu T. Effect of an endotoxin-removing column containing immobilized polymyxin B fiber in a patient with septic shock from Gram-positive infection. *Acta Anaesthesiol Scand* 1998; **42**: 1–4.
19. Schlievert PM. Enhancement of host susceptibility to lethal endotoxin shock by staphylococcal pyrogenic endotoxin type C. *Infect Immunol* 1981; **26**: 123–128.
20. Miwa K, Fukuyama M, Kunitomo T, Igarashi H. Rapid assay for detection of toxic shock syndrome toxin-1 from human sera. *J Clin Microbiol* 1994; **32**: 539–542.
21. Otsuka Y, Yoshibe T, Namioka M, Ezaki T. Combination effect of teicoplanin and beta-lactams on MRSA. *Jpn J Antibiot* 2000; **53**: 643–651.
22. Wilson APR, Gruneberg RN. Use of teicoplanin in community medicine. *Eur J Clin Microbiol Infect Dis* 1994; **13**: 701–710.
23. Davis RL, Smith AL, Koup JR. The red man syndrome and slow infusion of vancomycin. *Ann Intern Med* 1986; **104**: 285–286.
24. Babul N, Pasko M. Teicoplanin: a new glycopeptide antibiotic complex. *Drug Intelligence Clin Pharm* 1988; **22**: 218–226.

25. Knapp S, Thalhammer F, Locker GJ *et al*. Prognostic value of MIP-1-alpha, TGF-beta, sELAM-1, and sVCAM-1 in patients with Gram-positive sepsis. *Clin Immunol Immunopathol* 1998; **87**: 139–144.

26. Yoh K, Kobayashi M, Yamaguchi N *et al*. Cytokines and T-cell responses in superantigen-related glomerulonephritis following methicillin-resistant *Staphylococcus aureus* infection. *Nephrol Dial Transplant* 2000; **15**: 1170–1174.