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Development of a Competitive Direct Enzyme-Linked Immunosorbent Assay for Teicoplanin

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Abstract A competitive direct enzyme-linked immunosorbent assay (cdELISA) was developed for selective and rapid detection of a glycopeptide antibiotic, teicoplanin (TP). TP was conjugated to bovine serum albumin (BSA) for use as an immunogen. Repeated subcutaneous injections of 0.5 mg of the conjugate was effective in generating specific polyclonal antibody (PAb) toward TP in rabbits, as determined by cdELISA. TP-horseradish peroxidase conjugate (TP-HRP) was used as an enzyme marker. The cdELISA was developed based on a competition reaction between TP-BSA PAb and TP-HRP conjugate. The TP-BSA PAb was highly sensitive (detection limit, 0.3 ng/ml) and specific toward teicoplanin, showing no cross-reactivity to other glycopeptide antibiotics including vancomycin. There were good correlations (r^2=0.84 and 0.76, respectively) between cdELISA and microbiological assay, and high-performance liquid chromatography. The cdELISA system developed in this work is expected to be useful not only for selective and rapid monitoring of TP but also for study of TP pharmacokinetics.

Key words: *Actinoplanes teichomyceticus*, competitive enzyme-linked immunosorbent assay, polyclonal antibody, teicoplanin

Teicoplanin (TP), a group of antibiotics produced by *Actinoplanes teichomyceticus*, belongs to the vancomycin-ristocetin family of glycopeptide antibiotics [4, 8, 34]. It consists of five major components (A2-1 through A2-5), one hydrolysis component (A3-1), and four minor components (RS-1 through RS-4) [7, 9]. A2 components are used in clinic and A2-2 is the major component with the highest antibiotic potency [9, 30]. To this day, TP has been used in advanced clinical experimentation to treat severe infections caused by Gram-positive bacteria [2, 8, 9, 17, 30, 36]. It interferes with cell wall synthesis by inhibiting polymerization of peptidoglycan [32], and fights methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci, clostridia, and enterococci [10]. TP may be preferred to vancomycin (VM) in the treatment of Gram-positive infections [10], and world-wide problems with MRSA demand increased use of VM and TP, the only agents that effectively treat these infections. Recently, several studies on TP biosynthesis using the TP-producing strain of *Actinoplanes teichomyceticus* and TP therapeutic drug monitoring in patients have been performed [19, 32].

In chemical and analytical studies as well as in studies of pharmacokinetics and monitoring of theraputic concentrations, TP concentrations have been determined by various methods such as microbiological assay (MA) [11], high-performance liquid chromatography (HPLC) [1, 3, 20, 26, 27, 33, 35], liquid chromatography with fast atom bombardment mass spectrometry (LC-FAB-MS) [14], solid phase enzyme receptor assay (SPERA) [11], receptor-antibody sandwich assay (RASA) [12], and FPIA [15, 16, 25, 26, 27, 28], however, all of these analytical methods present some advantages and drawbacks, shown in the published reports.

To provide an analytical method with high specificity toward TP and low detection limit, to studies on TP pharmacokinetics and TP fermentation biotechnology in medical and veterinary fields, we developed a new method, a competitive direct enzyme-linked immunosorbent assay (cdELISA) system. We describe herein the cdELISA procedure for determination of TP levels. The precision level of the cdELISA was compared with various existing methods previously reported.

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MATERIALS AND METHODS

Chemicals

TP as a mixture of five closely related similar compounds, T-A2 complex, and a more polar T-A3 were purchased from TARGOCID Co. (Gruppo Lepetit SpA, Milan, Italy). The HPLC gradient chromatogram of the standard teicoplanin complex is shown in Fig. 1.

Horseradish peroxidase (HRP), TRIZMA^{TR} PRE-SET CRYSTALS [tris (hydroxymethyl) aminomethane, 0.05 M, pH 9.0] as a coating buffer, phosphate buffered saline with Tween 20 (PBST; 0.01 M phosphate buffer with 0.138 M NaCl, 0.0027 M KCl, 0.05% Tween 20) as a washing buffer, phosphate-citrate buffer tablets (0.05 M phosphate-citrate buffer, pH 5.0) as a substrate buffer, 3,3',5,5'-tetramethyl-benzidine (TMB) as a substrate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), goat anti-rabbit IgG-HRP, Freund's complete adjuvant and incomplete adjuvant, and phosphate buffered saline (PBS; 0.01 M phosphate buffer with 0.138 M NaCl, 0.0027 M KCl, pH 7.2) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Ultra Link™ Immobilized Protein A column, Slide-A-Lyzer Dialysis Cassette, and bovine serum albumin (BSA) for conjugation with TP were obtained from Pierce Co. (Rockford, IL, U.S.A.). All other reagents were of reagent grade or better.

Preparation of TP-BSA Conjugate

TP was conjugated to BSA for use as an immunogen. The conjugation (TP-BSA) of TP and BSA and a coating antigen for enzyme-linked immunosorbent assay (ELISA) was prepared according to Bauminger and Wilchek [6]. Briefly, 20 mg of freeze-dried BSA was solubilized in 2 ml of PBS and 20 mg of TP was solubilized in 0.5 ml of PBS, and 100 mg of EDC was added into the solution of TP and BSA which was slowly stirred. The reaction was kept for 24 h, and the reaction mixture was dialyzed against PBS. Conjugation degree between TP and BSA was determined by 10% SDS-PAGE according to Laemmli's method [23].

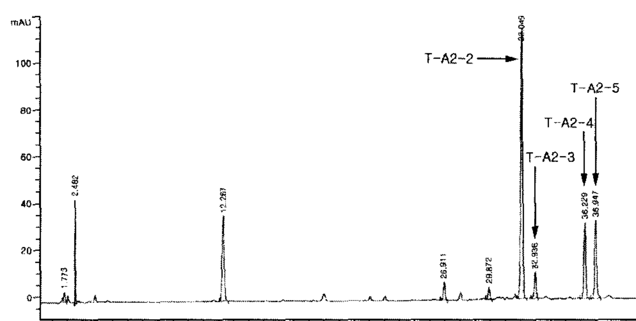

Fig. 1. HPLC gradient chromatogram of the standard teicoplanin complex.

Preparation of TP-HRP Conjugate

The TP-HRP conjugate for a competitive direct ELISA (cdELISA) was prepared as follows: 2.5 mg of TP in 0.5 ml PBS was added into 20 mg of HRP in 25% ethanol, and 188 mg of EDC was added into the solution and reacted for 30 min at room temperature. After keeping the solution at 4°C for 16 h, the TP-HRP solution was dialyzed against PBS. TP-HRP was precipitated by adding 2 volumes of cold acetone and stored at - 20°C for 10 min, and supernatant at 1,500 ×*g* centrifugation (10 min) was decanted and the pellet was solubilized in PBS. Acetone precipitation was repeated once, and the pellet obtained was solubilized in PBS. The solution was loaded onto Sephadex G-25 column, and the eluate was spectrophotometrically monitored at 280 nm. The fractions containing TP-HRP were stored at 4°C until used.

Production of Anti-TP-BSA Antibody

TP was conjugated to BSA for use as an immunogen. With repeated subcutaneous injections of 0.5 mg of the conjugate, TP-BSA was effective in generating specific polyclonal antibody (PAb) against TP-BSA in rabbits as determined by cdELISA. Immunization was done as follows: the concentration of immunogen was first adjusted to 1 mg/ml. For the first injection, the portions were emulsified with Freund's complete adjuvants using micro-mate interchangeable syringe, and 1 ml of emulsified immunogen (0.5 mg per animal) was injected to the rear footpad of rabbits. In the following injections, the portions were emulsified with Freund's incomplete adjuvants and were subcutaneously injected to the rear back part of rabbits. The animal was bled one week after each injection on the vein of its ear. The blood was kept at room temperature for 1 h and kept in refrigerator overnight. It was then centrifuged at 3,000 rpm for 20 min to obtain antisera, and 10% of NaN_3 was added to a final 0.02% concentration and stored at - 70°C.

Purification of Antibody

The purification of IgG type Ab from antisera was carried out with an Ultra Link™ Immobilized Protein A column. Two ml of each antisera and binding buffer were mixed and the mixture was applied onto a Protein A column pre-equilibrated with the binding buffer. After washing unbound antisera with the binding buffer, IgG was eluted with an elution buffer and the fractions were collected in test tubes which contained 100 μl of 1 M phosphate buffer, pH 7.6. The IgG was pooled and desalted with a G-25 column pre-equilibrated with PBS buffer and stored in a refrigerator.

Noncompetitive Indirect ELISA

To determine the specificity of Abs produced, noncompetitive indirect ELISA was performed. One-hundred μl (2 μg/ml) of TP-BSA in a coating buffer (0.05 M Trizma^{TR} PRE-SET

CRYSTALS, pH 9.0) were dispensed into microplate wells, and kept overnight at 4°C. After washing each well with 150 μl of a washing buffer three times and tapping the plate onto a paper towel to remove the remaining liquid, 100 μl of diluted solution of anti-TP-BSA antiserum with a washing buffer containing 1% BSA were added and reacted for 1 h at room temperature. After washing the wells, 100 μl of diluted goat anti-rabbit IgG-HRP conjugate were added into each well and reacted again for 1 h. After washing the wells, 100 μl of a fresh substrate solution (0.01% TMB, 0.05 M phosphate citrate buffer, pH 5.0, and 1% H_2O_2 added to a final concentration of 0.001% just before use) were added into each well and reacted for 30 min at room temperature. The enzyme reaction was stopped by adding 50 μl of a stop solution (2 M H_2SO_4), read at 450 nm with a microplate reader (THERMOmax^{TR}, Molecular Devices, U.S.A.), and an average value from three wells per treatment was obtained.

Competitive Direct ELISA (cdELISA)

TP-horseradish peroxidase conjugate (TP-HRP) was used as an enzyme marker. The cdELISA was established using the purified TP-BSA PAb and TP-HRP conjugate. The cdELISA for determining concentration of TP in the sample was performed as follows: 100 μl of purified Abs (2 μg/ml) in coating buffer was dispensed into the well of a microplate and kept overnight at 4°C for coating. After washing the wells as mentioned above, 100 μl of the mixture of 50 μl of TP standard diluted serially with PBST (10^{-1}- 10^{-4} ng/ml) or of sample and 50 μl of TP-HRP conjugate (diluted to 1:300 in PBST) were added into each well and reacted for 1 h at room temperature. The coloring reaction was the same as described for noncompetitive indirect ELISA. TP concentrations were calculated from a standard curve.

Cross-Reactivity of Anti-TP-BSA Antibody

To determine the specificity of antibody against TP, the cross-reactivity of anti-TP-BSA antibody with several glycopeptide or non-glycopeptide antibiotics were evaluated by cdELISA. Cross-reactivities (%) were determined as below,

$$\text{Cross-reactivity (\%)} = \frac{\text{Concentration of standard TP to inhibit 50\% of Ab binding}}{\text{Conc. of TP related antibiotics to 50\% of Ab binding}} \times 100$$

Effect of Methanol on ELISA

To determine methanol effect on cdELISA, methanol was serially diluted with PBST to final concentrations of 0.03, 1, 3, 10, 30, and 100%. TP was dissolved in each methanol solution to 1 mg/ml concentration, and each TP solution was serially diluted with the above methanol solutions. Each sample was tested to compete with TP-HRP toward the anti-TP-BSA antibody mentioned above in the cdELISA.

Preparation of Culture Samples

Actinoplanes teicomyceticus ATCC 31121 and mutants were maintained on Bennett's agar medium (pH 7.0) containing glucose 10 g, yeast extract 1 g, peptone 2 g, beef extract 1 g, and 15 g agar per liter of distilled water. The seed medium contained 10 g glucose, 4 g Bacto yeast extract (Difco), 4 g Bacto-peptone (Difco), 0.5 g $MgSO_4 \cdot 7H_2O$, 2 g KH_2PO_4, 4 g K_2HPO_4 per liter of distilled water. The production medium contained 30 g glucose, 5 g Bacto yeast extract (Difco), 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, and 0.1 g $CaCl_2 \cdot 2H_2O$ per liter of distilled water, and the combination of carbon and nitrogen source was varied to be 30 g glucose and 5 g yeast extract (Y.E.); 30 g dextrin and 5 g Y.E.; 30 g malto dextrin (DE 14-20) and 5 g Y.E.; and 50 g malto dextrin and 10 g Y.E. per liter of distilled water. Diaion HP-20 (Mitsubishi Chemical Industries Limited, Tokyo, Japan) was used in the fermentation as adsorbent resin according to the method of Lee *et al.* [24]. Prior to use, the adsorbent resin was soaked and swelled in 100% (v/v) methanol for 12 h to remove impurities and the solvent was removed by sufficiently washing with distilled water. The resin was added to the medium before sterilization. Frozen stock culture broths (1.0 ml) of *Actinoplanes teicomyceticus* and its mutants were inoculated in a 500-ml Erlenmeyer flask containing 50 ml of seed medium. After incubation on a rotary shaker with 150 rpm at 28°C for 48 h, 5 ml of seed culture broth were inoculated in a 500-ml Erlenmeyer flask containing 50 ml of production medium and adsorbent resin. The fermentations were performed for 5 days at 28°C and 300 rpm. After 5 days of fermentation, the resin was separated by centrifugation at 1,670 ×*g* for 10 min and teicoplanin was extracted from the resin with 80% (v/v) methanol at room temperature. It was diluted by 100, 1,000, and 10,000 times with PBST to remove the matrix effect.

HPLC Assay for TP

Instrumental analysis was based on HPLC with Hewlett Packard Series II 1090 instrument using YMC-Pack ODS-A column (4.6×250 mm). A gradient of 100% to 30% phase A (20 mM NaH_2PO_4/CH_3CN, 95:5, v/v) in a phase B (20 mM NaH_2PO_4/CH_3CN, 25:75, v/v) required 40 min at a flow rate of 1 ml/min. The UV detector was set at 254 nm. Targocid [(200 mg TP per vial, Gruppo Lepetit SpA, Milan, Italy); lyophilized teicoplanin used for injection] was used as a reference standard.

Microbiological Assay

As a microbiological assay (MA) of TP, the paper disc diffusion method was applied for detecting TP concentration, using *Bacillus subtilis* 6633 as the indicator strain and

Targocid diluted to final concentrations of 100, 250, 500, 750, and 1,000 μg/l as a standard. One % (v/v) of cultured *B. subtilis* 6633 was inoculated into LB medium containing 1.2% agar, and 20 ml of inoculated LB agar were poured into petri dish. Paper disc (8 mm) was placed on the surface of solidified LB agar and 50 μl of TP samples were added onto the paper disc and dried. The petri dish was incubated at 37°C for 12 h. The diameter of the inhibition zones obtained was measured, and the concentration of TP in samples was calculated from a standard curve.

RESULTS

Conjugation of TP and BSA

The result of SDS-PAGE of the TP-BSA conjugate is shown in Fig. 2. The BSA band was shifted to higher than 68 kDa and broader after conjugation, indicating that TP was covalently bound well to the NH_2 group of BSA molecules. The TP-BSA conjugate was used as an antigen for immunization.

Antibody Production and Purification

The antiserum for anti-PT-BSA antibodies which showed the highest titer by a noncompetitive indirect ELISA was used for the fourth antiserum of rabbit. This antiserum was purified with Protein A column according to the manufacturer's instructions. The purified anti-PT-BSA antibody was desalted and kept at 4°C for the following use.

Standard Curve of cdELISA

A cdELISA for TP was developed based on the competition between the purified TP-BSA Ab and TP-HRP conjugate. CdELISA was prepared by using the purified antibody and TP-HRP described in Materials and Methods, and a standard curve of established cdELISA is shown in Fig. 3. The standard curve on cdELISA shows that the detection limit for TP was 0.3 ng/ml. In comparison with SPERA, RASA, and FPIA, the cdELISA was most sensitive, showing the lowest detection limit value (approximately 10-fold lower than RASA). Therefore, our results showed that the TP-BSA Ab was highly sensitive and specific toward TP.

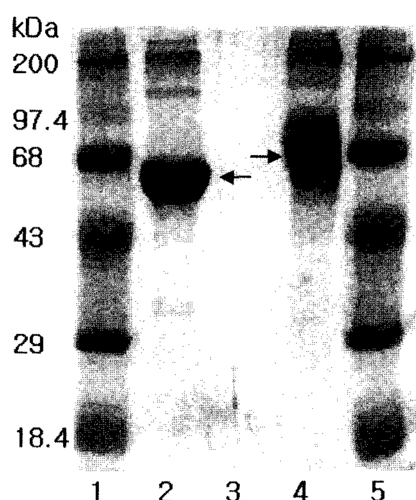

Fig. 2. SDS-PAGE pattern of the TP-BSA conjugate.
Lanes 1 and 5, molecular weight markers; lane 2, bovine serum albumin; lane 3, teicoplanin (TP); lane 4, TP-BSA conjugate.

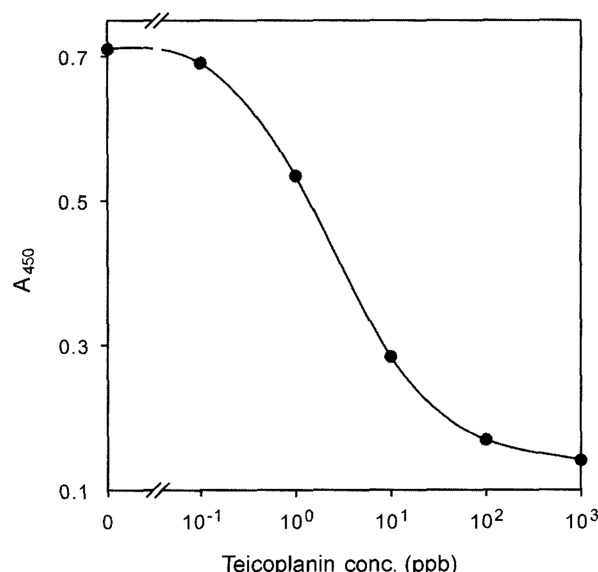

Fig. 3. Standard curve of cdELISA for TP with anti-TP-BSA antibody.
A purified polyclonal anti-TP-BSA Ab was adsorbed to the wells of microplates. Serially diluted TP and diluted TP-HRP conjugate were added to the wells and incubated at room temperature for 1 h. TMB/H_2O_2 substrate was then added and the mixture was reacted for 30 min. The reaction was stopped by 2 M H_2SO_4 and absorbance at 450 nm was measured.

Effect of Methanol on ELISA

The effect of methanol, which was employed as an extracting buffer for cdELISA, is shown in Fig. 4. The standard curve of TP, which was diluted with less than 0.3% methanol solution in PBST, was similar to that of TP in PBST. However, the overall color development of 10 and 30% methanol solution in PBST was lower than that of PBST, and there was no competition when TP was diluted in 100% methanol solution. Interference effect of methanol on cdELISA was not significant when extracting solution was used, as less than 3% methanol solution in PBST was used as the extracting solution. Thus, less than 3% methanol solution in PBST could be used for quantitative determination of TP by cdELISA.

Cross-Reactivity of Anti-TP-BSA Antibody

The cross-reactivity of anti-TP-BSA antibody towards antibiotics is shown in Fig. 5. The TP-BSA PAb was

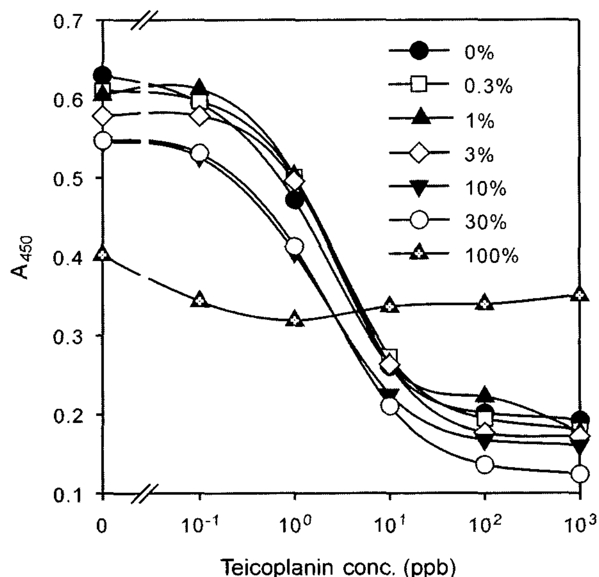

Fig. 4. Cross-reactivity of anti-TP-BSA antibody toward several antibiotics, as determined by cdELISA.
A purified polyclonal anti-TP-BSA Ab was absorbed to the wells of microplates. TP-HRP conjugate and serially diluted antibiotics were added to the anti-TP-BSA antibody-coated wells and incubated at room temperature for 1 h. The remaining reaction procedure was the same as in Fig. 3. TP, teicoplanin; R, ristocetin; V, vancomycin; L, lincomycin; E, erythromycin; A, amoxicillin; P, penicillin-G; S, spiramycin.

highly sensitive (detection limit, 0.3 ng/ml) and specific toward teicoplanin, indicating no cross-reactivity towards either VM, ristocetin as VM class, or non-glycopeptide antibiotics such as lincomycin, erythromycin, amoxicillin, penicillin-G, and spiramycin. cdELISA with the specific

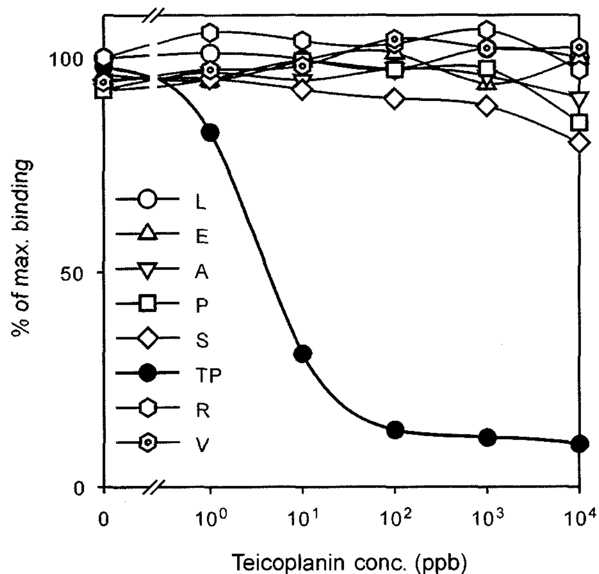

Fig. 5. Effect of methanol on cdELISA.
CdELISA was performed as in Fig. 3.

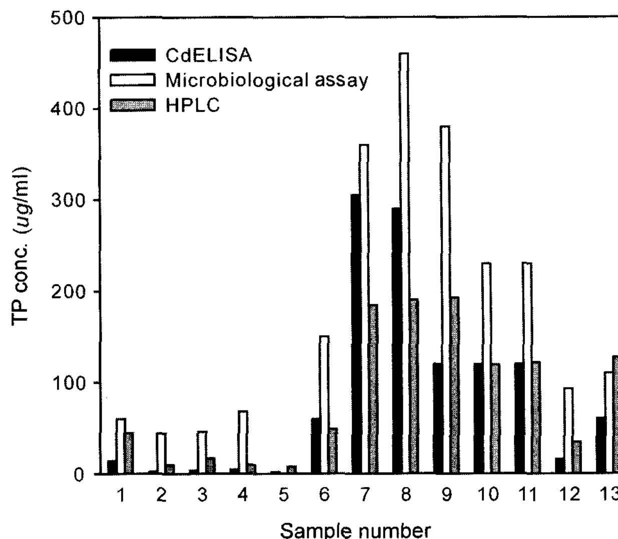

Fig. 6. Measurement of TP contents in culture samples, using TP-producing strains, as determined by cdELISA, MA, and HPLC.

TP-BSA Ab was more useful for detecting TP than SPERA, which showed a cross-reactivity toward glycopeptide antibiotics of VM class.

Comparison of cdELISA with Microbiological Assay and HPLC

TP contents in samples determined by cdELISA, MA, and HPLC varied in the range of 2-305 μg/ml, 0-460 μg/ml, and 7.5-192.8 μg/ml, respectively (Fig. 6). When TP was analyzed by the MA method, the TP content was slightly higher than those by the other two methods, indicating a low precision level. In contrast, when analyzed by cdELISA and HPLC methods, the TP content was somewhat higher than MA, showing a similar pattern between cdELISA and HPLC. Although the TP content was measured to higher extent than MA, there were good correlations between cdELISA and MA, between MA and HPLC, and between cdELISA and HPLC (r^2=0.84, 0.87, and 0.76, respectively) (Fig. 7).

DISCUSSION

The glycopeptide antibacterial teicoplanin (TP) has become increasingly popular in the last decade with the rise in infections related to methicillin-resistant *Staphylococcus aureus* [37]. TP has been determined or analyzed by various methods, such as HPLC, LC-MS, SPERA, RASA, and FPIA, with some advantages and drawbacks. Antibodies are used in numerous highly sensitive analytical techniques to detect antibiotics, proteins, and pesticides [21, 29]. The direct ELISA system developed for the first time in this

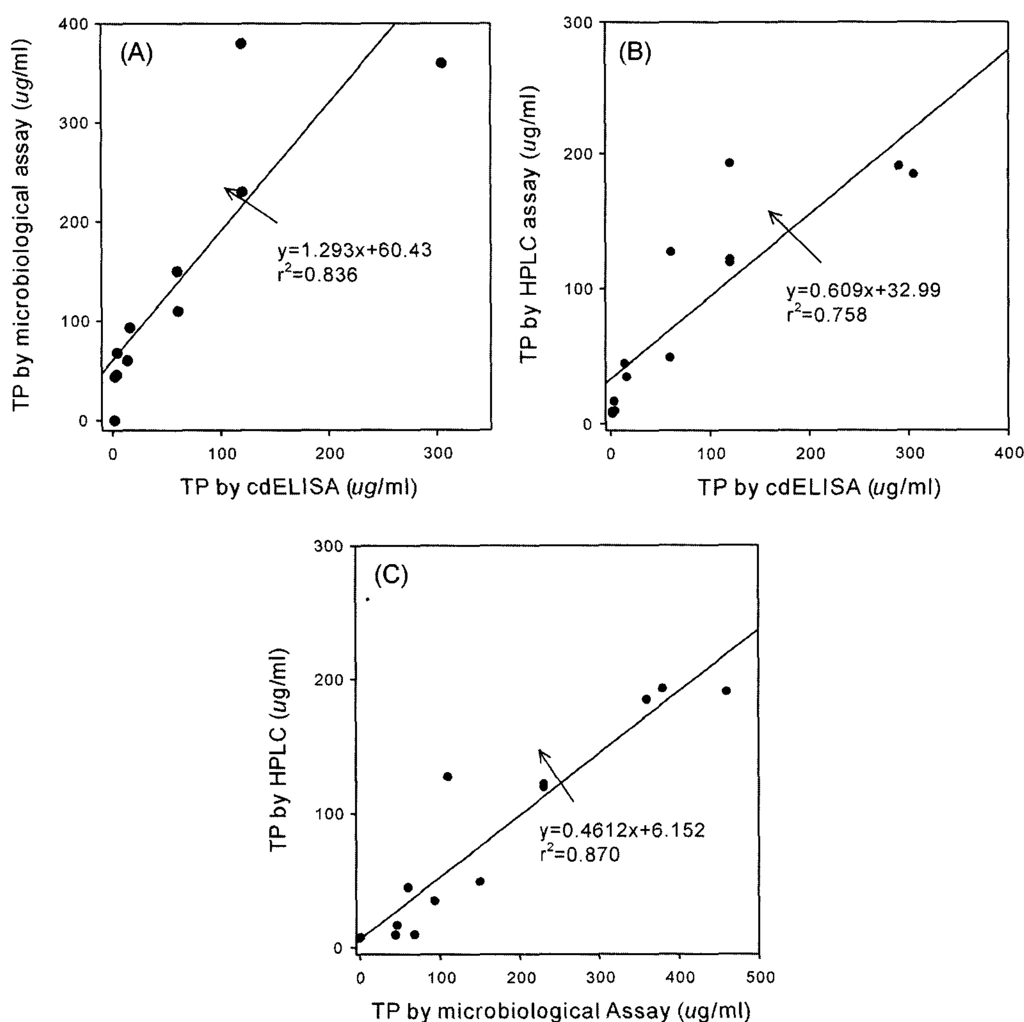

Fig. 7. Correlation curve between TP concentrations, as determined by cdELISA and/or microbiological assay and/or HPLC (n=13). Comparison between cdELISA and MA (A), between cdELISA and HPLC (B), and between MA and HPLC (C). r^2=correlation coefficient.

study is based on a competition between TP and TP-HRP on wells coated with specific TP-BSA antibody. The system would be useful for a method to rapidly evaluate up to pg/ml ordered materials in the solution, such as serum culture supernatant. Its detection limit (0.3 ng/ml) for TP was the lowest in comparison with previous methods; in comparison with SPERA (50 ng/ml) [10], RASA (3 ng/ml) [12], and FPIA (1,500 ng/ml) [25]. In addition, the cdELISA system could also detect free TP as well as complexes with other antibiotics including VM, ristocetin, erythromycin, and penicillin-G. Kureishi *et al.* [22] reported a modified agar diffusion bioassay for monitoring levels of either TP or VM in serum, and indicated that agar dilution, tube dilution, and broth microdilution susceptibility tests may be useful for both TP MIC determination and measurement of TP in serum containing rifampin or beta-lactam antibiotics, using rifampin-resistant *Bacillus subtilis* as an indicator organism.

On the other hand, the HPLC method has been most frequently used for determination of TP. The method requires equipments for analytical experiments and extraction procedures for sample preparation [3, 29]. Falcoz *et al.* [17] assessed the effect of renal failure on TP by using a highly specific chromatographic technique, and McCann *et al.* [26] indicated that a simple liquid extraction coupled with reverse HPLC and UV detection was shown to correlate well with fluorescence polarization immunoassay (FPIA) on the Abbot TD (x) analyzer. Awni *et al.* [3] mentioned that FPIA might be an optimal technique for therapeutic monitoring of TP in the clinical setting because of its simplicity, specificity, and good correlation to HPLC and MA. Of several methods available to measure TP concentrations in serum, FPIA has frequently been evaluated as a useful method in routine diagnostic laboratories [15]. Compared with bioassay, the assay demonstrated a good correlation in estimating concentrations of 5-100 mg/l.

McMullin *et al.* [27] compared the isocratic HPLC with FPIA and bioassay (agar diffusion). However, the competitive homogeneous FPIA, based on the ability of antibody to bind TP, showed detection limit higher than cdELISA [15, 26], although it sometimes showed low detection limit with 1-10 ng/ml pesticides [16].

On the other hand, the SPERA method, which is based on binding of TP to ε-aminocaproyl-D-alanyl-D-alanine conjugated to BSA, was not always specific in complex biological matrixes [11, 13]. The RASA method, based on bioselective adsorption of TP onto microtiter plates coated with a synthetic analog, represented detection limit lower than SPERA [12].

In conclusion, our results show that the cdELISA system with specific TP-BSA PAb may be useful not only for selective and rapid monitoring of TP in cultures or specimens, but also for studying teicoplanin pharmacokinetics and biosynthesis in medical and veterinary fields.

REFERENCES

1. Alet, P., O. Lortholary, F. Fauvelle, M. Tod, T. Genereau, M. Louchahi, A. Leon, L. Guillevin, and O. Petitjean. 1999. Pharmacokinetics of teicoplanin during plasma exchange. *Clin. Microbiol. Infect.* **5:** 213-218.
2. Auwera, P. V., M. Aoun, and F. Meunier. 1991. Randomized study of vancomycin versus teicoplanin for the treatment of Gram positive bacterial infections in immunocompromised hosts. *Antimicrob. Agents Chemother.* **35:** 451-457.
3. Awni, W. M., W. L. St Peter, D. R. Guay, M. T. Kenny, and G. R. Matzke. 1991. Teicoplanin measurement in patients with renal failure: Comparison of fluorescence polarization immunoassay, microbiological assay, and high-performance liquid chromatographic assay. *Ther. Drug Monit.* **13:** 511-517.
4. Bardone, M. R., M. Paternoster, and C. Coronelli. 1978. Teichomycins, new antibiotics from *Actinoplanes teicomyceticus* nov. sp. *J. Antibiot.* **31:** 170-177.
5. Barna, J. C. J. and D. H. William. 1984. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. *Annu. Rev. Microbiol.* **38:** 339-357.
6. Bauminger, S. and M. Wilchek. 1980. The use of carbodiimides in the preparation of immunizing conjugates. *Methods Enzymol.* **70:** 151-159.
7. Bernareggi, A., A. Borghi, M. Borgonovi, L. Cavenaghi, P. Ferrari, K. Vekey, M. Zanol, and L. F. Zerilli. 1992. Teicoplanin metabolism in humans. *Antimicrob. Agents Chemother.* **36:** 1744-1749.
8. Bibler, M. R., T. F. Peter, D. N. Hagler, R. B. Bode, J. L. Staneck, and V. Thamlikitkul. 1987. Clinical evaluation of efficacy, pharmacokinetics, and safety of teicoplanin for serious Gram-positive infections. *Antimicrob. Agents Chemother.* **31:** 207-212.
9. Borghi A., C. Coronelli, L. Faniuolo, G. Allievi, R. Pallanza, and G. G. Gallo. 1984. Teicomycins, new antibiotics from *Actinoplanes teicomyceticus* nov. sp. IV. Separation and characterization of the components of teicomycin (teicoplanin). *J. Antibiot.* **37:** 615-620.
10. Brogden, R. N., D. H. Peters, R. N. Brogden, and D. H. Peters. 1994. Teicoplanin. A reappraisal of its antimicrobial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* **47:** 823-854.
11. Cavenaghi, L., A. Corti, and G. Cassani. 1986. Comparison of the solid-phase enzyme-receptor assay (SPERA) and the microbiological assay for teicoplanin. *J. Hosp. Infect.* **7:** 85-89.
12. Corti, A., L. Cavenaghi, E. Giani, and G. Cassani. 1987. A receptor-antibody sandwich assay for teicoplanin. *Clin. Chem.* **33:** 1615-1618.
13. Corti, A., C. Rurali, A. Borghi, and G. Cassani. 1985. Solid-phase enzyme-receptor assay (SPERA): A competitive binding assay for antibiotics of the vancomycin class. *Clin. Chem.* **31:** 1606-1610.
14. Coutant, J. E., T. M. Chen, and B. L. Ackermann. 1990. Interfacing microbore and capillary liquid chromatography to continuous-flow fast atom bombardment mass spectrometry for the analysis of glycopeptides. *J. Chromatogr.* **529:** 265-275.
15. Cox, H., M. Whitby, G. Nimmo, and G. Williams. 1993. Evaluation of a novel fluorescence polarization immunoassay for teicoplanin. *Antimicrob. Agents Chemother.* **37:** 1924-1926.
16. Eremin, S. A. and D. S. Smith. 2003. Fluorescence polarization immunoassay for pesticides. *Comb. Chem. High Throughput Screen* **6:** 257-266.
17. Falcoz, C., N. Ferry, N. Pozet, G. Cuisinaud, P. Y. Zech, and J. Sassard. 1987. Pharmacokinetics of teicoplanin in renal failure. *Antimicrob. Agents Chemother.* **31:** 1255-1262.
18. Goldstein, F. W., A. Coutrot, A. Sieffer, and J. F. Acor. 1990. Percentages and distribution of teicoplanin and vancomycin resistant strains among coagulase negative Staphylococci. *Antimicrob. Agents Chemother.* **34:** 899-900.
19. Heydorn, A., B. O. Petersen, J. Ø. Duus, S. Bergmann, T. Suhr-Jessen, and J. Nielsen. 2000. Biosynthetic studies of the glycopeptide teicoplanin by ^{1}H and ^{13}C NMR. *J. Biol. Chem.* **275:** 6201-6206.
20. Joos, B. and R. Luthy. 1987. Determination of teicoplanin concentrations in serum by high-pressure liquid chromatography. *Antimicrob. Agents Chemother.* **31:** 1222-1224.
21. Jun, D. Y., S. W. Rue, B. W. Kim, and Y. H. Kim. 2003. Detection of mitotic centromere-associated kinesin (MCAK) during cell cycle progression of human jurkat T cells using polyclonal antibody raised against its N-terminal region overexpressed in *E. coli*. *J. Microbiol. Biotechnol.* **13:** 912-918.
22. Kureishi, A., P. J. Jewesson, K. H. Bartlett, C. D. Cole, and A. W. Chow. 1990. Application of a modified bioassay for monitoring serum teicoplanin and vancomycin in febrile neutropensic patients. *Antimicrob. Agents Chemother.* **34:** 1642-1647.
23. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680-685.
24. Lee, J. C., H. R. Park, D. J. Park, H. B. Lee, Y. B. Kim, and C. J. Kim. 2003. Improved production of teicoplanin using

adsorbent resin in fermentations. *Lett. Appl. Microbiol.* **37:** 189–274.

25. Mastin, S. H., R. L. Buck, and P. A. Mueggler. 1993. Performance of a fluorescence polarization immunoassay for teicoplanin in serum. *Diagn. Microbiol. Infect. Dis.* **16:** 17–24.
26. McCann, S. J., L. O. White, and B. Keevil. 2002. Assay of teicoplanin in serum: Comparison of high-performance liquid chromatography and fluorescence polarization immunoassay. *J. Antimicrob. Chemother.* **50:** 107–110.
27. McMullin, C. M., L. O. White, A. P. MacGowan, H. A. Holt, A. M. Lovering, and D. S. Reeves. 1994. Assay of serum teicoplanin concentrations in clinical specimens: A comparison of isocratic high performance liquid chromatography with polarisation fluoroimmunoassay and bioassay. *J. Antimicrob. Chemother.* **34:** 425–429.
28. Mastin, S. H., R. L. Buck, and P. A. Mueggler. 1993. Performance of a fluorescence polarization immunoassay for teicoplanin in serum. *Diagn. Microbiol. Infect. Dis.* **16:** 17–24.
29. Munro, A. J., J. Landon, and E. J. Shaw. 1982. The basis of immunoassay for antibiotics. *J. Antimicrob. Chemother.* **9:** 423–432.
30. Parenti, F., G. Beretta, M. Berti, and V. Arioli. 1978. Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. I. Description of the producer strain, fermentation studies and biological properties. *J. Antibiot.* **31:** 276–283.
31. Pea, F., L. Brollo, P. Viale, F. Pavan, and M. Furlanut. 2003. Teicoplanin therapeutic drug monitoring in critically ill patients: A retrospective study emphasizing the importance of a loading dose. *J. Antimicrob. Chemother.* **51:** 971–975.
32. Reynolds, P. E. 1989. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* **8:** 943–950.
33. Riva, E., N. Ferry, A. Cometti, G. Cuisinaud, G. G. Gallo, and J. Sassard. 1987. Determination of teicoplanin in human plasma and urine by affinity and reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **421:** 99–110.
34. Somma, S., L. Gastaddo, and A. Corti. 1984. Teicoplanin, a new antibiotic from *Actinoplanes teicomyceticus* sp. nov.: Mechanism of action. *Antimicrob. Agents Chemother.* **26:** 917–923.
35. Taylor, R. B., R. G. Reid, and I. M. Gould. 1991. Determination of teicoplanin in plasma using microbore high-performance liquid chromatography and injection-generated gradients. *J. Chromatogr.* **563:** 451–457.
36. Williams, A. H., R. N. Gruneberg, A. Webster, and G. I. Ridgway. 1986. Teicoplanin in the treatment of infections caused by Gram-positive organisms. *J. Hosp. Infect.* **7:** 101–103.
37. Wilson, A. P. 2000. Clinical pharmacokinetics of teicoplanin. *Clin. Pharmacokinet.* **39:** 167–183.