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COMMUNICATION

Synthesis and Properties of Dextran-Linked Ampicillin

Dong Sun Kim,[1] Yun Jin Jung,[2] and Young Mi Kim[2,*]

[1]*College of Medicine, KyungBuk National University, Taegu, Korea*
[2]*College of Pharmacy, Pusan National University, Pusan 609-735, Korea*

ABSTRACT

Ampicillin was coupled to dextran of average molecular weight 9,000 or 81,200 via the cyanogen bromide method. The degree of drug substituted per glucose unit (DSG) was varied from 0.104 to 0.028 (by weight bases: 22.1-5.9%) depending on the ratio of the reactants. Water solubility of dextran-linked ampicillin increased compared with free ampicillin, and the solubility decreased as the amount of ampicillin substituted increased. Plasma concentration of ampicillin, which appeared after intravenous administration of dextran-linked ampicillin in rats, was higher than when free ampicillin was administered, and the more so, the higher the molecular weight of dextran. Plasma half-life of dextran-linked ampicillin was two times longer than that of free ampicillin in rats. Antibacterial activities of dextran-linked ampicillin were evaluated against Staphylococcus aureus, Bacillus substillis, *and* Escherichia coli *at two concentration levels according to the cup-plate method by measuring the diameter of inhibition zone, which was comparable to that of free ampicillin.*

KEY WORDS: Ampicillin; Dextran-linked ampicillin; Polymeric ampicillin; Properties of dextran-linked ampicillin.

INTRODUCTION

A polymeric drug in which active substances are linked to polymeric matrices contains the merits that stem from the ability to incorporate multiple features into polymer chains more readily than into low-molecular-weight compounds. The expected advantages include sustained activity, specificity of action, and reduced side effects. A number of pharmacologically active dextran-associated substances have been prepared, and their properties were

*To whom correspondence should be addressed. Fax: 051-513-6754. E-mail: ymikim@hyowon.cc.pusan.ac.kr

reported (1–6). In most cases, combination of pharmacologically active substances to dextran resulted in longer biological half-life and lower toxicity compared with free drugs.

Ampicillin, D(-)-α-aminobenzylpenicillin, is relatively stable in acid and can be administered orally. It is susceptible to hydrolysis by penicillinase (β-lactamase) and is split by the amidase (acyclase) of coliform bacteria (7).

In this study, ampicillin was coupled to dextran via covalent bond to the hydroxyl groups of dextran by the cyanogen bromide method, with the expectation that their biological properties would be improved. Factors affecting the coupling yield of the reaction were studied, and biological properties of dextran-linked ampicillin were investigated.

MATERIALS AND METHODS

Materials

Dextran (MW: 9,000 and 81,200), ampicillin anhydrous, and cyanogen bromide were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO). The ingredients of medium used for antimicrobial test were received from DIFCO Laboratories. The sources of bacteria used were *S. aureus (ATCC 12228)*, *B. subtilis (NA-1)*, and *E. coli (TG-1)*. All other chemicals were of reagent grade, commercially available products and were used without further purification. UV and IR spectra were recorded with a Bomem MB 100 FT-IR spectrophotometer and a Shimadzu UV 2101-PC spectrophotometer, respectively. ^{1}H-NMR spectra were taken on a Bruker AC-200 spectrometer, and the chemical shifts are in parts per million downfield from tetramethylsilane. TLCs were performed on Merck Kiesegel 60 F_{254}, and an Orion 320 pH meter was used for the pH measurements.

Preparation of Dextran-Linked Ampicillin

Cyanogen bromide (0.65 g 6.13 mmole) was added in three portions at intervals of 15 min to 100 ml of a well-stirred solution of dextran (1 g) in distilled water that had been adjusted to pH 10.5. The reaction mixture was stirred vigorously using a mechanical stirrer and maintained the pH 10.5 to 10.7 by the addition of 3N-NaOH. After the addition was complete, the reaction mixture was stirred for 30 min, and 0.27 g of ampicillin (0.77 mmole) was added while maintaining the pH of the solution at 7.4 by the addition of 3N-NaOH. After stirring for 6 hr at 4°C, the reaction mixture was dialyzed against distilled water at 5°C and concentrated to 10% under reduced pressure. Ethanol (50 ml) was added to precipitate dextran-linked ampicillin, and the precipitates were collected by suction filtration and washed thoroughly with ethanol and dried in a vacuum desiccator. Derivatives of ampicillin prepared with dextran of average molecular weight (9,000 or 81,200) were named as Dex 9-A and Dex 81-A, respectively. TLCs were performed on Merck Kiesegel 60 F_{254} plate eluting with a solution of n-butanol/water/acetic acid (3/1/1), and the spots were identified with an UV detector and treated with iodine azide solution (8). Dextran-linked ampicillin appeared as a single spot at the originally spotted position, which was different from the free ampicillin ($R_f = 0.63$).

Determination of Ampicillin Coupled to Dextran-Linked Ampicillin

The amount of ampicillin attached to dextran was determined by the iodometric method (9,10). Two-milliliter aliquot of ampicillin solution in distilled water (2 mg/ml) was introduced into a glass-stoppered flask (50 ml) and 2.0 ml of 1N NaOH was added. After stirring for 15 min at room temperature, it was neutralized by the addition of 2.0 ml of 1N HCl, and 10.0 ml of 0.01N iodine solution was added. After 15 min, unconsumed iodine was titrated with 0.01 N sodium thiosulfate solution. A blank test was run to correct the effect of dextran on the determination of ampicillin by iodometry by the same procedure. A standard calibration curve was constructed from the series of results obtained from varied amount of ampicillin. Dex 9-A or Dex 81-A (100 mg) was analyzed by the iodometry as described above, and the amount of ampicillin attached in the sample was determined from the standard calibration curve.

Determination of Plasma Concentration-Time Profiles in Rats

Male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with urethane (1 g/kg) and cannulated with PE tubing (i.d., 0.6 mm) through the femoral artery, and administered a solution of ampicillin in 0.9% saline via the femoral vein at a dose level of 12.5 mg/kg. Blood samples were collected from the femoral artery with a heparinized syringe at appropriate time intervals and centrifuged immediately at 5000 rpm for 3 min to obtain plasma samples. Plasma samples for Dex 9-A and Dex 81-A were obtained as described above, administering at a dose level of 12.5 mg/kg equivalent of ampicillin. Plasma concentration of ampicillin was determined by fluorometry

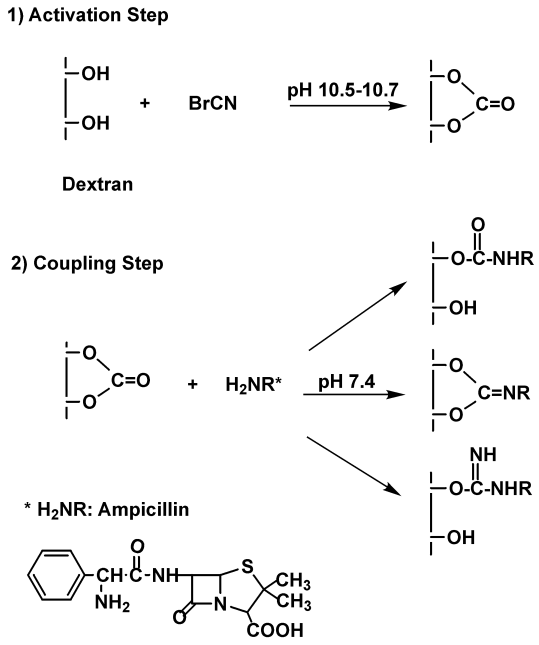
according to the method described by Jusko (11). Plasma sample (100 μl) and 2.0 ml of 10% trichloroacetic acid were placed in a cap tube. The mixture was vortexed for 1 min and centrifuged for 3 min at 5000 rpm. Supernatant (1.5 ml) was transferred to another cap tube, with 0.5 ml of 7% formaldehyde in 0.4M pH 2.0 citrate buffer added, heated in a water bath at 90°C for 2 hr, and cooled to room temperature. To this solution was added 7.0 ml of acetone/chloroform solution (1/1), and the mixture was shaken for 4 min. An aliquot (4.0 ml) of organic phase was removed and mixed with 3.0 ml of 1N-NaOH. After shaking for 2 min, the fluorescence of the solution was measured at 346 nm excitation and 422 nm emission wavelengths. A standard calibration curve for ampicillin was obtained over the range of 0.2 to 10 μg/ml by following the same procedure. The presence of dextran did not interfere with the analysis of ampicillin by this procedure. The concentrations of ampicillin in the plasma samples were determined from the standard calibration curve.

Antibacterial Activities of Dextran-Linked Ampicillin

The antibacterial activities were evaluated at two different concentration levels by measuring the diameters of inhibition zones using the cup-plate method (12,13). A loopful of *S. aureus* obtained from a recently grown slant culture was inoculated to the culture tube containing 10 ml of nutrient broth. It was incubated at 37°C for 24 hr, and the inoculum was prepared by diluting the incubated nutrient broth with 200 ml of medium I (USP XX) on the day of the test. Inoculums for *B. substilis*, and *E. coli* were prepared using the same procedure. To a culture plate, 21 ml of medium I (USP XX) was added and allowed to solidify to give a smooth base layer with uniform 3- to 4-mm depth. To this plate, 4.0 ml of inoculum was added and was tilted back and forth to spread the inoculum evenly and and entirely over the surface, and allowed to harden. Four stainless steel cylinders (o.d., 11 mm; i.d., 9 mm; height, 10 mm) were dropped from 24 mm above the inoculated surface for each culture plate and covered the plate to avoid contamination. After filling these cups with a test solution of ampicillin, Dex 9-A, or Dex 81-A, the culture plate was incubated at 37°C for 16 hr, the cups removed, and the diameter of inhibition zone measured.

RESULTS AND DISCUSSION

Dextran was activated by treating with cyanogen bromide and reacted with ampicillin to prepare dextran-linked ampicillin (14,15). The processes are shown in Scheme 1.



Scheme 1. Preparation of dextran-linked ampicillin.

Even though the coupling reaction between activated dextran and the compound with amino group is favored at a pH level of 9 to 11 in most cases, the reaction was carried out at a pH level of 7.4 considering that β-lactam ring of ampicillin was unstable at a high pH level. The structure of Dex 9-A or Dex 81-A was identified by the data from UV, IR, and NMR spectra. In UV spectrum, Dex 9-A or Dex 81-A showed λ_{max} at 195 nm, which was shifted from 208 nm of free ampicillin. IR spectrum showed absorption peaks at 1760 cm^{-1} and 1600 cm^{-1}, which were typical C=O stretching vibration and N-H bending vibration of the β-lactam ring, respectively. NMR spectrum showed that signals for aromatic protons (7.2 to 7.3 ppm) and methyl group (1.1 to 1.2 ppm) originated from ampicillin, and the protons of acylic ring (3.0 to 4.0 ppm) and hydroxyl group (4.0 to 5.0 ppm) originated from dextran were noticed. The amount of ampicillin coupled in dextran with intact β-lactam ring was determined by iodometric method (9). In iodometric assay, iodine is consumed not by the intact penicillin molecule but by the penicilloate ion, which forms after alkaline or penicillinase hydrolysis of penicillin. Physical mixtures of ampicillin and dextran were subjected to iodometry to check the effect of dextran on this assay method. The results were shown to be average of 103% in comparison with ampicillin itself.

Table 1.

Effect of Dextran/Ampicillin Ratio on the Coupling Yield

Gm Ampicillin/ Gm Dextran	% Ampicillin		Coupling Yield		DSG[a]	
	Dex 9-A	Dex 81-A	Dex 9-A	Dex 81-A	Dex 9-A	Dex 81-A
2.131	22.1	21.0	32.5	30.8	0.104	0.099
1.066	16.1	15.6	31.3	30.2	0.076	0.073
0.533	10.2	10.1	29.3	29.1	0.048	0.047
0.266	6.0	5.9	28.6	28.2	0.028	0.028

[a] Degree of substitution per glucose unit.

This correction factor was applied for the determination of ampicillin attached in dextran by iodometry.

Coupling reactions were carried out with a fixed amount of activated dextran while increasing the amount of ampicillin successively, and the reactions were terminated at varied reaction times. The reaction time was optimal at approximately 6 hr because the coupling yield decreased by the prolongation of the reaction time more than 6 hr. The coupling yield increased from 5.9 to 22.1% on the weight bases by increasing the ratio of ampicillin in the starting materials, as shown in Table 1. Water solubility of Dex 9-A or Dex 81-A increased compared with free ampicillin, and the one containing 6% ampicillin was freely soluble in water. As expected, solubility decreased as the degree of substitution increased.

The plasma concentration-time profiles were determined at varied time intervals after the administration of the drugs intravenously in rats, and the results are shown in Figure 1. Plasma concentration of ampicillin, which appeared after administration of Dex 9-A or Dex 81-A, was higher than when free ampicillin was administered, and the more so, the higher the molecular weight of dextran. This might suggest that the linkage between ampicillin and dextran was relatively stable in the plasma and that the elimination of dextran-linked ampicillin was slower than that of free ampicillin. Plasma half-life (hr) of free ampicillin, Dex 9-A, and Dex 81-A was 1.7, 3.6, and 3.5, respectively, which was calculated from the slope of each plot in Figure 2.

The antimicrobial activities of Dex 9-A, Dex 81-A, and free ampicillin were evaluated by measuring the diameter of inhibition zone according to the cup-plate method at two different concentration levels (12,13). In general, the antimicrobial activities of Dex 9-A and Dex 81-A were comparable with that of ampicillin, as shown in Table 2.

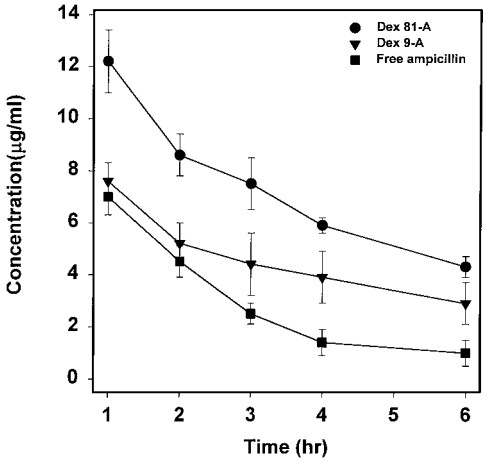


Figure 1. Plasma concentration profiles of ampicillin after intravenous administration of free ampicillin, Dex 9-A, and Dex 81-A in rats. Data are mean ±SE ($n = 5$).

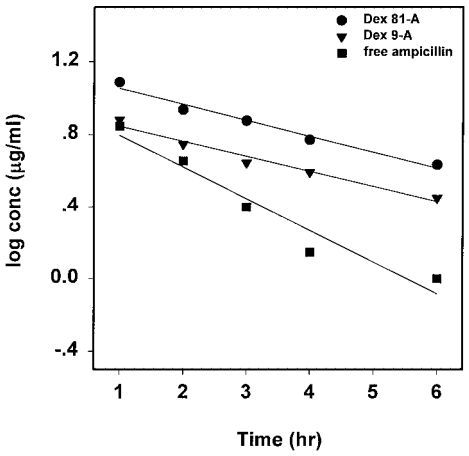


Figure 2. Log plasma concentration vs time after intravenous administration of free ampicillin, Dex 9-A, and Dex 81-A in rats.

Table 2.

Antimicrobial Activity of Dextran-Linked Ampicillin[a]

	Ampicillin		Dex 9-A		Dex 81-A	
Strain	20	50	20	50	20	50
S. aureus	13	16	13	15	13	14
B. subtilis	23	26	21	24	19	22
E. coli	28	31	27	30	25	27

[a] Diameter of inhibition zone (mm) at concentration level of 20 or 50 μg/ml equivalent of ampicillin.

In summary, coupling of ampicillin to dextran with varying degrees of substitution was achieved via cyanogen bromide method. Dextran-linked ampicillin showed a higher level of ampicillin concentration in the plasma than did free ampicillin after intravenous administration during the entire range of the experimental period. Plasma half-life of dextran-linked ampicillin was two times longer than that of free ampicillin in rats. Antimicrobial activities of dextran-linked ampicillin were comparable with those of free ampicillin.

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