Antibacterial Activity and Nephrotoxicity of Two Novel 2"-Amino Derivatives of Arbekacin

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The antibacterial activity of 2"-amino-2"-deoxyarbekacin (AmABK) and 2"-amino-5,2"-dideoxy-5-epiaminoarbekacin (Am2ABK) was comparable to, or slightly less than, that of arbekacin (ABK) against gram-positive and gram-negative bacteria, including 60 stock cultures and 50 clinical isolates of Pseudomonas aeruginosa, but more potent against 31 isolates of MRSA possessing an aminoglycoside-modifying enzyme APH(2")/AAC(6'). AmABK and Am2ABK showed in vivo activity which paralleled in vitro MICs, and were less toxic than ABK in acute toxicity in mice and nephrotoxicity in rats. These results indicate that the 2"-amino group introduced to ABK confers high stabilization to the aminoglycoside-modifying enzymes, while reducing acute and renal toxicities.

Key words: arbekacin, 2"-amino-arbekacin derivatives, aminoglycoside-modifying enzymes, antibacterial activity, acute toxicity, nephrotoxicity

INTRODUCTION

Arbekacin (ABK), a semi-synthetic aminoglycoside antibiotic, has broad antibacterial activity against gram-positive and gram-negative bacteria, and is stable to most aminoglycoside-modifying enzymes produced by aminoglycoside-resistant bacteria including methicillin-resistant Staphylococcus aureus (MRSA).1-3 In 1990, ABK was introduced in Japan as a useful chemotherapeutic agent for the treatment of infections caused by MRSA. Very few MRSA strains have been isolated that have a moderate level (6.25-25 µg/mL) of ABK resistance due to a bifunctional enzyme, APH(2")/AAC(6') (2"-O-phosphorylation and 6'-N-acetylation).4 Introduction of an amino group instead of the 2"-hydroxy group in ABK resulted in much higher stabilization of the derivative against aminoglycoside-modifying enzymes including APH(2")/AAC(6'). Among the several available 2"-amino derivatives of ABK,5,6 2"-amino-2"-deoxyarbekacin (AmABK) and 2"-amino-5,2"-dideoxy-5-epiaminoarbekacin (Am2ABK) were selected for further evaluation as chemotherapeutic agents (Fig. 1). This paper describes the in vitro and in vivo antibacterial activities of these derivatives, including animal toxicity studies.

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MATERIALS AND METHODS

Antibiotics

ABK was obtained from Meiji Seika Kaisha Ltd, Tokyo, Japan and AmABK and Am2ABK were prepared starting from dibekacin as previously reported,7,8 and were used in the sulfated form. The concentrations and doses of antibiotics were determined from the free base molecular weights. Each antibiotic was dissolved in saline at a concentration of 10 mg/mL, and used after ultrafiltration through a 0.45 µm disposable syringe filter (Gelman Sciences, Ann Arbor, MI, USA).

Bacterial strains

The standard bacterial strains used for the antibacterial studies were the stock cultures of the Institute of Microbial Chemistry, Tokyo, Japan. Thirty-one strains of MRSA possessing APH(2")/AAC(6')5,9 clinical isolates from 1986 to 1990 from a hospital at Osaka obtained from Takeda Analytical Research Laboratories Ltd, Osaka, Japan, and 50 strains of Pseudomonas aeruginosa isolated clinically from hospitals in 1992 in Japan were also used in these experiments. Two clinically-isolated strains of S. aureus, MS16526 (MRSA, moderately resistant to ABK)6 and P. aeruginosa GN10362 used for in vivo studies were kindly supplied by Prof. Susumu Mitsuhashi, Episome Institute, Gunma, Japan.

In vitro antibacterial activity

MICs were determined by a two-fold agar dilution
Activity and toxicity of ABK derivatives

Fig. 1. Structures of arbekacin and its 2”-amino derivatives.

method using Bacto Mueller-Hinton Medium (Difco Laboratories, Detroit, MI, USA) incubated at 37°C for 18 hours, according to the standard method of the Japan Society of Chemotherapy.

In vivo antibacterial activity

Four-week old ICR-Jcl male mice (CLEA, Japan Inc, Tokyo, Japan) weighing 18 to 21 g were used, with 8 mice in each group. Bacterial cells, which were pre-incubated on heart infusion agar (Difco) at 37°C overnight, were suspended in saline and mixed with an equal volume of 5% mucin (Difco). The suspension (0.5 mL total) was inoculated intraperitoneally into mice. The challenge dose (MRSA, 1.7 × 10⁵ or 7.1 × 10⁵ CFU/mouse; P. aeruginosa, 4.9 × 10⁴ CFU/mouse) was adjusted to kill the mice within 48 hours. In the case of MRSA infection, the mice were pre-treated intraperitoneally with 200 mg/kg of cyclophosphamide 4 days before inoculation. An antibiotic solution (0.2 mL) was administered subcutaneously into the mouse 1 hour after bacterial inoculation as a single dose. The numbers of surviving mice after 1 week post-inoculation were counted, and the 50% effective dose (ED₅₀) and 95% confidence limits were calculated using the probit method.

Acute toxicity

Four-week old ICR-Jcl male mice were used, with 5 in each group. An antibiotic solution (0.2 mL) was administered intravenously into each mouse, and the LD₅₀ value was calculated from the mice surviving after 2 weeks using the Behrens-Karber method.

Nephrotoxicity

Male F344/Ducr Fischer rats (SPF, Nippon Charles River, Kanagawa, Japan), 6 weeks old were used. These animals were divided at random into experimental groups depending on the body weight. Each group consisted of 8 animals, and the average weight range in each group was 109–132 g. Two animals were housed per cage (20 × 18 × 25 cm), which was kept in a room at 21–25°C, 45–65% humidity, and the rats were fed with solid NMF food (Oriental Kobo Kogyo, Tokyo, Japan) and tap water ad libitum. The lighting cycle was set from 7 AM to 7 PM. Animals received an antibiotic solution (0.5 mL) intramuscularly twice a day (10 AM and 4 PM) for a total dose of 100 mg/kg/day for 9 days. The control animals received saline injections (0.5 mL each).

Nephrotoxicity was determined by observing the number of fatalities on a daily basis, measuring body weight 1 day before, on the fourth day, and on the last day after the first dose, and obtaining blood from the inferior vena cava under anaesthesia with pentobarbital the day after the final dose. Blood urea nitrogen (BUN) and serum creatinine (Cre) were determined using an autoanalyzer (Roche Cobas Fara, Hoffman-La Roche, Basel, Switzerland) and expressed as mg/dL. After exsanguinating the animal by cutting the axillary artery, the kidneys were removed, weighed and visually inspected. The kidney specimens were stained with hematoxylin-eosin and subjected to microscopic pathological inspection.

Statistical analysis

The biochemical parameters were analyzed for significance between test sample and the control, and between test sample and ABK using the F test.

RESULTS

Antibacterial activity

The antibacterial activity of AmABK and Am₂ABK against 60 strains including the standard bacterial strains and resistant strains having the aminoglycoside-modifying enzymes are shown in Table 1. AmABK and Am₂ABK showed antibacterial activity similar to that of ABK, but differences were observed with the MRSA strains. Two 2”-amino derivatives exhibited stronger activity than ABK against 2 strains of MRSA possessing APH(2”)/AAC(6’) and AAD(4’,4”). For most strains, AmABK and Am₂ABK were comparable to ABK in potency, but weaker in potency against some strains such as Micrococcus flavus FDA16, Sarcina lutea PCI1001, Corynebacterium bovis 1810, Escherichia coli K-12 C600 R135 and P. aeruginosa A3.

Table 3 shows the in vivo activity of AmABK and Am₂ABK against MRSA and pseudomonal infections. AmABK was comparable to ABK against MRSA infection with the MS16526 strain, but Am₂ABK was more...