In Vitro and In Vivo Antibacterial Activities of KB-5246, a New Tetracyclic Quinolone

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The in vitro and in vivo antibacterial activities of KB-5246, a tetracyclic quinolone, were compared with those of ciprofloxacin, ofloxacin, and norfloxacin. KB-5246 demonstrated a broad antibacterial spectrum. The in vitro activity of KB-5246 against gram-negative bacteria was higher than that of ofloxacin or norfloxacin and was comparable to that of ciprofloxacin. KB-5246 demonstrated the greatest activity against gram-positive bacteria of the four agents tested. Among Streptococcus pyogenes strains resistant to 1.56 μg of norfloxacin per ml, there were 26 strains susceptible to 0.2 μg of KB-5246 per ml. Similarly, among the Staphylococcus aureus and Staphylococcus epidermidis strains resistant to 3.13 μg of norfloxacin per ml, there were 23 S. aureus and 11 S. epidermidis strains susceptible to 0.39 μg of KB-5246 per ml. Among the Streptococcus pneumoniae and Enterococcus faecalis strains resistant to 12.5 μg of norfloxacin per ml, there were 5 S. pneumoniae and 10 E. faecalis strains susceptible to 0.39 μg of KB-5246 per ml. KB-5246 had bactericidal activity at the MIC. KB-5246 demonstrated excellent antibacterial activity against various systemic infections in mice. After oral administration, KB-5246 was as active as ofloxacin and about two times more active than norfloxacin.

Since nalidixic acid was developed in 1963 (1), many nalidixic acid analogs, such as pipemidic acid (8), have been introduced into clinical use, primarily against urinary tract infections. In 1980 (4), norfloxacin was reported and drew attention because of its broad antibacterial spectrum and potent antibacterial activity. This heralded the appearance of new quinolones. Since the introduction of norfloxacin, new quinolones have been developed and their clinical use has been expanded (2, 5, 7, 9).

KB-5246, or 9,1-epoxymethano-7-fluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5H-thiazolo[3,2-a]quinoline-4-carboxylic acid hydrochloride, was synthesized at the Pharmaceuticals Research Center of Kaneko, Ltd., Osaka, Japan. KB-5246 is a new quinolone which has a tetracyclic structure (Fig. 1).

To evaluate the antibiotic value of KB-5246, we compared its antibacterial activities with the in vitro and in vivo activities of ciprofloxacin, ofloxacin, and norfloxacin.

Determination of MICs. The MICs were determined by the twofold agar dilution method. The media used for preculture and MIC determination were brain heart infusion broth (Nissui Seiyaku) and heart infusion agar (Nissui Seiyaku) supplemented with 10% defibrinated horse blood for streptococci, brain heart infusion broth and agar supplemented with 5% Filde's enrichment (Difco Laboratories, Detroit, Mich.) for Haemophilus influenzae, GC agar (Difco Laboratories) supplemented with 1% hemoglobin (Difco Laboratories) and 1% IsoVitalE (Becton Dickinson and Co., Parsippany, N.J.) for Neisseria gonorrhoeae, GAM broth and agar (Nissui Seiyaku) for obligate anaerobes, and sensitivity test broth and sensitivity disk agar for the other microorganisms. An overnight broth culture (about 10⁸ CFU/ml) was diluted 100 times, and about 10⁴ CFU was inoculated onto a drug-containing agar surface. The MIC was evaluated after 18 to 20 h of incubation at 37°C. H. influenzae and N. gonorrhoeae were incubated in a candle jar, and obligate anaerobes were incubated in an anaerobic glove box. The MIC was defined as the lowest drug concentration which prevented visible growth of microorganisms.

Bactericidal activity. Bactericidal activity was tested by evaluating the reduction of viable cells during exposure to the drug for 24 h. An overnight culture of microorganisms in sensitivity test broth was diluted to about 10⁴ CFU/ml in the same medium and incubated at 37°C on a shaker. After 2 h of incubation, KB-5246 was added to the cultures at final concentrations of one half, one, two, or four times the MIC.

Materials and Methods

Drugs. KB-5246 was obtained from the Pharmaceuticals Research Center of Kaneko, Ltd. Other antimicrobial agents used were obtained as follows: ciprofloxacin, Bayer Yakuhin Co., Ltd., Osaka, Japan; norfloxacin, Kyorin Seiyaku, Tokyo, Japan; ofloxacin, Daiichi Seiyaku Co., Ltd., Tokyo, Japan.

Test strains. The bacterial strains used in this study were acquired from the Laboratory of Drug Resistance in Bacteria, School of Medicine, Gunma University, and from the Episome Institute, Gunma-ken, Japan.

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| Organism (no. of strains) | Drug     | MIC (μg/ml) |   |   |
|--------------------------|----------|-------------|---|---|
|                          |          | Range       | 50% | 90% |
| *Staphylococcus aureus* (145) | KB-5246 | 0.05-0.39   | 0.10 | 0.10 |
|                          | Ciprofloxacin | 0.10-3.13 | 0.39 | 0.78 |
|                          | Ofloxacin | 0.20-1.56  | 0.39 | 0.78 |
|                          | Norfloxacin | 0.20-25    | 1.56 | 3.13 |
| *Staphylococcus epidermidis* (97) | KB-5246 | 0.05-0.20   | 0.10 | 0.20 |
|                          | Ciprofloxacin | 0.10-0.78 | 0.39 | 0.78 |
|                          | Ofloxacin | 0.20-3.13  | 0.39 | 0.78 |
|                          | Norfloxacin | 0.20-6.25  | 1.56 | 3.13 |
| *Streptococcus pneumoniae* (22) | KB-5246 | 0.39-0.39   | 0.39 | 0.39 |
|                          | Ciprofloxacin | 0.78-3.13 | 1.56 | 1.56 |
|                          | Ofloxacin | 1.56-3.13  | 1.56 | 3.13 |
|                          | Norfloxacin | 3.13-25    | 6.25 | 12.5 |
| *Streptococcus pyogenes* (93) | KB-5246 | 0.05-0.20   | 0.10 | 0.20 |
|                          | Ciprofloxacin | 0.10-0.39 | 0.20 | 0.39 |
|                          | Ofloxacin | 0.39-1.56  | 0.78 | 0.78 |
|                          | Norfloxacin | 0.39-6.25  | 0.78 | 1.56 |
| *Enterococcus faecalis* (78) | KB-5246 | 0.20-6.25   | 0.39 | 0.78 |
|                          | Ciprofloxacin | 0.39-25    | 0.78 | 3.13 |
|                          | Ofloxacin | 0.78-25    | 3.13 | 12.5 |
|                          | Norfloxacin | 1.56-50    | 3.13 | 12.5 |
| *Escherichia coli* (108) | KB-5246 | 0.013-6.25  | 0.05 | 0.05 |
|                          | Ciprofloxacin | 0.006-3.13 | 0.025 | 0.05 |
|                          | Ofloxacin | 0.05-25    | 0.10 | 0.20 |
|                          | Norfloxacin | 0.05-6.25  | 0.10 | 0.20 |
| *Klebsiella pneumoniae* (50) | KB-5246 | 0.025-0.78  | 0.05 | 0.10 |
|                          | Ciprofloxacin | 0.013-0.78 | 0.05 | 0.10 |
|                          | Ofloxacin | 0.025-3.13 | 0.20 | 0.20 |
|                          | Norfloxacin | 0.05-3.13  | 0.20 | 0.39 |
| *Klebsiella oxytoca* (56) | KB-5246 | 0.013-0.05  | 0.025 | 0.025 |
|                          | Ciprofloxacin | 0.006-0.025 | 0.025 | 0.025 |
|                          | Ofloxacin | 0.05-0.20  | 0.10 | 0.20 |
|                          | Norfloxacin | 0.05-0.20  | 0.10 | 0.20 |
| *Citrobacter freundii* (76) | KB-5246 | 0.013-3.13  | 0.05 | 0.39 |
|                          | Ciprofloxacin | 0.006-3.13 | 0.05 | 0.39 |
|                          | Ofloxacin | 0.025-12.5 | 0.20 | 1.56 |
|                          | Norfloxacin | 0.025-12.5 | 0.10 | 1.56 |
| *Shigella spp.* (107) | KB-5246 | 0.006-0.39  | 0.013 | 0.025 |
|                          | Ciprofloxacin | 0.006-0.39 | 0.025 | 0.025 |
|                          | Ofloxacin | 0.025-1.56 | 0.05 | 0.10 |
|                          | Norfloxacin | 0.025-0.78 | 0.05 | 0.10 |
| *Salmonella spp.* (107) | KB-5246 | 0.025-0.10  | 0.05 | 0.10 |
|                          | Ciprofloxacin | 0.013-0.05 | 0.025 | 0.05 |
|                          | Ofloxacin | 0.05-0.20  | 0.20 | 0.20 |
|                          | Norfloxacin | 0.05-0.39  | 0.20 | 0.20 |
| *Enterobacter cloacae* (100) | KB-5246 | 0.006-25    | 0.05 | 0.20 |
|                          | Ciprofloxacin | 0.006-25   | 0.025 | 0.20 |
|                          | Ofloxacin | 0.025-25   | 0.20 | 0.78 |
|                          | Norfloxacin | 0.05-25    | 0.10 | 0.78 |
| *Proteus mirabilis* (99) | KB-5246 | 0.025-0.39  | 0.05 | 0.10 |
|                          | Ciprofloxacin | 0.025-0.78 | 0.05 | 0.10 |
|                          | Ofloxacin | 0.10-3.13  | 0.20 | 0.39 |
|                          | Norfloxacin | 0.10-0.78  | 0.10 | 0.20 |
TABLE 1—Continued

| Organism (no. of strains) | Drug       | MIC (µg/ml)*  |
|---------------------------|------------|---------------|
|                           |            | Range         | 50%          | 90%          |
| **Proteus vulgaris (99)** | KB-5246    | 0.013–0.10    | 0.025        | 0.025        |
|                           | Ciprofloxacin | 0.013–0.10    | 0.025        | 0.025        |
|                           | Ofloxacín  | 0.025–0.39    | 0.10         | 0.10         |
|                           | Norfloxacin | 0.025–0.20    | 0.05         | 0.10         |
| **Morganella morganii (50)** | KB-5246    | 0.013–0.78    | 0.025        | 0.05         |
|                           | Ciprofloxacin | 0.006–1.56    | 0.013        | 0.025        |
|                           | Ofloxacín  | 0.025–6.25    | 0.10         | 0.20         |
|                           | Norfloxacin | 0.013–3.13    | 0.05         | 0.10         |
| **Providencia rettgeri (100)** | KB-5246    | 0.025–25      | 0.20         | 1.56         |
|                           | Ciprofloxacin | 0.006–12.5    | 0.10         | 0.78         |
|                           | Ofloxacín  | 0.05–25       | 0.78         | 6.25         |
|                           | Norfloxacin | 0.05–12.5     | 0.39         | 3.13         |
| **Serratia marcescens (100)** | KB-5246    | 0.025–50      | 0.20         | 6.25         |
|                           | Ciprofloxacin | 0.013–25      | 0.39         | 6.25         |
|                           | Ofloxacín  | 0.05–100      | 0.78         | 12.5         |
|                           | Norfloxacin | 0.05–100      | 0.78         | 12.5         |
| **Pseudomonas aeruginosa (99)** | KB-5246    | 0.20–6.25     | 0.39         | 3.13         |
|                           | Ciprofloxacin | 0.10–3.13     | 0.20         | 0.78         |
|                           | Ofloxacín  | 0.078–12.5    | 1.56         | 6.25         |
|                           | Norfloxacin | 0.39–12.5     | 0.78         | 6.25         |
| **Pseudomonas cepacia (51)** | KB-5246    | 0.39–50       | 1.56         | 3.13         |
|                           | Ciprofloxacin | 0.78–12.5     | 6.25         | 6.25         |
|                           | Ofloxacín  | 3.13–25       | 12.5         | 25           |
|                           | Norfloxacin | 6.25–50       | 25           | 25           |
| **Pseudomonas maltophilia (50)** | KB-5246    | 0.10–25       | 3.13         | 6.25         |
|                           | Ciprofloxacin | 0.39–50       | 12.5         | 25           |
|                           | Ofloxacín  | 0.39–25       | 6.25         | 12.5         |
|                           | Norfloxacin | 1.56–100      | 25           | 50           |
| **Haemophilus influenzae (72)** | KB-5246    | 0.013–0.20    | 0.025        | 0.025        |
|                           | Ciprofloxacin | 0.006–0.05    | 0.013        | 0.013        |
|                           | Ofloxacín  | 0.025–0.20    | 0.025        | 0.05         |
|                           | Norfloxacin | 0.025–0.39    | 0.05         | 0.10         |
| **Neisseria gonorrhoeae (17)** | KB-5246    | 0.006–0.025   | 0.013        | 0.025        |
|                           | Ciprofloxacin | 0.006–0.013   | 0.006        | 0.013        |
|                           | Ofloxacín  | 0.013–0.05    | 0.013        | 0.05         |
|                           | Norfloxacin | 0.013–0.10    | 0.025        | 0.10         |
| **Bacteroides fragilis (27)** | KB-5246    | 1.56–12.5     | 3.13         | 6.25         |
|                           | Ciprofloxacin | 3.13–100      | 12.5         | 50           |
|                           | Ofloxacín  | 1.56–25       | 3.13         | 12.5         |
|                           | Norfloxacin | 25–>100       | 50           | >100         |
| **Clostridium perfringens (16)** | KB-5246    | 0.20–1.56     | 0.20         | 1.56         |
|                           | Ciprofloxacin | 0.39–1.56     | 0.39         | 1.56         |
|                           | Ofloxacín  | 0.39–3.13     | 0.78         | 1.56         |
|                           | Norfloxacin | 0.78–6.25     | 1.56         | 3.13         |
| **Clostridium difficile (27)** | KB-5246    | 6.25–12.5     | 6.25         | 6.25         |
|                           | Ciprofloxacin | 12.5–12.5     | 12.5         | 12.5         |
|                           | Ofloxacín  | 6.25–12.5     | 12.5         | 12.5         |
|                           | Norfloxacin | 25–50         | 50           | 50           |

* Determined by the agar dilution method.

Samples (0.1 ml) of each culture were taken periodically, diluted appropriately with buffered saline gelatin (NaCl, 8.5 g/liter; KH₂PO₄, 0.3 g/liter; NaH₂PO₄, 0.6 g/liter; gelatin, 0.1 g/liter), and plated on drug-free sensitivity disk agar. After incubation at 37°C for 18 h, the CFU were numerated and regarded as viable cells. The limit of the assay was about 10² CFU/ml.

**In vivo test.** In vivo antibacterial activity against systemic infections in mice was determined. Ten male ddY mice weighing 19 to 23 g each were used for each dose level. An
TABLE 2. Activity of KB-5246 against norfloxacin-resistant gram-positive bacteria in clinical isolates

| Organism                      | No. of strains | MIC (µg/ml)*a | Norfloxacinb | KB-5246 | Ciprofloxacin | Ofloxacin |
|-------------------------------|----------------|--------------|--------------|---------|---------------|-----------|
| Staphylococcus aureus         | 22             | 3.13-6.25    | 0.05-0.20    | 0.78-1.56 | 0.39-0.78     |           |
|                               | 21             | 3.13-6.25    | 0.39         | 1       | 1.56          |           |
|                               | 1              | 3.13-6.25    | 1.13         | 25      |               |           |
| Staphylococcus epidermidis    | 11             | 3.13-6.25    | 0.05-0.20    | 0.39-0.78 | 0.78          |           |
| Streptococcus pneumoniae      | 5              | 12.5-25      | 0.39         | 1.56-3.13 | 1.56-3.13     |           |
| Streptococcus pyogenes        | 25             | 1.56-3.13    | 0.10-0.20    | 0.20-0.39 | 0.78-1.56     |           |
|                               | 1              | 6.25         | 0.20         | 0.39    | 1.56          |           |
| Enterococcus faecalis         | 14             | 12.5-25      | 0.39-6.25    | 1.56-12.5 | 3.13-25       |           |
|                               | 1              | 50           | 6.25         | 25      |               |           |

* Determined by the agar dilution method.

a Norfloxacin-resistant strains were selected from clinical isolates identified as bacteria with susceptibilities to norfloxacin at concentrations ≥MIC₉₀.

overnight culture in brain heart infusion broth (Nissui Seiyaku) at 37°C was diluted appropriately in the same medium with 4% gastrin mucin (Difco Laboratories). A 0.2-ml sample of a bacterial suspension, corresponding to a dose 1 to 25 times higher than the minimal lethal dose, was injected intraperitoneally. Immediately after infection, mice were treated orally with a single dose of KB-5246, ciprofloxacin, ofloxacin, or norfloxacin through intragastric tubes. All untreated mice died within 3 days after bacterial inoculation. The number of mice surviving at each dose was counted 7 days after infection. The 50% effective dose was calculated by the probit method (6).

RESULTS

Susceptibility of clinical isolates. The in vitro antibacterial activities of KB-5246 against clinical isolates are shown in Table 1. The MICs for 90% of isolates tested (MIC₉₀) of KB-5246 against gram-positive microorganisms such as Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, and Streptococcus pyogenes, were ≤0.39 µg/ml. The MIC for 50% of isolates tested (MIC₅₀) of KB-5246 against Enterococcus faecalis was 0.39 µg/ml. The activity of KB-5246 against S. pyogenes was comparable to that of ciprofloxacin. KB-5246 demonstrated a more potent activity against all gram-positive bacteria except S. pyogenes than ciprofloxacin, ofloxacin, or norfloxacin did.

The activity of KB-5246 against various species of Enterobacteriaceae was comparable to that of ciprofloxacin. KB-5246 inhibited 90% of isolates of Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Morganella morgani, Proteus vulgaris, and Proteus mirabilis at a concentration of 0.10 µg/ml or less. Citrobacter freundii, Enterobacter cloacae, Providencia rettgeri, and Serratia marcescens were less susceptible to KB-5246 than other species of Enterobacteriaceae.

The activities of KB-5246, ofloxacin, and norfloxacin against non-glucose-fermenting gram-negative rods were lower than those against other microorganisms. KB-5246 was more active than ofloxacin against Pseudomonas aeruginosa, and it was the most active against Pseudomonas cepacia and Pseudomonas maltophilia.

N. gonorrhoeae was extremely susceptible to KB-5246; the MIC₉₀ was 0.025 µg/ml. H. influenzae was also susceptible to this compound, with a MIC₉₀ of 0.025 µg/ml.

At the MIC₅₀ level, KB-5246 was the most active against Bacteroides fragilis, Clostridium perfringens, and Clostridium difficile.

Table 2 shows the antibacterial activities of KB-5246 against norfloxacin-resistant gram-positive bacteria in clinical isolates. Norfloxacin-resistant strains were defined as bacteria with susceptibilities to norfloxacin at concentrations greater than or equal to the MIC₉₀. Among S. pyogenes strains resistant to 1.56 µg of norfloxacin per ml, there were 26 strains susceptible to 0.2 µg of KB-5246 per ml. Similarly, among S. aureus and S. epidermidis strains resistant to 3.13 µg of norfloxacin per ml, there were 23 S. aureus and 11 S. epidermidis strains susceptible to 0.39 µg of KB-5246 per ml. Among S. pneumoniae and E. faecalis strains resistant to 12.5 µg of norfloxacin per ml, there were 5 S. pneumoniae and 10 E. faecalis strains susceptible to 0.39 µg of KB-5246 per ml.

Bactericidal activity. After the addition of KB-5246 at the MICs, the numbers of viable cells of S. aureus Smith and E. coli ML4707 decreased rapidly. When a concentration of KB-5246 at the MIC or higher was added, no regrowth after 24 h of incubation was observed (Fig. 2). The susceptibility of S. aureus Smith to KB-5246 after 24 h of incubation with one-half the MIC of KB-5246 was unchanged.

In vivo antibacterial activity. The antibiotic effects of KB-5246 on systemic infections in mice are shown in Table

FIG. 2. Bactericidal activity of KB-5246. (A) S. aureus Smith (MIC, 0.10 µg/ml); (B) E. coli ML4707 (MIC, 0.05 µg/ml). The number of viable cells was counted on drug-free disks at appropriate time intervals after addition of KB-5246 (arrows). The MIC was defined as the lowest drug concentration which prevented visible growth after 24 h of shaking at 37°C.
TABLE 3. In vivo antibacterial activities of KB-5246, ciprofloxacin, ofloxacin, and norfloxacin against systemic infections in mice.

| Organism          | Drug^a       | MIC (µg/ml) | ED50^b (mg/kg per dose) | 95% Confidence limit |
|-------------------|--------------|-------------|-------------------------|----------------------|
| Staphylococcus aureus Smith | KB-5246 | 0.05 | 5.1 | 3.6-7.1 |
|                   | Ciprofloxacin | 0.2 | 5.7 | 3.8-4.2 |
|                   | Ofloxacin    | 0.2 | 4.3 | 3.1-5.8 |
|                   | Norfloxacin  | 0.39 | 13.1 | 9.4-17.8 |
| Streptococcus pneumonia 2132 | KB-5246 | 0.78 | 50.5 | 33.6-68.4 |
|                   | Ciprofloxacin | 1.56 | >100 | -     |
|                   | Ofloxacin    | 3.13 | 66.7 | 7.9-196 |
|                   | Norfloxacin  | 25 | >100 | -     |
| Serratia marcescens GN7577 | KB-5246 | 1.56 | 10.8 | 7.7-14.7 |
|                   | Ciprofloxacin | 3.13 | 6.6 | 4.3-9.6 |
|                   | Ofloxacin    | 3.13 | 11.2 | 8.8-14.2 |
|                   | Norfloxacin  | 3.13 | 23.5 | 18.0-30.6 |
| Pseudomonas aeruginosa GN11189 | KB-5246 | 0.78 | 21.4 | 14.9-31.2 |
|                   | Ciprofloxacin | 0.2 | 5.9 | 4.4-7.7 |
|                   | Ofloxacin    | 1.56 | 21.8 | 17.1-27.8 |
|                   | Norfloxacin  | 0.78 | 29.8 | 21.1-44.5 |

^a Single oral administration immediately after infection.
^b ED50, 50% effective dose.

3. The 50% effective dose values of KB-5246 against S. pneumoniae 2132 infections were 50.5 mg/kg of body weight, while the 50% effective dose values of ciprofloxacin, ofloxacin, and norfloxacin were >100, 66.7, and >100 mg/kg, respectively. The activities of KB-5246 against S. aureus Smith, P. aeruginosa GN11189, and Serratia marcescens GN7577 infections were comparable to those of ofloxacin and greater than those of norfloxacin.

DISCUSSION

KB-5246 has a broad spectrum of activity and high antibacterial activity, especially against gram-positive organisms, and a strong bactericidal effect. KB-5246 exhibited potent activity against several norfloxacin-resistant gram-positive bacteria in clinical isolates. The susceptibility of S. aureus to KB-5246 after 24 h of incubation with one-half the MIC of KB-5246 was unchanged. KB-5246 is characterized by its excellent activity against gram-positive bacteria.

It was reported that new quinolones, norfloxacin and enoxacin, were taken up by E. coli through porin(s) on the outer membrane. Hirai reported that the hydrophilic property of norfloxacin should contribute to the permeability through OmpF porin and that the hydrophobic property of nalidixic acid should contribute to its permeability through the lipid bilayer (2). In our preliminary data, the hydrophobicity of KB-5246 was higher than that of norfloxacin or nalidixic acid, as estimated by the partition between n-octanol and 0.1 M phosphate buffer (pH 7.2) or by a comparison of retention time on a high-pressure liquid chromatography column (Inertsil ODS-2 [Gasukuro Kogyo]; developing solvent, 2/1 [vol/vol] 0.1 mM phosphate buffer-acetonitrile). The hydrophobicity of KB-5246 may be one reason for the potent activity against gram-positive bacteria which have no outer membrane. The mechanism of KB-5246 uptake in gram-positive bacteria is under investigation.

The activity of KB-5246 against gram-negative bacteria was higher than that of ofloxacin or norfloxacin and was comparable to that of ciprofloxacin. The in vivo antibacterial activity of KB-5246 was comparable to that of ofloxacin. The toxicity and side effects of KB-5246 appeared to be minimal; no abnormal findings were observed (G. Tsukamoto, Y. Inoue, T. Hamada, N. Awata, Y. Koteru, and S. Mitsuhashi, Program Abstr. 28th ICAAC, abstr. no. 1492, 1988.).

These results justify additional studies to determine the clinical usefulness of KB-5246. Phase I studies on KB-5246 are in progress.

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