IN VITRO METABOLISM OF A NEW ANTICANCER AGENT, 6-N-FORMYLAMINO-12,13-DIHYDRO-1,11-DIHYDROXY-13-(β-D-GLUCOPYRANOSIL)5H-INDOLE [2,3-A]PYRROLO [3,4-C]CARBAZOLE-5,7(6H)-DIONE (NB-506), IN MICE, RATS, DOGS, AND HUMANS

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ABSTRACT:

The metabolism of 6-N-formylamino-12,13-dihydro-1,11-dihydroxy-13-(β-D-glucopyranosil)5H-indole [2,3-a]pyrrolo [3,4-c]carbazole-5,7(6H)-dione (NB-506), a potent inhibitor of DNA topoisomerase I, was characterized in mice, rats, dogs, and humans in vitro. NB-506 was deamidylated to ED-501 in mouse and rat plasma with enzyme activity of 140 and 116 pmol/min/mg protein, respectively. The enzyme activity in dog and human plasma was found to be less than 2 pmol/min/mg protein. In liver S9 and small intestine S9 samples from mice and rats, activity of the enzyme was very low. Also, there was no activity in the liver or small intestine of dogs and humans. The enzyme involved in the conversion of NB-506 to ED-501 in rat plasma is a rodent-specific serine enzyme with a molecular mass of 138KDa. The ED-501 in rat plasma is a rodent-specific serine enzyme with a molecular mass of 138KDa. The enzyme activity in rodent plasma was 6.3 mmol/min/ml plasma and 54 μM at an optimum pH of 7.4, respectively. Although NB-506 was converted to ED-551 in dog and human plasma in vitro, no conversion was observed in mouse and rat plasma. In human plasma this conversion was not affected by heat treatment (100°C for 1 min), but was inhibited completely by 50 mM EDTA, indicating that the reaction is a chemical reaction catalyzed by metal ions. Although NB-506 was not metabolized by cytochrome P-450 isozymes in liver, this drug was glucuronized in mice, rats, and humans, but not in dogs. These results suggest that a species difference in the metabolism of NB-506 occurred in the liver as well as in plasma. There appeared to be species differences in the metabolism of NB-506 in vitro, correlating well with the species-dependent pharmacokinetics of this drug in vivo.

6-N-formylamino-12,13-dihydro-1,11-dihydroxy-13-(β-D-glucopyranosil)5H-indole [2,3-a]pyrrolo [3,4-c]carbazole-5,7(6H)-dione (NB-506)1 (Fig. 1) shows antitumor activity and is currently being developed as an anticancer agent (Arakawa et al., 1995). This compound was derived from a novel indolocarbazole antibiotic produced by Actinomyces. NB-506 acts as a potent inhibitor of topoisomerase I, and also inhibits the activity of DNA polymerase α and RNA polymerase II (Yoshinari et al., 1995).

As we described in a previous report, nonlinearity in the pharmacokinetics of NB-506 was observed in rodents, and species-specific metabolism was noted. ED-501, an NB-506 metabolite that also has antitumor activity, was identified in rat plasma after i.v. administration of NB-506. In contrast, ED-501 levels were negligible in dogs and humans (Sasaki et al., 1995; Takenaga et al., 1995). These results suggest that these observed species differences result from the presence of rodent-specific enzyme-converting NB-506 to ED-501. Therefore, it is important to elucidate the mechanism underlying species differences in the metabolism of NB-506.

The present report describes the in vitro species-specific plasma metabolism of NB-506 in mice, rats, dogs, and humans and the metabolism of NB-506 in liver microsomes. In addition, because NB-506 glucuronide (ED-594) was identified as one of the main metabolites in rat bile, the glucuronidation of NB-506 in human, dog, rat, and mouse liver microsomes was investigated.

Materials and Methods

Materials. NB-506 and its metabolites (ED-501, ED-551, and ED-594; Fig. 1) were synthesized at Banyu Tsukuba Research Institute (Tsukuba, Japan). [14C]NB-506 was synthesized at Dai-ichi Pure Chemicals (Ibaraki, Japan). Acetonitrile, methanol, trifluoroacetic acid (TFA), N,N-dimethylformamide [which has high-performance liquid chromatography (HPLC) grade], and EDTA were purchased from Wako Pure Chemicals (Osaka, Japan). Water was purified with a Milli-Q system (Millipore-Tokyo, Japan). Disopropyl fluorophosphate (DFP), phenylmethylsulfonyl fluoride (PMSF), monoiodoacetamide (MIA), and iodoacetamide (IAA) were purchased from Wako Pure Chemicals (Osaka, Japan). MgCl2 was purchased from Junsei Chemical Co., Ltd (Tokyo, Japan). Mouse (male, ICR, 7 weeks old) and dog (male, beagle, 7 months old) plasma was purchased from Charles River Breeding Laboratories Japan, Inc. (Kanagawa, Japan).
Japan). Rat (male, Sprague-Dawley, 7–8 weeks old) and human plasma were prepared in our laboratory. Dog (male, beagle, 9–14 months old) liver S9 fraction was purchased from Charles River Breeding Laboratories Japan Inc. Human liver S9 fraction and microsomes, and small intestine S9 fraction were purchased from Keystone Skin Bank (Exton, PA), imported by KAC (Kyoto, Japan). S9 fractions from mouse liver and small intestine, rat liver and small intestine, and dog small intestine were prepared in our laboratory. Mouse, rat, and dog liver microsomes were prepared in our laboratory.

**NB-506 Deformylase Activity Assay.** The incubation mixtures consisted of 240 μl of mouse, rat, dog, or human plasma (1 mg of protein in 50 mM Tris-HCl buffer, pH 7.4) or liver or small intestine S9 fraction (1 mg of protein in 50 mM Tris-HCl buffer, pH 7.4). After 5 min of preincubation at 37°C, the reaction was initiated by the addition of 10 μl of NB-506 (2 mM in dimethylformamide) to make a final concentration of 80 μM. After incubation for 30 min, the reaction was terminated by the addition of 500 μl of methanol containing 0.1% TFA. After centrifugation (1800 g for 5 min), an aliquot of supernatant was assayed for ED-501 by HPLC equipped with an UV detector as described below.

For the inhibition study, the same incubation mixture as described above was used. The inhibitor was added to the reaction mixture and incubated for 1 h at room temperature. After 5 min of preincubation at 37°C, the reaction was initiated by the addition of 10 μl of NB-506 (2 mM in dimethylformamide) to make a final concentration of 80 μM. After incubation for 30 min, the remaining activity was measured.

For the determination of $K_m$ and $V_{max}$ values of rat plasma, NB-506 methanol solution was evaporated to dryness and reaction was initiated by adding rat plasma warmed at 37°C before addition to yield final concentrations ranging from 10 to 250 μM. After incubation at 37°C for 3 min, the reaction was terminated by adding a 2-fold volume of DMF/methanol, 1:1 (v/v). After centrifugation (9000 g, 4°C, 15 min), the supernatant was filtered and injected into an HPLC system for quantitation of ED-501. $K_m$ and $V_{max}$ were calculated from a Michaelis-Menten plot using nonlinear regression.

**In Vitro Metabolism in Plasma.** NB-506 (10 mM, in dimethylformamide) was added to mouse, rat, dog, and human plasma samples at a final concentration of 50 μM. After incubation for 0, 5, 10, 20, 30, 60, 120, and 180 min, a 100-μl aliquot of each sample was deproteinized by adding 100 μl of 0.2 M TFA and 1 ml of acetonitrile. After 3 h of incubation at room temperature for shifting the equilibrium of ED-551 between anhydride form and open acid form to the anhydride form, each sample was centrifuged. The supernatant was evaporated to dryness and the residue was reconstituted in 300 μl of a mixture of acetonitrile/0.1 M TFA, 1:1 (v/v). The solution was filtered through a 0.5-μm filter (Millipore), and an aliquot of the sample was injected into an HPLC system.

For the heat treatment study to determine the effects of temperature on the metabolism of NB-506, rat and human plasma samples were heated at 100°C for 1 min. NB-506 was then added to the heat-treated samples at a final concentration of 50 μM. After incubation for 0, 0.5, 1, and 2 h at 37°C, an aliquot of each sample was analyzed by HPLC.

EDTA was added to human plasma at final concentrations of 1, 10, and 50 mM, and these samples were incubated for 3 h at 37°C with NB-506 (50 μM). An aliquot of each sample was analyzed by HPLC.

**NB-506 Oxidation in Human Liver Microsomes under NADPH-Generating System.** The reaction mixture (200 μl final volume) contained 0.1 M potassium-phosphate buffer (pH 7.4), 3 mM MgCl$_2$, 1 mM β-NADP$^+$, 10 mM G-6-P, 2 μM G-6-PDH, 1 mg/ml microsomal protein, and 100 μM [14C]NB-506 (added 10 mM dimethylformamide solution). After a 5-min preincubation at 37°C with microsomes and [14C]NB-506 in potassium-phosphate buffer, reactions were initiated by adding to the NADPH-generating system (MgCl$_2$, β-NADP$^+$, G-6-P, G-6-PDH). All reactions were conducted at 37°C for 60 min and were terminated by adding 200 μl of ethanol. Proteins were removed.
by centrifugation at 9000g for 5 min; aliquots of the supernatant were analyzed by HPLC.

For checking the oxidative activity of microsomes used in these experiments, aniline p-hydroxylation activity was determined as positive control. **NB-506 UDP-Glucuronyltransferase Activity.** The reaction mixture (500 \( \mu \)l final volume) contained 0.1 M Tris-HCl (pH 7.4), 10 mM MgCl\(_2\), 1 mg/ml microsomal protein of human, dog, and rat liver (or 0.5 mg/ml microsomal protein of mouse liver), and NB-506. NB-506 was dissolved in dimethylformamide and added to reaction mixture to make a 1% final organic solution. For activation, microsomes were preincubated with 0.5 mg of CHAPS/mg protein (for human and dog liver microsomes) or 0.3 mg CHAPS/mg protein (for mouse and rat liver microsomes) at 4°C for 20 min. Reactions were started by adding 4 mM UDPGA. All reactions were conducted at 37°C for 15 min for rat, 20 min for mouse, and 30 min for dog and human, and were terminated by adding 500 \( \mu \)l of ethanol. Proteins were removed by centrifugation at 9000g for 5 min; aliquots of the supernatant were analyzed by HPLC.

**Analytical Procedures, HPLC method.** The concentrations of NB-506, ED-501, and ED-551 were quantified by HPLC with some modification of the previously described method (Takenaga et al., 1995). To determine the concentrations of NB-506, ED-501, and ED-551 simultaneously, the mobile phase consisted of 1) water/acetonitrile/methanol/TFA (66:19:15:0.1, v/v/v/v) and 2) water/acetonitrile/methanol/TFA (45:40:15:0.1, v/v/v/v). A gradient scheme was used as follows: 0 to 5 min, 100% 1 and 0% 2; 5 to 20 min, linear gradient to 75% 2; and 20 to 26 min, 75% 2. The flow rate was 1 ml/min at 40°C. A Shiseido Superiorex ODS (Tokyo, Japan) (4.6 \( \times \) 250 mm, 5-\( \mu \)m) column was used for analysis and an endcapped LiChrospher RP-8 (E. Merck, Darmstadt, Germany) was used as a guard column. To determine **ED-501** formation, the mobile phase consisted of water/acetonitrile/methanol/TFA (66:19:15:0.1 or 65:20:15:0.1 v/v/v/v) with a flow rate of 1 ml/min at 40°C. A Superiorex ODS

**TABLE 1**

<table>
<thead>
<tr>
<th>Specific Activity</th>
<th>Plasma ( \mu )mol/min/mg protein</th>
<th>Liver ( \mu )mol/min/mg protein</th>
<th>Small intestine ( \mu )mol/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>140 ± 3</td>
<td>17 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Rat</td>
<td>116 ± 4</td>
<td>2 ± 0</td>
<td>N.D.</td>
</tr>
<tr>
<td>Dog</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Human</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. <1.67 \( \mu \)mol/min/mg protein. Each value represents the mean ± S.D. (n = 4).

**TABLE 2**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Remaining Activity %</th>
<th>Remaining Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100.0 ± 7.88</td>
<td>N.D.</td>
</tr>
<tr>
<td>0.1mM DFP</td>
<td>16.0 ± 0.64</td>
<td>N.D.</td>
</tr>
<tr>
<td>0.1mM PMSF</td>
<td>94.0 ± 2.75</td>
<td>N.D.</td>
</tr>
<tr>
<td>0.1mM MIA</td>
<td>94.2 ± 0.96</td>
<td>N.D.</td>
</tr>
<tr>
<td>0.1mM IAA</td>
<td>98.8 ± 2.95</td>
<td>N.D.</td>
</tr>
<tr>
<td>0.1mM EDTA</td>
<td>99.6 ± 1.22</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Rat plasma was preincubated with each inhibitor in 50 mM Tris-HCl buffer, pH 7.4, at room temperature for 1 h, and then a residual activity was assayed. Each value represents the mean ± S.D. (n = 4). N.D. <1.4%.

**FIG. 2.** Metabolism of NB-506 in mouse, rat, dog, and human plasma.

NB-506 was incubated in plasma at 37°C, and the concentrations of NB-506 (\( \bullet \)), ED-501 (\( \square \)), and ED-551 (\( \square \)) were measured. Each value represents the mean ± S.D. (n = 3).
(4.6 × 150 mm or 4.6 × 250 mm, 5-μm) column was used for analysis and a NewGuard RP-18 3.2 × 15 mm (Applied Biosystems, Foster City, CA) was used as a guard column. The column effluent was monitored by ultraviolet absorption at 305 nm. To quantitate ED-594, the mobile phase consisted of water/acetonitrile/methanol/TFA (71:19:10:0.1, v/v/v/v) with a flow rate of 1 ml/min at 40°C. A Supelcosil ABZ+Plus (4.6 × 250 mm, 5-μm) column (Supelco, Bellefonte, PA) was used for analysis. The column effluent was also monitored by UV absorption at 305 nm.

**Protein Concentration.** Tissue S9 fractions, liver microsomes, and plasma protein concentrations were measured using a BCA protein assay kit (Pierce Chemical Co., Rockford, IL).

**Molecular Weight Determination.** Rat plasma was applied to a Sephadex G150 column (2.5 × 94 cm) with a mobile phase consisting of 50 mM Tris-HCl buffer, pH 7.4, at a flow rate of 0.15 ml/min. A gel filtration calibration kit (Pharmacia Biotech, Tokyo, Japan) was used as a molecular weight marker.

**Results**

**In Vitro Metabolism of NB-506 in Plasma.** *Species-specific conversion of NB-506 to ED-501 and ED-551.* To study the conversion of NB-506 to ED-501 (the deformyl form of NB-506) in plasma, NB-506 was incubated with mouse, rat, dog, and human plasma (Table 1). ED-501 was detected in mouse and rat plasma; the NB-506 deformylase activity for mice and rats was 140 and 116 pmol/min/mg protein, respectively. However, ED-501 was not detected (<1.67 pmol/min/mg protein) in dog and human plasma.

When NB-506 was incubated with mouse, rat, dog, and human plasma for various incubation times (Fig. 2), NB-506 rapidly disappeared and was completely converted to ED-501 in mouse and rat plasma. Although ED-501 was not noted in dog or human plasma, another metabolite, ED-551 (the deformylhydrazine form of NB-506) was detected.

**NB-506 deformylation kinetics in rat plasma.** The effects of pH on NB-506 deformylase activity were examined. The maximal activity was found at pH 7.4 in rat plasma (data not shown), and all subsequent studies were carried out at pH 7.4. Figure 3 shows the enzyme kinetics for the deformylation of NB-506 in rat plasma. The kinetic constants were calculated from the Michaelis-Menten plot (a); the \( V_{max} \) was 6.3 nmol/min/ml plasma and the \( K_m \) was 54 μM. The Eadie-Hofstee plot (b) shows one reaction involving in the deformylation.
Effects of inhibitors on NB-506 deformylase activity in rat plasma.

The inhibition of NB-506 deformylase activity by DFP, PMSF, MIA, IAA, and EDTA is shown in Table 2. DFP and PMSF, a potent serine enzyme inhibitor, inhibited the enzyme activity. MIA and IAA, which are SH reagents, did not affect NB-506 deformylase activity, and neither did EDTA.

Molecular mass determination of the enzyme involved in the deformylation reaction in rat plasma.

The molecular mass of the enzyme with deformylation activity was determined to be 138 KDa using gel filtration methods (data not shown).

Effects of heat treatment on the conversion of NB-506 to ED-501 and ED-551.

The effects of heat treatment (100°C for 1 min) on the metabolism of NB-506 in rat and human plasma were studied (Fig. 4). The deformylation of NB-506 to ED-501 in rat plasma was completely abolished by heat treatment. NB-506 was converted to ED-551 instead of metabolizing to ED-501. In contrast, the conversion of NB-506 to ED-551 in human plasma was not affected by heat treatment.

Effects of EDTA on the conversion of NB-506 to ED-551.

When NB-506 was incubated in human plasma containing 1, 5, and 50 mM EDTA, the conversion of NB-506 to ED-551 was completely inhibited by 50 mM EDTA (Fig. 5).

In Vitro Metabolism of NB-506 in Tissue.

The conversion of NB-506 to ED-501 in animal and human tissues was examined (Table 1). The NB-506 deformylase activity in liver S9 (17 and 2 pmol/min/mg protein in mice and rats, respectively) and small intestine S9 (11 and <1.67 pmol/min/mg protein in mice and rats, respectively) was very low as compared with that in plasma. No conversion of NB-506 to ED-501 was observed (<1.67 pmol/min/mg protein) in dog and human liver and small intestine.

Metabolism of NB-506 by human, dog, rat, and mouse liver microsomes. When [14C]NB-506 was incubated with human or rat liver microsomes in the presence of an NADPH-generating system, no radioactive peaks corresponding to metabolites were detected in either sample (Fig. 6).

### Table 3

<table>
<thead>
<tr>
<th>Liver Microsomes</th>
<th>Apparent $K_m$ (µM)</th>
<th>Apparent $V_{max}$ (pmol/min/mg)</th>
<th>Apparent $V_{max}/K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>6.6 ± 0.3</td>
<td>40.6 ± 6.4</td>
<td>6.1 ± 0.7</td>
</tr>
<tr>
<td>Rat</td>
<td>5.8 ± 0.6</td>
<td>52.4 ± 6.6</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td>Human</td>
<td>75.1 ± 1.5</td>
<td>641.7 ± 298</td>
<td>8.5 ± 3.8</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of three experiments. Glucuronidation of NB-506 was not observed in dog liver microsomes.
ED-594 (the glucuronide of NB-506) was isolated and identified from rat bile using mass spectra and NMR spectra (accompanying manuscript). The glucuronidation of NB-506 by mouse, rat, dog, and human liver microsomes was examined in vitro. ED-594 was formed in incubation mixture with human, rat, and mouse liver microsomes, but was not detected with dog liver microsomes (Table 3). To study the effects of detergents on the UDPGA-glucuronosyltransferase activity toward NB-506, we examined several different types of detergent (e.g., Triton X-100, CHAPS) incubated with human and rat liver microsomes. CHAPS was shown to be the most effective of the detergents and was therefore used in the kinetic studies. Eadie-Hofstee plots (Figs. 7 and 8) showed that the glucuronidation of NB-506 by human and rat liver microsomes was monophasic reaction. But, in mouse liver microsomes, glucuronidation of NB-506 catalyzed by two kinds of enzyme (Fig. 9).

**Discussion**

After i.v. administration to mice, rats, dogs, and humans, NB-506 was converted to ED-501 in mice and rats, but not in dogs and humans (Sasaki et al., 1995; Takenaga et al., 1995; accompanying manuscript). ED-551 was formed from NB-506 in dogs and humans, but not in mice and rats. Although either ED-501 or ED-551 was formed in plasma, NB-506 was not metabolized by human and rat liver microsomes in the presence of NADPH. This result indicates that a hepatic mixed function oxidase involving cytochrome P-450 is not responsible for NB-506 metabolism. To elucidate the species-dependent metabolism of NB-506 in vivo, the in vitro metabolism of NB-506 was studied in the above-mentioned species.

NB-506 deformylation in plasma appeared to be species-dependent and to be catalyzed by a rodent-specific enzyme with a molecular mass of 138 KDa. The deformylase activity is inhibited by typical serine enzyme inhibitor (DFP and PMSF), but not by SH reagents and EDTA. The properties of this enzyme were compared with those of amidase and esterase, which have been reported to undergo enzymatic cleavage of the C-N bond. p-Amidase purified from rat liver has a molecular mass of 58 KDa, and its activity is inhibited by SH reagents (Hersh, 1971). Formamidase is distributed in various mammalian tissues and can be purified from rat liver (molecular mass = 35 KDa) (Shinohara and Ishiguro, 1970; Bailey and Wagner, 1974; Krisch et al., 1975). Aryl acylamidase and amidase activity are reported to be
present in tissues of certain animals (Bray et al., 1950; Nimmo-Smith, 1960; Oommen and Balasubramanian, 1979). These comparisons suggest that the rodent-specific NB-506 deformylation enzyme is clearly different from these amidase and esterase enzymes with respect to biochemical and physical properties. To identify the rodent-specific enzyme, purification, cDNA cloning, and sequence determination would be useful.

Traceable NB-506 deformylase activity was observed in mouse liver and small intestine and in rat liver, probably because of contamination of blood during the preparation of the liver and intestine samples from mice and rats. The enzyme responsible for the deformylation of NB-506 may be present in the liver and small intestines of mice and rats, but its activity is very low compared with that in plasma.

NB-506 was also converted to ED-551 in dog and human plasma in vitro. The conversion of NB-506 to ED-551 was not affected by heat treatment, but was inhibited by EDTA. NB-506 was found to be stable in 50 mM Tris-HCl buffer, pH 7.4, for 3 h at 37°C (data not shown). These data suggest that this chemical reaction is catalyzed by metal ions. A possible mechanism of the reaction involves a π-complex.

**FIG. 9.** Enzyme kinetics of the glucuronidation of NB-506 by mouse liver microsomes.

- velocity (v) versus substrate concentration ([S]) plot.
- corresponding Eadie-Hofstee plot. Each value represents the mean of three experiments.

**FIG. 10.** Proposed mechanism for the formation of ED-551.

M⁺: Metal ion
formed by π electrons of NB-506 and a transition metal (Fig. 10). Thereby, NB-506 is converted to the acid anhydride form of NB-506, which is designated as ED-551.

The chemical conversion of NB-506 to ED-551 was also observed in rat plasma, but the formation of ED-551 was less than in dog and human plasma. However, after heat treatment of rat plasma, the formation of ED-551 was approximately the same as in dog and human plasma. This phenomenon can be explained as follows: 1) rat plasma has the ability to convert NB-506 to ED-551 by the same chemical reaction observed in dog or human plasma; 2) NB-506 is converted to ED-501 by an enzymatic reaction, and under physiological conditions, the enzymatic conversion is faster than the chemical reaction, resulting in a minimal conversion of NB-506 to ED-551; and 3) in heat-treated plasma, the enzymatic reaction is abolished and only the heat-stable chemical reaction occurs, resulting in the same activity as in dog and human plasma.

The glucuronidation of NB-506 by mouse, rat, and human liver microsomes was studied in vitro. One of the main metabolites of NB-506 was isolated from rat bile and identified as a glucuronide form of NB-506, which was designated as ED-594 (accompanying manuscript). The glucuronidation of NB-506 was also observed in mouse, rat, and human liver microsomes in vitro. Kinetic studies revealed that relative catalytic efficiency (apparent \( V_{\text{max}}/K_m \)) toward NB-506 in rats was similar to that in humans. ED-594 was detected in plasma samples obtained from human in phase I studies, and also in mouse and rat plasma after i.v. administration of NB-506 (unpublished observation). In dog studies, no glucuronidation of NB-506 was observed using liver microsomes and in vivo (unpublished observation).

In conclusion, NB-506 was metabolized to ED-501 in rodent plasma by an enzymatic reaction, whereas little or no conversion of NB-506 to ED-501 was observed in dog and human plasma. ED-551 was formed in dogs and humans by a chemical reaction catalyzed by metal ions. No metabolism of NB-506 by cytochrome P-450 isozymes was observed in the liver. NB-506 was glucuronized in mice, rats, and humans, but it was not glucuronidated in dogs. These data show that the metabolism of NB-506 in plasma and its glucuronidation in the liver are species-dependent. There appeared to be species differences in the metabolism of NB-506 in vitro, correlating well with the species-dependent pharmacokinetics of this drug in vivo.

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References


