A Phase I Pharmacokinetic and Pharmacodynamic Study of S-3304, a Novel Matrix Metalloproteinase Inhibitor, in Patients with Advanced and Refractory Solid Tumors

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The matrix metalloproteinases (MMP) are a large family of zinc-dependent endopeptidases (1), which play a fundamental role in cancer progression because of their ability to cleave virtually any component of the extracellular matrix (ECM) and basement membranes, thereby allowing cancer cells to penetrate and infiltrate the underlying stromal matrix and to access the vascular and lymphatic systems, which support tumor growth (angiogenesis) and constitute an escape route for further dissemination (invasion and metastasis; refs. 2, 3).

Elevated levels of MMPs can be detected in tumor tissue or serum of patients with advanced cancer, and their role as prognostic indicators in cancer has been widely examined (1). Among the MMPs, MMP-2 and MMP-9 degrade type IV collagen, which is a major component of the basement membranes, and their expression has been reported to correlate with tumor metastasis and angiogenesis in various cancers such as gastric, lung, bladder, nasopharyngeal, ovarian, breast, and head and neck (4).

A number of MMP inhibitors (MMPI) have been tested in clinical trials. Unfortunately, results of initial clinical trials with MMPIs in advanced cancer patients have been disappointing (5, 6) probably because the role of MMPs in cancer progression is much more complex than their degradative action on extracellular matrix components (2). This activity alone does not account for the diversity of biological responses modulated by these enzymes (7), particularly their relevance to the early steps of cancer progression such as angiogenesis. Better understanding of these processes has provided a strong impetus for the development of newer MMPIs (8).

A limitation of the early trials of MMPIs was the lack of a biological marker of activity. Recently, however, MMP activity has been successfully visualized, in terms of gelatinolytic activity, by film in situ zymography (FIZ) in human cancer xenografts implanted in nude mice, a technique that has also been used to evaluate the inhibitory effects of MMPIs (9). The

Abstract

Purpose: Matrix metalloproteinases (MMP) play a fundamental role in cancer development and progression. S-3304 is a potent, orally active, noncytotoxic inhibitor of MMPs, primarily MMP-2 and MMP-9, that prolongs survival in mice xenografts and is well tolerated in healthy volunteers.

Experimental Design: The aims of this phase I clinical trial were to determine the maximum tolerated dose, dose-limiting toxicities, pharmacokinetic profile, and intratumoral MMP inhibitory activity of single-agent S-3304 in advanced and refractory solid tumors. MMP activity was determined by film in situ zymography (FIZ). Patients had tumor biopsies before and after S-3304 administration and were also evaluated for response and survival.

Results: Four dose levels were explored [DL1-DL4 or 800, 1,600, 2,400, and 3200 mg twice daily (BID), respectively], and 32 patients were enrolled. Toxicities were mostly gastrointestinal. The maximum tolerated dose was not reached, but dose escalations beyond DL4 were impractical (number of capsules needed). S-3304 steady-state concentrations were reached by day 8, and day 1 mean Cmax and AUC0-8 increases were less than dose proportional. After S-3304 administration, 17 of 18 patients experienced inhibition of MMP activity by FIZ. Strong mean inhibition of MMP activity was observed in DL1 to DL3. The negative mean inhibitory activity calculated for DL4 was due to one patient with a 397% MMP activity increase.

Conclusion: S-3304 is safe, well tolerated, and achieves plasma concentrations above those required to inhibit MMP-2 and MMP-9. Its intratumoral MMP inhibitory activity has been shown using FIZ, which is useful as a biomarker with this and other MMP inhibitors.

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S-3304 is a novel D-tryptophan derivative (N\(^\text{\textsuperscript{\text{a}}}\)\{-2-[5-\{4-methylphenyl\}ethyl\}thienyl\}{sulfonyl}\}-D-tryptophan; Fig. 1). It is a potent, orally active, and noncytotoxic MMP inhibitor. Biochemical studies showed that S-3304 most potently inhibits the activities of MMP-2 and MMP-9 but does not inhibit MMP-1, MMP-3, or MMP-7 and may, therefore, lack the musculoskeletal side effects seen with nonspecific inhibitors. In vivo pharmacologic studies have shown that the oral administration of S-3304, at a dose range of 20 to 200 mg/kg, inhibited angiogenesis, artificially induced in mice by the dorsal air-sac method (10). Similar oral doses of S-3304 resulted in potent inhibition of metastatic lung colonization of Lewis murine lung carcinoma injected via tail vein and liver metastasis of C-111 human colon cancer implanted into the spleen (11). These results suggest that the antiangiogenic and antimetastatic activity of S-3304 could be due to its intrinsic inhibition of MMP-2 and MMP-9.

In healthy volunteers, S-3304 has shown a good safety profile and good systemic exposure when administered orally in doses up to 800 mg twice daily (BID) for 10 to 17 days. At this dose level, S-3304 was devoid of musculoskeletal symptoms, and the most commonly reported adverse events were headache and somnolence (12). A second, double blind, placebo-controlled healthy-volunteer study has also been completed. At daily doses up to 3,200 mg BID, no clinically significant changes in laboratory tests were observed, except for reversible grade 1 or 2 elevations of hepatic transaminases and one grade 3 creatine phosphokinase elevation. Clinically, this dose was well tolerated with a minority of subjects reporting myalgia/arthralgia (13).

We report here the first phase I study of single-agent S-3304 in patients with advanced solid tumors, designed to determine the dose-limiting toxicities (DLT) and other major toxicities, maximum tolerated dose (MTD), and pharmacokinetic profile of this drug. We also report results on the activity of S-3304 in inducing MMP inhibition (using FIZ as surrogate marker) in paired tumor biopsies.

**Patients and Methods**

**Patient selection**

Patients with histologically documented, advanced solid tumors, refractory to standard therapy or for whom no effective therapy existed, were eligible for this trial. Other eligibility criteria included (a) age \( \geq 18 \) years; (b) Eastern Cooperative Oncology Group performance status \( \leq 2 \) and a life expectancy \( > 6 \) weeks; (c) adequate bone marrow (hemoglobin \( \geq 9.0 \) g/dL, absolute neutrophil count \( > 1,500 \) per \( \mu \)L, and platelets \( \geq 100,000 \) per \( \mu \)L), renal (serum creatinine \( < 2.0 \) mg/dL), and hepatic (bilirubin \( \leq 2 \) times upper limit of normal and alanine aminotransferase and aspartate aminotransferase \( < 2.5 \) times upper limit of normal) functions; (d) no therapy 4 weeks before study entry; and (e) no serious inter-current illnesses, including HIV (AIDS). Eligible patients also had to be able to tolerate oral medications (no underlying gastrointestinal disorders such as recurrent vomiting, inflammatory bowel disease, or significant gastric resection), to have recovered from the toxicities of prior cancer therapies (all toxicities to be below grade 2 assessed by the National Cancer Institute Common Toxicity Criteria, version 2.0) (3) and to have signed informed consent according to federal and institutional guidelines. Patients with untreated or symptomatic brain metastasis and women who were pregnant, lactating, or not willing to use acceptable means of contraception, if of childbearing potential were excluded. Written informed consent was obtained from each subject, and an Institutional Review Board reviewed and approved the study protocol at each institution.

S-3304 was administered as an oral, BID dose for 28 days (one course) at four dose levels. Patients were allowed to continue on therapy for an indefinite number of courses in the absence of disease progression or unacceptable toxicity. Patients who did not complete one course of therapy for reasons other than a DLT could be replaced.

**Pretreatment evaluation**

Consisted of a history and physical examination, including the determination of the Eastern Cooperative Oncology Group performance status and a review of concomitant medications, pregnancy test, electrocardiogram, chest radiograph, complete blood count, serum chemistries (electrolytes, glucose, blood urea nitrogen, creatinine, magnesium, calcium, phosphate, albumin, alkaline phosphatase, total bilirubin, and transaminases), and a specific assessment of the patient’s tumor status with the documentation of measurable disease, using appropriate imaging studies (computed tomography and/or magnetic resonance imaging).

Patients were evaluated weekly for safety and toxicities, with a brief history and physical examination (vital signs and Eastern Cooperative Oncology Group performance status), clinical laboratory studies (similar to pretreatment studies), and a review of their drug accountability log (record of date/time and morning/evening dose). Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, v2.0. For the purpose of this study, MTD

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**Fig. 1.** Chemical structure of S-3304 metalloproteinase inhibitor and its metabolites. Parent compound S-3304 and its metabolites 4’-OH-methyl S-3304, 5-OH S-3304, and 6-OH S-3304.

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6 http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf
was defined as the highest dose level at which <33% of the patients experienced a DLT. A DLT was defined as \( g \) any grade 4 toxicity or \( h \) nonhematologic grade 3 toxicities (except for nausea and vomiting for which optimal anti-emetic therapy had not been given). For the purposes of determining MTD, only DLTs experienced during the first 28 days of therapy were considered.

Urine and blood samples as well as pretreatment and posttreatment tumor biopsies were obtained during the first cycle of therapy for pharmacokinetics and pharmacodynamics by FIZ analyses, respectively. Evaluation of objective tumor response was done after the first two cycles of therapy and every two cycles thereafter, using Standard Response Evaluation Criteria in Solid Tumors (14). The best overall response was recorded from the start of treatment until disease progression or recurrence.

S-3304 was manufactured by Shionogi & Co. Ltd. (Osaka, Japan), as a 200-mg hard gelatin capsule and was packaged and distributed by Quintiles, Inc. (Kansas City, MO). The study drug was stored at room temperature protected from light.

### Dose escalation and drug administration

Four dose levels were evaluated: 1,600 mg/d (800 mg BID, DL1), 3,200 mg/d (1,600 mg BID, DL2), 4,800 mg/d (2,400 mg BID, DL3), and 6,400 mg/d (3,200 mg BID, DL4). The starting dose of 800 mg BID was selected because it is two dose levels lower than the MTD of the healthy volunteer study and was generally well tolerated. There was no intrapatient dose escalation. The planned enrollment was six patients each at DL1 and DL2 and eight patients each at DL3 and DL4. At a given dose level, when all patients had completed 14 days of treatment and at least half had completed 28 days, escalation proceeded to the next level, if the MTD had not been reached.

If two patients at DL2 or three patients at DL3 or DL4 experienced a DLT, then escalation was stopped, and MTD was declared at the previous level. If two patients experienced a DLT at DL1, an additional dose level at 75% dosage, enrolling a total of six patients, was to be opened. This level was then to be declared the MTD if less than two patients experienced a DLT. If two or more patients at this new level still experienced a DLT, a decision would be made at that time to terminate the study or to enroll additional patients at 50% of DL1.

### Pharmacokinetics

Serial blood samples were drawn on days 1 and 28 (pre-dose and 1, 2, 3, 4, 5, 6, 8, and 12 h post-dose) and on days 8, 15, and 22 (trough or pre-dose only) for the plasma assay of S-3304 and its metabolites [4'-hydroxy-methyl S-3304 (4'-OH-methyl S-3304), 5-hydroxy S-3304 (5-OH S-3304), and 6-hydroxy S-3304 (6-OH S-3304)]. The S-3304 metabolites 5-OH S-3304 and 6-OH S-3304 were assayed at all dose levels. The 4'-OH-methyl S-3304 metabolite was only assayed at or near the MTD. In addition, 10-mL blood samples were drawn for serum binding assay at select times. Urine samples were collected, divided into aliquots, and stored in a refrigerator at less than or equal to −70°C, until the end of the collection period. Urine and protein binding analyses were limited to the parent compound.

The plasma and urine sample concentrations were determined using high-performance liquid chromatography with tandem mass spectrometric detection, and this method was validated by Quintiles. The validated standard curve ranges of this method for S-3304 (plasma and urine) and its metabolites (plasma) were 0.1 to 100 μg/mL, 10 to

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>6 (18.8%)</td>
</tr>
<tr>
<td>Renal</td>
<td>6 (18.8%)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>5 (15.6%)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>3 (9.4%)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2 (6.3%)</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>2 (6.3%)</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>2 (6.3%)</td>
</tr>
<tr>
<td>GIST</td>
<td>2 (6.3%)</td>
</tr>
<tr>
<td>Immune/Invest</td>
<td>17 (53.1%)</td>
</tr>
</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NSCLC, non–small cell lung cancer; GIST, gastrointestinal stromal tumors.

### Table 2. Drug-related adverse events (occurring in ≥2 patients, all courses)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>S-3304 dose group (mg BID)</th>
<th>Total, % (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G 1</td>
<td>G 2</td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Increased CPK</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Increased LDH</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: G, grade; CPK, creatine phosphokinase; LDH, lactose dehydrogenase.
10,000 ng/mL, and 1.00 to 200 ng/mL, respectively. Pharmacokinetic variables were computed from the plasma concentrations for days 1 and 28 on WinNonlin Professional version 4.01 (Pharsight Corp., Mountain View, CA) using noncompartmental methods.

**Pharmacodynamics**

**Biopsy sampling and tissue slide preparation.** Paired tumor biopsies were obtained from each patient. The samples were obtained from the same tumor before (day 0) and after (days 27-31) S-3304 administration (second biopsy obtained 3±2 h after the morning drug dose). Once obtained, samples were placed in Tissue-Tek crymold plastic containers poured with optimum cutting temperature compound (Miles, Inc., Elkhart, IN) and immediately frozen on dry ice and kept at −70°C or below until use. They were shipped to Shionogi & Co. Ltd. for processing and assay. The slides were used in immunohistochemical staining for MMP-2 and MMP-9 immunohistochemical staining.

**H&E and MMP-2/MMP-9 immunohistochemical staining.** H&E staining was conducted according to conventional methods (13). These slides were used for morphologic and pathologic investigations. MMP immunohistochemical staining was conducted according to previously reported procedures (16). Briefly, slides were incubated with MMP-2 and MMP-9 anti-human polyclonal antibodies and normal rabbit serum (MMP immunohistochemistry−negative control) diluted in PBS containing 0.1% bovine serum albumin for 1 h at room temperature. After washing with PBS, these slides were then reincubated with biotin-conjugated goat anti-rabbit IgG antibody followed by treatment with horse radish peroxidase avidin-biotin complex reagent. The exogenous peroxidase activity was visualized as dark brownish color deposits with 10 min of drenching in 50 mmol/L Tris-HCl (pH 7.6) containing 200 μg/mL 3,3′-diaminobenzidine and 0.003% H2O2 in the dark. It should be noted that the antibodies used in this study do not distinguish between proenzyme and proteolytically processed forms of MMPs; thus, both latent and active forms of MMP-2/MMP-9 were immunostained in this study.

**Photomicrographic evaluation.** The H&E slides were first observed under light microscope to investigate tumor morphopathology (i.e., sample size, % tumor cell area in the total tissue, and % necrotic area within the lesions). The sample size was calculated from the length and width of each optical image. The tumor cell area was calculated according to microscopic criteria, such as anaplasia, florid hyperplasia, nucleolar multiplication, etc. Necrosis was judged by criteria such as loss of nucleus, degeneration of cellular organelles, etc. The localization of MMP-2/MMP-9 with immunohistochemistry and the intensity of the immunoreactivity in each preparation were analyzed using Optimus version 6.0, and the resultant scores were graded as “−” (<10), “+” (10-40), and “++” (40-100).

**FIH.** This method has been previously described (9). Briefly, one tumor section used to detect net gelatinolytic activity was mounted on a polyethylene terephthalate base-film coated with homogeneous 7-µm gelatin layer, (gelatin film) provided by Fuji Photo Film Co. Ltd. (Tokyo, Japan). The other, used as reference was mounted on a film coated with 7-µm gelatin layer containing 1 mmol/L 1,10-phenanthroline metal chelator (gelatin with inhibitor film). They were then immediately incubated in a moist chamber (Cosmo Bio Co. Ltd., Tokyo, Japan) at 37°C for 3.5 h. After the incubation, the preparations were

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**Table 3. Days 1 and 28 S-3304 and metabolite pharmacokinetic variables**

<table>
<thead>
<tr>
<th>Compound/dose (mg BID)</th>
<th>S-3304*</th>
<th>4′-OH-methyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>800 (N = 6)</td>
<td>1,600 (N = 6)</td>
</tr>
<tr>
<td>Mean AUC0-12 h (ng.h/mL)</td>
<td>199</td>
<td>210</td>
</tr>
<tr>
<td>CV (%)</td>
<td>58.9</td>
<td>36.0</td>
</tr>
<tr>
<td>Median T max (h)</td>
<td>2.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Day 28 (steady state)

| Mean AUC0-12 h (ng.h/mL) | — | — | 569 | 602 | — | — | 172 | 217 |
| CV (%) | — | — | 50.3 | 21.4 | — | — | 48.7 | 35.0 |
| Median T max (h) | 3.29 | 2.92 | 3.00 | 3.00 | 2.21 | 2.00 | 2.53 | 3.00 |

NOTE: N, the total number of patients in each group; n, number of patients included for the calculation of pharmacokinetic variables (variable between doses and parameters analyzed). If n < 3, then descriptive statistics were not reported. Abbreviation: CV, coefficient of variation.

*Units for the parent compound in μg.

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**Abbreviation:** CV, coefficient of variation.
stained for 15 min in a coplin jar with 1.0% amido black 10B dye and diluted to 1.0% with 70% methanol and 10% acetic acid. Then the films with tissue specimens were destained for 10 min with a 70% methanol and 10% acetic acid solution. For the evaluation of the performance of gelatin with inhibitor film, xenograft of Ma44 human tumor cells in nude mice was dissected, and FIZ was conducted with the same method described above.

FIZ was done and the gelatinolytic activity determined in a tumor area confirmed by H&E and immunohistochemical staining. Both gelatin film and gelatin with inhibitor film specimens were observed in parallel by focusing on the gelatinolysis of the tumor lesions. For semiquantitative analysis of the local gelatinolytic activity, the average optical densitometric brightness (range, 0-255) was measured by Optimus version 6.0 image analyzing software. The value of brightness was used to estimate the gelatin-digesting enzymatic activity (MMP activity), and the following two variables were used as end points of MMP inhibition by S-3304:

\[
\frac{\text{Change}}{\text{C}_{138}} = \frac{\text{Brightness}_{\text{day0}} - \text{Brightness}_{\text{day28}}}{\text{Brightness}_{\text{day0}}}
\]

\[
\% \text{Inhibition} = \left(\frac{\text{Brightness}_{\text{day0}} - \text{Brightness}_{\text{day28}}}{\text{Brightness}_{\text{day0}}}ight) \times 100
\]

The stronger the MMP activity, the higher the value recorded as brightness and vice versa. Therefore, it was expected that by inhibiting MMP activity, S-3304 would cause a reduction in the tumor sample brightness.

**Statistical method**

Because one of the objectives of this study was to investigate the MMP inhibition activity of S-3304, a typical 3 + 3 phase I design was not considered adequate; thus, six evaluable patients were planned for enrollment at DL1 and DL2 and eight patients at DL3 and DL4. This design satisfied toxicity evaluation requirements, allowed for the ethical study of the biological effect in more patients of S-3304, and finished the study in a reasonable time span.

The pharmacokinetic population consisted of all patients who received S-3304 and have evaluable plasma data, whereas the safety population included all patients who received at least one dose of study drug. The pharmacodynamic population included patients with pre- and post-S-3304 tumor biopsies and evaluable FIZ results. Safety variables were reported in absolute numbers or as a fraction of the total population. The pharmacokinetic variables were summarized by mean and coefficient of variation for AUC and \(C_{max}\) and by median for \(T_{max}\). Summary statistics were not reported for groups of less than three patients. Pharmacodynamic variables were reported as mean FIZ % inhibition. Due to the reduced sample size, no statistical test was done, and the mean FIZ % inhibitions were only compared among the different dose levels.

**Results**

**Patients**

Thirty-two patients were treated at four institutions; 6 each at DL1 and DL2, 9 at DL3, and 11 at DL4 (two additional patients were registered but did not receive drug). Twenty-six patients completed one course of therapy (28 days) and were evaluable for toxicity. Of the six patients who did not complete course 1 (one each at DL1 and DL3 and four at DL4, four had progressive disease, one withdrew for social and logistic reasons, and one did not return and was lost to follow-up). No patient failed to complete course 1 for reasons related to toxicity. Patient characteristics are outlined in Table 1.

**Treatment**

Twenty-six patients completed at least one course of the treatment (5 at DL1, 6 at DL2, 8 at DL3, and 7 at DL4). Eleven
patients received one course only; seven patients received two courses; and eight patients received more than two courses (3–8). A total of 65 courses of therapy were administered: 11 at DL1 and 18 in each of DL2-4; one patient at each dose level received more than four courses of therapy. The median durations of therapy were 44, 57, 44, and 29 days for DL1 to DL4, respectively. For 23 patients, treatment was discontinued for progressive disease. Four patients elected to discontinue treatment. Only two patients were taken off treatment for clearly drug-related toxicity. Two patients developed a DLT during the first course: one at DL3 (grade 3 hyponatremia) and one at DL4 (grade 3 anorexia).

Toxicity
The drug was very well tolerated with few serious side effects (Table 2). No grade 4 or 5 toxicities were seen. The majority of side effects were gastrointestinal grade 1 or 2, including anorexia, nausea, vomiting, diarrhea, and abdominal pain. A grade 1 increase in creatine phosphokinase was noted in three patients; fatigue also occurred frequently. Grade 3 toxicities were infrequent, consisting of anorexia, nausea, vomiting, fatigue, and hyponatremia. Except for gastrointestinal toxicities, there was no clear relation of toxicity to dose level. Two patients (one at DL3 and one at DL4) had a DLT in course 1. These were respectively a grade 3 hyponatremia considered possibly drug related and a grade 3 anorexia considered definitely drug related.

Pharmacokinetics
Table 3 and Fig. 2 show the derived pharmacokinetic variables for S-3304 and its metabolites (4′-OH-methyl S-3304, 5-OH S-3304, and 6-OH S-3304) and the median plasma concentration-time profile of S-3304 on days 1 and 28, for all dose groups, respectively. Given that the 12-h sampling period used was inadequate to define the terminal $t_{1/2}$, the calculated AUC$_{0-\infty}$ may have been underestimated as the estimated terminal $t_{1/2}$ could actually have represented the distribution/elimination $t_{1/2}$. In view of this, some of the data related to AUC$_{0-\infty}$ and terminal $t_{1/2}$ would need to be interpreted with caution and are not reported. Reliable variables, such as $T_{\text{max}}$, $C_{\text{max}}$, and AUC$_{0-12}$, are described. Because AUC$_{0-\infty}$ at steady state is equal to AUC$_{0-\infty}$, and because $\tau$ (the dosing interval) is 12 h, the AUC$_{0-12}$ on day 28 should be equivalent to AUC$_{0-\infty}$ and is also reported (Table 3).

S-3304 parent compound. Pharmacokinetic variables are shown in Table 3. S-3304 steady-state concentration was achieved by day 8. $T_{\text{max}}$ was 1.00 to 2.03 h on day 1 and 2.92 to 3.29 h on day 28. $C_{\text{max}}$ was 44.3 to 91.9 $\mu$g/mL on day 1 and 48.5 to 103 $\mu$g/mL on day 28. AUC was 219 to 565 $\mu$g mL$^{-1}$ h for DL1 to DL4 (Fig. 2). For all dose levels, increase in dose resulted in less than proportionate increase in exposure as measured by $C_{\text{max}}$ and AUC.

The mean $t_{1/2}$ calculated from the 12-h sampling period is from 2.05 to 3.34 on DL1 and was not dose dependent. On day 28, it was 4.67 and 3.34 h for the two higher dose levels. However, it should be noted that because of the 12-h sampling period, these data do not represent a true terminal phase $t_{1/2}$. S-3304 was highly bound to serum protein, with 0.001% to 0.01% of S-3304 available as unbound drug. The renal elimination of unchanged drug was <0.1% of the dose.

The interindividual variability was <50% for most variables on days 1 and 28 and ranged from 15.2% to 116.8%.

S-3304 metabolites. The data for the three metabolites 4′-OH-methyl S-3304, 5-OH S-3304, and 6-OH S-3304 are given in Table 3. All concentrations remained above the quantitation limit at the end of the sampling period. Although there is a trend towards higher levels at higher doses, there is no dose proportionality, and the data are extremely variable.

Trough concentrations. The mean and individual trough concentrations of S-3304 on days 8, 15, 22, and 28 for DL1 and DL2 were similar and seemed to reach a steady state on or by day 8. The trough concentrations achieved for DL3 and DL4 were higher and generally variable. The trough concentrations seemed to increase with dose but were highly variable for all the metabolites on days 8, 15, and 22 (data not shown).

Efficacy
Twenty-six patients were evaluable for response. There were no complete or partial responses. Seven patients had stable disease, of which four had renal cell carcinoma, and one each...
had soft tissue sarcoma, mesothelioma, and head and neck carcinoma.

**Pharmacodynamic evaluation**

Biopsies were obtained pretreatment and on day 28 from 18 patients (4 at DL1, 5 at DL2, 6 at DL3, and 3 at DL4). Stained biopsy material from a typical case is shown in Fig. 3, with the pretreatment (day 0) sample shown in Fig. 3A, and the day 28 biopsy material shown in Fig. 3B. Gelatinolytic activity was detected in high frequency in the areas of tumor as confirmed by H&E staining and immunohistochemistry for MMP-2 and MMP-9, although with considerable variability. Of the 18 sample pairs, 17 showed inhibition ranging from 20.8% to 93.7%. Only one patient, at DL4, with the lowest pretreatment value, showed an increase of 397.4% on day 28 compared with the pretreatment sample; therefore, the mean FIZ % inhibition for DL4 was negative (−109.2%). Mean FIZ % inhibition ranged from 51.0% to 69.9% in DL1 to DL3. There was no trend toward a dose-response relationship; the median values for the inhibition for DL1 to DL3 were as follows: DL1, 60.4% (range, 46.7-70.0%; n = 4); DL2, 65.2% (range, 20.8-73.1%; n = 5); DL3, 72.0% (range, 38.4-93.7%; n = 6). At DL4, the two patients whose samples showed inhibition had 32.6% and 37.2% inhibition.

Immunohistochemistry for MMP-2 and MMP-9 showed that dense 3,3'-diaminobenzidine immunodeposits were present in regions exhibiting gelatin-digestive activity in FIZ GN films in most samples, suggesting that these MMPs are involved in the observed gelatin digestion. The semiquantitative analysis showed that none of the samples was negative for MMP-2 and MMP-9 immunoreactivity. In general, immunoreactive level of MMP-9 was higher than MMP-2. The immunoreactive...
levels of both MMP-9 and MMP-2 after S-3304 administration were generally similar to or lower than those observed pretreatment.

**Discussion**

S-3304 is a new low-molecular-weight inhibitor specific for type IV collagenases, and biochemical studies have shown that it most potently inhibits the activities of MMP-2 and MMP-9 but does not inhibit MMP-1, MMP-3, or MMP-7 and, therefore, should not exhibit the musculoskeletal side effects seen with nonspecific inhibitors. The present study was designed to identify the MTD of S-3304, but, with the recognition that the number of capsules required would limit the dose to 6,400 mg/d (16 capsules BID), only four pre-established dose groups were tested (800, 1,600, 2,400, and 3,200 mg BID). In addition to the traditional toxicity and pharmacokinetic analysis, pharmacodynamic testing of tumor samples before and after exposure to S-3304 was done, using as surrogate biomarker of MMP inhibition, the shown ability of FIZ to determine MMP activity, in terms of gelatinolytic activity, in human cancer xenografts (9).

S-3304 was extremely well tolerated, with gastrointestinal symptoms grade 1 or 2 being the predominant side effects; these may have been partly related to the number of capsules required at the higher doses. Only two patients had a DLT, both grade 3 hyponatremia and anorexia at DL3 and DL4, respectively. A dosage of up to 3,200 mg BID could be safely given to a refractory cancer patient population like this.

Pharmacokinetic variables were estimated for S-3304 and its metabolites on days 1 and 28 using a noncompartmental analysis. The plasma concentration time profiles for both S-3304 and the metabolites indicated concentrations well above the limit of quantitation, and dosages up to 3,200 mg BID achieved levels above the IC_{50} observed for S-3304, against MMP-2 and MMP-9, in preclinical models (10). Evaluation of the trough concentrations indicated that, consistent with previous data (12, 13), S-3304 achieved steady state on or before day 8, and the concentrations increased with dose but were highly variable.

The pharmacokinetic analysis was done on data collected over a 12-hr sampling period (the dosing interval): this sampling period may not have been adequate to characterize the exposure and elimination of S-3304 and its metabolites. Therefore, some of the data related to AUC_{0-\infty} and terminal t_{1/2} would need to be interpreted with caution, including the S-3304 estimated elimination half-life of 2 to 4 h on both days 1 and 28, well below the known terminal phase half-life for a single dose of S-3304 of ~12 h following multiple dosing with S-3304 (12, 13).

Additionally, dose proportionality, measured by AUC_{0-12} and C_{max} was inconclusive for both the parent compound and its metabolites, and the pattern of exposure to the metabolites after varying doses of S-3004 showed no clear trend. The reasons for the pharmacokinetic differences among the metabolites may be due to high variability in the data and to a significant number of data failing to meet criteria for analysis. Similarly, comparison of AUC_{0-\infty} on day 1 with AUC_{0-12} could not be interpreted conclusively due to high variability, an unequal sample number, and the limitations imposed by a sampling period within the dosing interval.

S-3304 was highly bound to serum protein with <0.001% to 0.01% available as free drug. There was an increase in binding and an increase in free concentration, with an increase in dose, as assessed by a dose-proportionality test on days 1 and 28 at the 3- and 8-h sampling time points. However, both percent unbound and free concentration were highly variable. Generally, it is known that the concentrations of plasma proteins change over time in cancer patients, and this, coupled with the range of concomitant medications used in these patients, could have contributed to the large variability in the data. In normal volunteers receiving 800 mg/d S-3304 (12, 13) as single dose, data on protein binding were more consistent. Renal elimination of the parent drug was negligible, with <0.1% of dose eliminated in urine. Neither renal clearance nor the percentage of unchanged drug excreted in urine seemed to be affected by dose.

The clinical efficacy results showed no complete or partial responses; seven patients achieved stable disease. The biological efficacy (assessed by FIZ) analysis, however, indicated very encouraging results, not only with direct evidence of S-3304 induced inhibition of MMP activity but also with evidence that supports the use of FIZ as a surrogate biomarker of MMP activity. The FIZ results showed that MMP activity at tumor sites was inhibited from 20% to >90% in 17 of 18 patients. Thus, these observations strongly suggest that MMP-2 and MMP-9 activities are suppressed at the tumor tissue by orally administered S-3304. No dose-dependent inhibitory effect of gelatinase activity was observed among the four dose groups, which may indicate a plateau phenomenon where a full effect of S3304 can be achieved by the lowest (and less toxic) dose tested (800 mg BID, effective biological dose less than the MTD). The preliminary nature of the FIZ evaluations and the fact that the gelatinolytic activity detected by FIZ is due not only to MMPs but also to non-MMP proteinases make these results suggestive but not conclusive. However, given that the immunohistochemical stains indicated that MMP-2/MMP-9 immunoreactivity in all samples generally coincided with the gelatinolytic activity, and that S-3304 is a specific inhibitor of MMP-2 and MMP-9, the decrease of gelatinolytic activity after drug administration is likely to be due to inhibition by S-3304 of these two MMPs. There was no obvious correlation between stable disease and the degree of inhibition as measured by FIZ.

In summary, the present study was designed to investigate the DLTs and MTD and to detect the inhibition of MMP-2 and MMP-9 activities at the tumor tissue after oral S-3304 administration in patients with solid tumors. The substantial inhibition of local gelatinolytic activity in tumor lesions by FIZ observed with S-3304, even at the lowest dose tested, is encouraging, particularly as this dose is virtually nontoxic. This indicates that further testing of this agent is indicated in renal cell carcinoma and possibly also in sarcoma.

**Conclusion**

S-3304 is a novel MMPI specific for MMP-2 and MMP-9. In a phase I clinical trial, it was very well tolerated and produced inhibition of gelatinase activity in tumor biopsies at the lowest dose tested, a dose that produced very little toxicity. Four of eight patients with renal cell carcinoma had stable disease; further testing in this disease is indicated.

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References


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