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Transarterial Sorafenib Chemoembolization: Preliminary Study of Technical Feasibility in a Rabbit Model

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Abstract

Purpose—To test the feasibility of targeted intra-arterial administration of the tyrosine kinase inhibitor chemotherapeutic agent sorafenib to inhibit embolotherapy-induced tumor angiogenesis and reduce systemic drug side effects.

Materials and Methods—The left hepatic lobes of five New Zealand White rabbits (mean weight 2.7 ± 0.2 kg) were treated with chemoembolization using sorafenib and ethiodized oil emulsion, followed by immediate euthanasia. Post-procedure noncontrast computed tomography (CT) was used to evaluate intrahepatic chemotherapy mixture distribution. Liquid chromatography/tandem mass spectrometry (LC-MS/MS) was then used to directly measure sorafenib concentration in the treated liver tissue. Histopathological assessment of treated left lobes was performed to identify any immediate toxic effects of the sorafenib solution.

Results—Lobar sorafenib chemoembolization was successfully performed in all cases via the left hepatic artery. Sorafenib and ethiodized oil (mean $6.4 \text{ mg} \pm 3.8$ and $0.95 \text{ mL} \pm 0.7 \text{ mL}$, respectively) were injected, and CT confirmed targeted left hepatic lobe sorafenib emulsion delivery in all cases. Corresponding LC-MS/MS analysis yielded a mean sorafenib concentration of $94.2 \text{ } \mu\text{g/mL} \pm 48.3$ in treated left lobe samples ($n = 5$), significantly greater than typical therapeutic drug levels ($2\text{--}10 \text{ } \mu\text{g/mL}$) achieved with oral sorafenib systemic therapy. Histopathological assessment showed only mild or moderate non-specific ballooning degeneration in zone 3 hepatocytes, without tissue necrosis.

Conclusions—Targeted transarterial sorafenib delivery is feasible and results in higher tissue drug levels than that reported for systemic sorafenib therapy, without immediate histopathological tissue toxicity. Future studies should aim to determine the utility of sorafenib chemoembolization in reducing hypoxia-induced vasculogenesis in liver tumors.

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INTRODUCTION

Transarterial chemoembolization takes advantage of the hepatic arterial derivation of hepatocellular carcinoma (HCC) perfusion for targeted chemotherapeutic agent delivery and tumor devascularization (1). Although embolization of hepatic arteries supplying liver malignancies results in hypoxia and tumor necrosis, this process also induces angiogenesis (2). Induction of ischemia within liver neoplasms has been shown, in rabbit models, to result in increased intratumoral expression of hypoxia-inducible factor (HIF)-1 α among residual surviving cells (3). When combined in cellular nuclei with HIF-1 β , operational HIF-1 is formed and initiates a cascade of gene expression of proangiogenic factors and altered glucose metabolism to counteract nutritional deprivation incipient with hypoxia (2). The array of upregulated factors is diverse, but prominently features vascular endothelial growth factor (VEGF), which results in aggressive tumor ontogenesis and vascularity (4) and connotes increased tumoral propensity for metastasis and invasive behavior (5). VEGF exerts its downstream effects by interaction with a tyrosine kinase receptor, designated VEGF receptor (VEGFR).

Receptor tyrosine kinase inhibitors are a class of drugs that interrupt signaling pathways involved in tumor progression and angiogenesis (6). In biochemical assays and murine models, sorafenib (BAY 43-9006, Nexavar; Bayer Pharmaceuticals, Leverkusen Germany), has been shown to potently inhibit multiple receptor tyrosine kinases, including VEGFR subtypes, involved in tumor angiogenesis. Immunohistological correlation with murine tumors in mice treated with sorafenib demonstrates decrease in tumoral microvessel density (6), and in vitro assays and murine models have demonstrated suppression of HCC cell proliferation and induction of apoptosis in a dose-dependent manner (7). Clinically, sorafenib has been used in the treatment of patients with advanced HCC. Double blind randomized controlled phase 3 clinical trials of sorafenib in such patients have shown delay in time to progression and increase in overall survival (8, 9). However, the adverse effect profile of this agent, which is at present commercially available only as an oral formulation for systemic delivery, limits medication compliance, with notable side effects including diarrhea and hand-foot syndrome that occur at a statistically significantly higher rate than in placebo comparisons (8). To date, other routes of sorafenib instillation have not been widely explored; the ability to infuse sorafenib by using a transcatheter intraarterial route has the potential to deliver high localized drug concentrations directly to tumor in order to reduce the proangiogenic cascade stimulated by hypoxic conditions precipitated during chemoembolization, while decreasing systemic drug side effects. As such, the goal of the present project was to assess the feasibility of transarterial hepatic delivery of a lipid-emulsified preparation of sorafenib using a rabbit model.

MATERIALS AND METHODS

Animal care and use committee approval was obtained for this prospective study. The experimental protocol consisted of several steps: (i) production of a lipid-emulsified sorafenib preparation, (ii) in vivo intravascular delivery of the agent into New Zealand White rabbit livers, (iii) noninvasive assessment of ethiodized oil and drug emulsion delivery by computed tomography (CT) imaging, (iv) liver explantation and liquid

chromatography (LC)/tandem mass spectrometry (LC-MS/MS) direct tissue chemotherapeutic analysis to determine local drug concentrations, and (v) histopathological analysis to assess for possible toxic effects of sorafenib solution on hepatic parenchyma.

Intra-arterial Dosing and Preparation of Sorafenib Solution

In devising a dosing regimen for intraarterial sorafenib, peak plasma concentrations ($C_{\max}$) and therapeutic drug levels for both humans and rabbits were considered. In human trials, the $C_{\max}$ for sorafenib, as demonstrated in multiple discontinuous (10) and continuous (11) dosing trials that used the maximum tolerated dosage consisting of oral sorafenib 400 mg twice daily (a regimen widely utilized clinically) ranged from approximately 2 to 10 $\mu\text{g/mL}$; these plasma concentrations represent therapeutic levels of sorafenib, which inhibits components of the Raf-mitogen-activated protein kinase kinase-extracellular signal-related kinase signaling pathway and receptor tyrosine kinases at concentrations ranging between approximately 3 and 270 ng/mL (10). In rabbits, plasma sorafenib levels approximating 5 $\mu\text{g/mL}$ (a concentration comparable to $C_{\max}$ achieved in clinical studies) may be reasonably attained by using an oral dosing regimen of 30 mg/kg/day (Bayer, unpublished data, February 2011). Considering that chemoembolization generally results in local drug concentrations 10–100 times greater than systemic administration (1), intraarterial dosing was therefore empirically targeted at approximately 3 mg/kg. The selected chemoembolization protocol consisted of a 6–12-mg/mL-concentration sorafenib solution emulsified with an equal volume of ethiodized oil (Lipiodol; Guerbet, Villepinte France), and required injection of 1.5–3 mL total chemotherapeutic emulsion volume for complete dose administration in a 3.0-kg rabbit.

Sorafenib liquid solution formulation was prepared by using a solvent of 12.5% Cremophor EL (Sigma-Aldrich, St. Louis, Missouri), 12.5% ethyl alcohol (Sigma-Aldrich), and 75% distilled water (Sigma-Aldrich) (6). For solution preparation, sorafenib powder (provided by Bayer) was dissolved in a 50% Cremophor EL and 50% ethyl alcohol mixture at 12–24 mg/mL. Heating of the mixture up to 60°C was necessary to get the sorafenib into solution. When the compound was in solution, distilled water was added gradually with mixing to generate the 6–12 mg/mL dosing solution. The sorafenib solution was then allowed to cool to room temperature before use in chemoembolization procedures.

Transarterial Sorafenib Chemoembolization

Five male New Zealand White rabbits (mean weight $2.7 \text{ kg} \pm 0.2$) underwent sorafenib chemoembolization procedures, which were performed by a single operator (R.C.G.) after rabbits were intubated and maintained under general anesthesia (induction with intramuscular ketamine 50–65 mg/kg and intramuscular xylazine 5 mg/kg, maintenance with inhaled isoflurane 1–3%). Angiography was performed with a C-arm unit (OEC Medical Systems series 9600; GE Healthcare, Milwaukee, Wisconsin). The femoral artery was accessed through a surgical cut-down and catheterized with a 3-F vascular sheath (Cook, Bloomington, Indiana). A 2-F JB1 catheter (Cook) was advanced over a guide wire, and the celiac artery was selectively catheterized. The catheter was then advanced into the left hepatic artery. Celiac and hepatic arteriography was performed via injections of iohexol (Omnipaque-300; Amersham, Princeton, New Jersey). After catheter position was

confirmed in the left hepatic artery, selective left hepatic lobe chemoembolization was performed by injection of a 1:1 volumetric emulsion of 6–12 mg/mL sorafenib solution and ethiodized oil (Lipiodol; Guerbet). Under fluoroscopic visualization, the chemotherapy emulsion was injected by hand into the catheter. Chemoembolization was performed to a static angiographic endpoint in all cases, at which point administration of the chemotherapy emulsion was ceased, regardless of administered volume. Immediately after completion of chemoembolization, rabbits were euthanized using a lethal intravenous dose of 390 mg/mL pentobarbital sodium solution (Schering-Plough, Kenilworth New Jersey).

Of note, a sixth rabbit (a 3.0-kg female) underwent bland liver embolization using 12.5% Cremophor EL (Sigma-Aldrich), 12.5% ethyl alcohol, and 75% distilled water solution mixed with ethiodized oil in a 1:1 volumetric ratio for the purposes of serving as an experimental control for histologic analysis. As in the chemoembolization cohort, the emulsion was injected by hand into a hepatic artery catheter under fluoroscopic visualization, and the embolization was performed to a static angiographic endpoint.

CT Imaging

CT scans were obtained within 30 minutes of euthanasia using a BrightSpeed 16 slice scanner (GE Healthcare) to delineate the anatomic distribution of injected sorafenib mixture, which was radiopaque as a result of incorporated ethiodized oil. The CT protocol included helical acquisition with a current of 300–400 mA, voltage of 120 kV, pitch = 1.375:1, and 0.625-mm acquisition slice thickness.

Animal Necropsy and Tissue Harvest

Within 30 minutes of CT scan completion, rabbit necropsy was performed and livers were harvested and processed for LC-MS/MS sorafenib analysis as well as histopathologic assessment. The explanted livers were separated into left and right hepatic lobes. Hepatic tissue was harvested for LC-MS/MS analysis; specimens consisted of two representative 2-cm³ samples of treated left hepatic lobe parenchyma, one from the left medial segment and one from the left lateral segment, assuming homogeneous distribution of sorafenib and ethiodized oil emulsion within the left hepatic lobe as seen on CT imaging. Collected specimens were stored in 1 mL of sterile saline and were frozen in liquid nitrogen at –80°C until the time of LC-MS/MS analysis.

Measurement of Tissue Sorafenib Concentration

A calibration curve was first created for the drug. Standard sorafenib solutions ranging from 4 µg/mL to 200 µg/mL (4, 8, 12.5, 25, 50, 100, and 200 µg/mL) were used to create the standard curve, which was linear with an $R^2 = 0.999$. Sample preparation was based on the method of Römisch-Margl et al (12) and modified for the analysis of sorafenib by using ultra-high-pressure LC/MS-MS (UHPLC/MS-MS). Briefly, approximately 100 mg of liver tissue was homogenized in phosphate buffer (10 µM, pH 7.4) containing 8% ethanol volumetric ratio (ie, vol/vol) to give a homogenate containing 100 mg tissue per milliliter. N-(4-phenoxyphenyl)-N'-phenylurea (Sigma-Aldrich) was added at a concentration of 10 µg/mL as an internal standard. Protein precipitation was carried out using 100 µL of homogenized tissue by adding 400 µL of acetonitrile/ethanol (4:1 vol/vol). After

centrifugation at 12,000g for 20 minutes (4°C), the supernatant was removed and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 50 µL of methanol/water (1:1 vol/vol).

UHPLC/MS-MS was carried out using a Nexera UHPLC system (Shimadzu, Kyoto, Japan) interfaced with a model LCMS 8040 triple-quadrupole mass spectrometer (Shimadzu). Separations were carried out by using a BEH Shielded C₁₈ column (2.0 mm × 50 mm i.d.; internal diameter, 1.7 µm; Waters, Milford, Massachusetts) at 45°C and a 1-minute gradient from 25% to 75% acetonitrile in 0.1% aqueous formic acid. The injection volume was 1 µL, and the flow rate was 0.6 mL/min. The column was reequilibrated for 1 minute between injections. Sorafenib and N-(4-phenoxyphenyl)-N'-phenylurea were measured by using positive ion electrospray with selected reaction monitoring (SRM) of the transitions from mass-to-charge ratios 465 to 252 and from 305 to 186, respectively. The SRM dwell time was 10 ms per transition. Samples were analyzed in triplicate.

Tissue Histopathological Assessment

After removal of representative specimens for LC-MS/MS analysis, the remainder of treated left lobes was fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological analysis. The reviewing pathologist (G.G.) was blinded to the procedure performed, and specimens were evaluated with the intent of ensuring that the sorafenib solution, which contained a low concentration of ethyl alcohol, did not exhibit immediate hepatotoxic properties. Stained sections were examined at low power to identify any regions of gross lobular ballooning degeneration or coagulative necrosis. Ballooning degeneration is a form of hepatocyte injury characterized by cellular enlargement and paleness as a result of the presence of irregular wispy or clumpy cytoplasm (13). This was followed by high-power examination of hepatic lobules with particular attention to zone 3 hepatocytes, central veins, and large bile ducts. Hepatocyte ballooning degeneration, when present, was graded as absent, mild, moderate, or severe, and tissue necrosis, when present, was estimated by visual inspection and expressed as a percentage of liver area for each slice.

Statistical Analysis

Statistical analysis was implemented using a commercially available statistics program (SPSS Statistics version 17.0; SPSS, Chicago Illinois). Results are reported as means, ± standard deviation.

RESULTS

Sorafenib solution was easily prepared in 6–12-mg/mL concentrations. Sorafenib chemoembolization was successfully performed in all five rabbits (Fig 1) and selective administration was performed from the left hepatic artery in all five cases. A mean of 6.4 mg ± 3.8 of sorafenib was administered, and a mean of 0.85 mL ± 0.7 of ethiodized oil was injected (Table). Postprocedure CT showed targeted left hepatic lobe chemotherapy emulsion delivery in all cases (Fig 1).

Tissue harvesting and processing was successfully performed in all five chemoembolized rabbits. LC-MS/MS analysis of the tissue specimens for sorafenib measurement was technically successful in all cases (Table, Fig 2). Mean overall tissue sorafenib concentration was $94.2 \mu\text{g/mL} \pm 48.3$ among all five rabbits. Mean left medial and left lateral lobe tissue sorafenib concentrations were $92.2 \pm 46.8 \mu\text{g/mL}$ and $96.3 \pm 55.1 \mu\text{g/mL}$, respectively. Variability between sorafenib levels in the left medial and left lateral liver segments was $9\% \pm 7$ (range 1%–17%) (Table). Tissue histopathological examination showed only mild ($n = 3$) or moderate ($n = 2$) non-specific ballooning degeneration in zone 3 hepatocytes (Table, Fig 3). No tissue necrosis was observed. Of note, histologic findings in the control rabbit treated with bland oily embolization comprised hepatocyte ballooning degeneration. These findings were in line with histopathologic changes reported in the literature, namely hepatocellular degeneration (14), as well as the findings observed in the sorafenib chemoembolization cohort.

DISCUSSION

The efficacy of transarterial chemoembolization is based at least in part on the induction of tumoral hypoxia, which both prompts ischemic tumor necrosis and facilitates intracellular transit of chemotherapeutic agents (15). Although tumor response rates after chemoembolization are generally favorable, treatment may be incomplete in as many as 40% of cases, with such tumors showing partial necrosis (16). In these instances, residual cancer cells are able to contribute to the angiogenic diathesis that may play a role in the inception of HCC recurrence. Hypoxia stimulates HCC angiogenesis by promoting transcription of VEGF (17), a potent mitogen that facilitates cellular migration during angiogenesis. Clinically, increased serum VEGF levels have been shown to correlate with poor prognostic outcomes after chemoembolization (18), and histopathological analyses of embolized tumors have demonstrated an increase in proliferative activity within residual neoplastic cells (19). Likewise, a retrospective analysis of HCC patients treated with transarterial embolization revealed that partial tumor necrosis not only increased the risk for HCC recurrence, but was also associated with worse survival when compared with patients in whom complete necrosis or no necrosis was achieved (20).

Sorafenib is a multikinase inhibitor approved by the United States Food and Drug Administration for treatment of unresectable HCC. This drug appears to exert its effects by abrogating neovascularization, and targets VEGFR among multiple receptor tyrosine kinases (6, 21). Patients with advanced HCC treated with sorafenib were shown to have a median survival benefit of 3 months over placebo in the Sorafenib HCC Assessment Randomized Protocol trial (8), and a recent metaanalysis of three major sorafenib trials (21) demonstrated prolongation in time to progression and overall survival. Unfortunately, significant side effects of sorafenib include skin toxicity (rash and hand-foot syndrome), gastrointestinal toxicity (nausea and diarrhea), and fatigue (15); these adverse outcomes are frequently cited as reasons for dose limitation and noncompliance with therapy. To this point, a recent study assessing the combination of oral sorafenib and drug-eluting bead chemoembolization for treatment of advanced HCC required 40 sorafenib dose interruptions and 25 sorafenib dose reductions due to drug side effects among 35 patients treated with 128 total therapeutic cycles over median 71 days (22). The current study thus aimed to translate the high local

drug concentrations and low systemic drug levels conferred by targeted transarterial chemoembolization (23) toward intra-hepatic delivery of sorafenib, with the notion that such administration could potentially reduce unfavorable systemic side effects.

In this investigation, transarterial sorafenib chemoembolization was successfully performed in a rabbit liver model, and effective high local drug delivery was confirmed using LC-MS/MS analysis methodologies. An approximately 3-mg/kg prescribed dose resulted in tissue sorafenib levels greater than 90 $\mu\text{g/mL}$, which is significantly higher than the C_{max} achieved with typical oral systemic sorafenib dosing (400 mg twice daily) applied clinically (10,11). These findings demonstrate successful proof of concept for targeted transarterial sorafenib delivery, and form a basis for future continued exploration of an approach that has the potential to offer a powerful adjuvant to the armamentarium of intraarterial therapies currently offered by interventional radiologists; sorafenib administration via an intraarterial route may limit propagation of tumor cells after chemoembolization by curtailing the angiogenic process spurred by therapeutic embolization. This method may theoretically temper pathways leading to HCC recurrence, and thereby potentiate the effectiveness of chemoembolization therapy. Additionally, the intraarterial administration route has the hypothetical advantage of delivering high doses of sorafenib that could not otherwise be tolerated via the conventional oral route, while reducing systemic drug levels, thereby potentially reducing undesirable systemic side effects. From a clinical standpoint, it is envisioned that sorafenib could be added to a conventional chemoembolization drug cocktail containing other chemotherapeutic agents including anthracycline (eg, doxorubicin), platinum (eg, carboplatin) and aziridine (eg, mitomycin) agents, to suppress tumor angiogenesis from the time of HCC embolotherapy.

Conventional chemoembolization was selected for sorafenib delivery in the present study based on its widespread use as the most commonly employed transarterial therapeutic technique worldwide (24), as well as its perceived adaptability to addition of new drugs into a polychemotherapy emulsion cocktail. As a result of the hydrophobic nature of sorafenib, preparation of a chemoembolization emulsion in this study required use of a solvent solution consisting of a low volumetric concentration of a polyethoxylated castor oil and ethanol, a previously developed and employed mixture (6). In administering these agents as part of the sorafenib chemotherapeutic emulsion, no directly attributable immediate toxic tissue effects were found. Histologic analysis of treated tissue specimens demonstrated nonspecific mild hepatocyte degenerative changes located near central veins, which may be observed after tissue devascularization with bland ethiodized oil embolization (14); no overt tissue necrosis was identified, and parenchymal changes were similar to those seen in a control animal embolized with bland 12.5% Cremophor EL, 12.5% ethyl alcohol, and 75% distilled water solution mixed with ethiodized oil. Interestingly, use of ethanol-based therapeutic emulsions has precedent in the interventional oncology literature: Gu et al (25) safely treated HCC in 15 patients using transarterial delivery of an ethiodized oil and ethanol solution mixed in a 1:1 volumetric ratio.

There are several limitations to the present investigation. First, this is a small, preliminary feasibility study that utilized nontumorous liver parenchyma for sorafenib chemoembolization. Use of a tumor model, such as VX2 carcinoma, would provide a system

more comparable to human HCC, and may further increase preferential deposition and local levels of sorafenib, as cancerous tissue siphons arterially injected therapeutic agent in a 3:1–20:1 ratio as compared with normal liver parenchyma (26). Second, empiric intraarterial sorafenib dosing based on peak and therapeutic plasma concentrations was applied herein. Third, assessment of intrahepatic chemotherapy agent content and tissue histological alterations was performed at only a single time point, without prospective temporal analysis. The acute nature of the histopathologic analysis herein therefore does not rule out toxic effects being observed at later times. Fourth, circulating systemic plasma levels of sorafenib were not assessed. Fifth, the current study did not attempt to investigate the effects of sorafenib chemoembolization on tissue HIF-1 α levels.

In conclusion, the present study confirms technical feasibility of sorafenib chemoembolization in rabbits. The findings herein indicate that intrahepatic sorafenib concentrations are significantly higher than the levels typically achieved with oral systemic sorafenib dosing, without resulting in immediate histopathologic hepatotoxicity beyond that typically seen in embolotherapy procedures. The ability to deliver sorafenib from an intraarterial approach may potentially counteract the hypoxia-induced angiogenesis and tumor growth after interventional oncologic transcatheter embolotherapy procedures, while possibly reducing the adverse effects of systemic sorafenib administration.

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Figure 1.

Sorafenib chemoembolization and CT imaging. Left hepatic arteriogram (a) from rabbit liver sorafenib chemoembolization shows catheter tip (arrowhead) at left, where 8 mg sorafenib was administered. Subsequent CT image in same rabbit (b) reveals left hepatic lobe (white arrowheads) distribution of radiopaque chemotherapy emulsion (black arrowheads), without nontarget right hepatic lobe (arrows) delivery.

Figure 2. LC-MS/MS tissue sorafenib analysis. SRM chromatogram from LC-MS/MS analysis of left hepatic lobe tissue from the same rabbit shown in Figure 1 demonstrates sorafenib peak elution at approximately 0.4 minutes. Area-under-curve analysis yielded sorafenib concentration of $132.9 \mu\text{g/mL} \pm 10.9$ in the treated left hepatic lobe medial segment.

Figure 3.

Histopathologic assessment of left hepatic lobe tissue from the same rabbit in Figures 1 and 2. Low-magnification image (a) shows mild nonspecific zone 3 hepatocyte ballooning degeneration, evidenced by relative cellular pallor (arrowheads) surrounding central vein (arrow). (Hematoxylin and eosin stain; original magnification, $\times 2.5$.) Higher magnification (b) better demonstrates hepatocyte ballooning degeneration (arrowheads), evidenced by cellular enlargement and pallor. (Hematoxylin and eosin stain; original magnification, $\times 20$.)

Table 1**Tissue Sorafenib Concentration and Histologic Analysis**

Rabbit*	Sorafenib Dose (mg)	Ethiodized Oil Volume (mL)	Histologic Alterations	Sorafenib Level (µg/mL)	
				MS	LS
1	3	0.5	Zone 3 mild degeneration	51.1 ± 9.1	44.1 ± 3.7
2	8	2	Zone 3 mild degeneration	132.9 ± 10.9	160.6 ± 2.3
3	12	1	Zone 3 moderate degeneration	152.4 ± 3.3	151.3 ± 6.7
4	6	0.5	Zone 3 moderate degeneration	60.8 ± 2.6	68.6 ± 3.1
5	3	0.25	Zone 3 mild degeneration	63.6 ± 3.4	60.6 ± 6.7

Values presented as means ± standard deviation where applicable.

MS = medial segment, LS = lateral segment

* All animals received treatment to the left liver lobe.