Research Article

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# Sorafenib attenuates monocrotaline-induced sinusoidal obstruction syndrome in rats through suppression of JNK and MMP-9

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**Background & Aims**: Sinusoidal obstruction syndrome (SOS) is a drug-induced liver injury that occurs with oxaliplatin treatment and is associated with postoperative morbidity after hepatectomy. The aim of this study was to investigate the effects of sorafenib in a monocrotaline (MCT)-induced model of SOS in rats.
**Methods**: Rats were divided into groups treated with sorafenib (2 mg/kg) or vehicle, 36 h and 12 h before MCT (90 mg/kg) administration by gavage. Liver tissues and blood were sampled 48 h after MCT administration to evaluate SOS. Survival after hepatectomy was examined and immunohistochemistry and electron microscopy were performed to assess sinusoidal injury.
**Results**: In the vehicle group, liver histology showed sinusoidal dilatation, coagulative necrosis of hepatocytes, endothelial damage of the central vein, and sinusoidal hemorrhage. In the sorafenib group, these changes were significantly suppressed, total SOS scores were significantly decreased, and the elevation of serum transaminase levels observed in the vehicle group was significantly reduced. Survival after hepatectomy was significantly higher in the sorafenib group compared to the vehicle group (45% *vs.* 20%, $p = 0.0137$). Immunohistochemistry and electron microscopy revealed a protective effect of sorafenib on sinusoidal endothelial cells at 6 h after MCT treatment. Sorafenib also attenuated the activity of metallopeptidase-9 (MMP-9) and phosphorylation of c-Jun N-terminal kinase (JNK).
**Conclusions**: Sorafenib reduced the severity of MCT-induced SOS in rats through suppression of MMP-9 and JNK activity, resulting in improvement of survival after hepatectomy.


Keywords: Sorafenib; Sinusoidal obstruction syndrome; MMP-9; JNK.
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*Abbreviations:* SOS, sinusoidal obstruction syndrome; SORA, sorafenib; MCT, monocrotaline; VHC, vehicle; MMP-9, matrix metallopeptidase-9; JNK, c-Jun N-terminal kinase; CRLM, colorectal liver metastasis; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; Alb, albumin; TEM, transmission electron microscopy; SEM, scanning electron microscopy; SEC, sinusoidal endothelial cell; RECA-1, rat endothelial cell antigen 1; p-JNK, phosphor-c-Jun N-terminal kinase; SD, the space of Disse; ECM, extracellular matrix.

## Introduction

The liver is a frequent site of metastasis for colorectal cancer and spread to the liver is associated with poor prognosis. Unlike systemic therapy, hepatectomy offers the potential for cure to a subset of colorectal cancer patients with limited liver metastases [1]. Adam *et al.* reported that these chemotherapy regimens allowed 12.5% of patients with unresectable colorectal liver metastases (CRLM) to be rescued by liver surgery [2]. Karoui *et al.* found that 60–70% of patients who underwent initial curative hepatic resection for CRLM developed recurrent disease and 10–15% were candidates for repeated hepatic resection [3]. These findings suggest that cases of hepatectomy for CRLM with preoperative and/or postoperative chemotherapy will increase in the future.

Sinusoidal obstruction syndrome (SOS) is a drug-induced liver injury [4] that has mostly been associated with therapy for hematopoietic stem cell transplantation, with an incidence of up to 70% and mortality up to 67% [4,5]. Use of oxaliplatin in chemotherapy for advanced colorectal cancer has also recently been associated with SOS [6], and prolonged preoperative chemotherapy including oxaliplatin before hepatectomy for CRLM, may cause sinusoidal injury and increase morbidity [7,8]. Thus, prevention and treatment of SOS are required to improve the safety of hepatectomy and perioperative chemotherapy for CRLM. Several studies have shown that anticoagulants prevent hematopoietic stem cell transplantation-related SOS [9–11]. An increase in serum vascular endothelial growth factor (VEGF) has been correlated with development of SOS [12] and recent retrospective studies [13–15] have found attenuation of SOS by addition of bevacizumab, a monoclonal humanized antibody directed against VEGF, to oxaliplatin-based chemotherapy.

These results lead to the hypothesis that antiangiogenic agents have a protective role against SOS. Sorafenib is a multiple receptor tyrosine kinase inhibitor targeting Ras/Raf kinase that also inhibits certain tyrosine kinases such as VEGF receptor-2 (VEGFR-2), platelet-derived growth factor receptor β, and VEGFR-3. Sorafenib is used as a standard treatment for hepatocellular carcinoma and renal cell carcinoma, and the efficacy of sorafenib for colorectal cancer is currently under investigation in clinical trials. In addition to its anticancer action, several articles have shown effects of sorafenib in the liver, including an antifibrotic effect and the prevention of portal hypertension [16,17]. The purpose of this study was to investigate the impact of sorafenib in a monocrotaline (MCT)-induced SOS model in rats.

## Materials and methods

*Reagents*

MCT was purchased from Sigma Aldrich (St. Louis, MO) and used as a 10 mg/ml solution [18]. Sorafenib was obtained from commercial tablets (Nexavar®, 200 mg). After removal of the outer coat, the tablets were dissolved in a vehicle composed of Cremophol EL (Sigma Aldrich), ethanol, and water (1:1:6) [17].

*Animals*

Male Sprague–Dawley rats of 8–9 weeks of age and weighing 300 ± 50 g were obtained from SLC (Shizuoka, Japan). All experiments were approved by the animal research committee of Kyoto University. Animals received humane care according to NIH Guidelines for the Care and Use of Laboratory Animals.

*Experimental protocol*

MCT-treated rats were used as an experimental model of SOS [19,20]. The protocol is shown in Fig. 1. Rats were fasted for 12 h before oral administration of MCT (90 mg/kg), but allowed water *ad libitum*. Subsequently, rats were allowed food and water *ad libitum*. To evaluate the impact of sorafenib on SOS, the rats were divided into groups (n = 20 in each group) treated with sorafenib (2 mg/kg) or vehicle, orally by gavage, 12 h and 36 h before MCT treatment. A total of 4 mg/kg was given to each rat in the sorafenib group divided into administration at −36 h and −12 h, while rats in the vehicle group were administered the same amount of vehicle [16]. Histopathological changes at 48 h after MCT treatment in rats are similar to those in human SOS [20]. Thus, rats were sacrificed 48 h after MCT administration and blood and liver samples were collected. Some animals were sacrificed at earlier time points. To investigate the impact of preoperative sorafenib on hepatectomy for the SOS liver, 30% partial hepatectomy was performed in another 20 rats each in the sorafenib and vehicle groups 48 h after MCT treatment. These animals were monitored for 14 days, and those that lived until the end of this period were considered to be survivors.

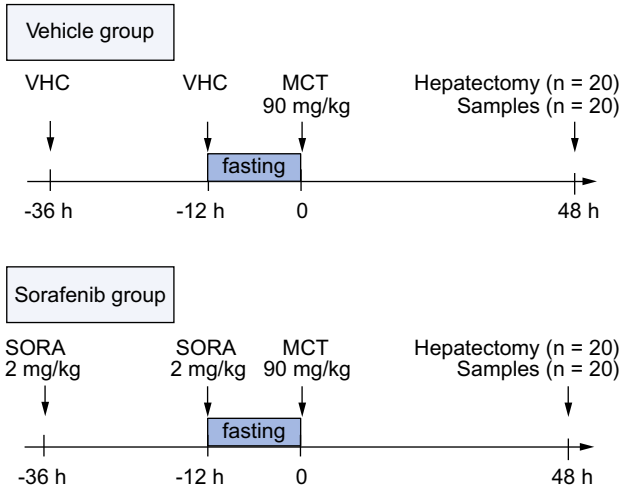


**Fig. 1. Experimental protocol for assessment of sinusoidal obstruction syndrome (SOS) and 30% partial hepatectomy.** MCT, monocrotaline; VHC, vehicle; SORA, sorafenib.

*Serum biochemistry*

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil) and albumin (Alb) levels were evaluated in serum samples.

*Liver histology*

Liver tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4 μm. The slides were stained with hematoxylin and eosin and histological assessment of SOS was performed blindly by a pathologist (A.M-H.). Histological changes were reviewed for sinusoidal dilatation, coagulative necrosis of hepatocytes, endothelial damage of the central vein, and sinusoidal hemorrhage [19,20]. Each of these features was graded on a 4-point scale: 0 = absent; 1 = mild; 2 = moderate; 3 = severe. The total SOS score was calculated as the sum of individual scores. Areas of necrosis were measured using Image J (National Institutes of Health) in 10 randomly selected images per low power field (magnification 100×).

*Electron microscopy*

Fixation of liver samples was performed as previously reported [21]. Sections were observed by transmission electron microscopy (TEM) (Hitachi H-7650) and scanning electron microscopy (SEM) (Hitachi S-4700).

*Assessment of injury to sinusoidal endothelial cells (SECs)*

Assessment of damage in sinusoidal endothelial cells (SECs) was performed by immunostaining for rat endothelial cell antigen 1 (RECA-1: MCA-970R, Sterotec, Oxford, UK) [20,21]. The staining area was morphometrically quantified using Image J. The total area of the endothelial cells was calculated from randomly selected images per high power field (magnification 200×).

*Zymography*

Matrix metallopeptidase-9 (MMP-9) activity was measured using a gelatin zymography kit (Primary Cell Co., Sapporo, Japan) [22]. After sample (20 μg) electrophoresis, gels were washed, incubated, stained, and destained according to the manufacturer's instructions. The intensity of each gelatinolytic band was quantified using Scion Image software (Scion Corp., Frederick, MD).

*Western blot analysis*

Western blot analysis was conducted as previously reported [21] using primary antibodies recognizing c-Jun N-terminal kinase (JNK) (#9252; Cell Signaling), phosphor-JNK (p-JNK) (#9251; Cell Signaling), and β-actin (sc-47778; Santa Cruz Biotechnology Inc., Santa Cruz, CA) at 1:1000 dilution. The intensity of the bands was quantified with Scion Image software. Relative ratios of p-JNK were calculated by dividing densitometric values of p-JNK by those of JNK.

*Quantification of liver tissue pyrroles*

Liver tissue pyrrole content was examined using a previously reported method [23].

*Statistical analysis*

Data are expressed as means ± SD. Differences in measured variables between each group were assessed by Student *t*-test. The probability of survival was calculated with Kaplan–Meier method and examined by log-rank test. $p < 0.05$ was