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Sorafenib prolongs liver regeneration after hepatic resection in rats

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ABSTRACT

Background: The multikinase inhibitor sorafenib inhibits angiogenesis and tumor cell proliferation. Sorafenib targets signaling pathways involved in liver regeneration. Previous works on regenerating mouse liver show differing results. We asked to which degree different lengths of sorafenib treatment would influence liver regeneration after hepatic resection in rats.

Methods: Fischer-344 rats received intragastric injections of sorafenib (5–15 mg/kg/d), underwent a two-thirds partial hepatectomy (PH), and were sacrificed at different time points thereafter. Sorafenib treatment was stopped 0, 3, or 14 d after PH. Serum levels of aminotransferases and labeling indices of S-phase nuclei (bromodeoxyuridine and MIB-5) were analyzed, body and liver weights measured, and levels of phospho-ERK determined by Western blot.

Results: Sorafenib increased aminotransferases and the number of S-phase nuclei at baseline, but decreased liver weights and levels of phospho-ERK 24 h after PH. The number of S-phase nuclei and mitotic indices decreased 48 h after PH and increased 7 d after PH in animals on sorafenib treatment. Relative liver weights were restored 5 d after PH in control rats, at 7 d in animals receiving sorafenib prior to surgery, at 10 d in rats where sorafenib was stopped 3 d after surgery, and after 14 d in rats on continuous treatment.

Conclusions: In this rat model, the regenerating liver adapted to the proliferation-inhibitory effect of sorafenib during continuous treatment. Sorafenib given after hepatic resection did not completely inhibit liver regeneration, but it prolonged the regenerative phase in proportion to the length of treatment.

1. Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide [1], and the incidence of HCC in the western world is rising [2]. Patients with HCC diagnosed at an early stage may be subject to potentially curative therapies, such as hepatic resection, transplantation, or local ablation. However, in spite of increased ultrasound surveillance of patients with liver cirrhosis, the majority of tumors are still diagnosed at an intermediate or advanced stage [3]. Intermediate-stage HCCs may be treated with transarterial chemoembolization [4], whereas for advanced

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HCC the only treatment proven to prolong survival is systemic treatment with the multikinase inhibitor sorafenib [5]. Sorafenib inhibits both angiogenesis and tumor cell proliferation by receptor tyrosine kinases, such as VEGFR (vascular endothelial growth factor receptor), PDGFR-β (platelet-derived growth factor-beta) and the serine/threonine RAF kinases [6].

In general, HCCs are resistant to conventional chemotherapy, and therefore the efficacy of adjuvant chemotherapy is poor [7]. Sorafenib has been suggested as an adjuvant treatment for early HCC in conjunction with curative therapies, as a means to prolong survival [8]. One concern has been that patients receiving multitargeted therapies could experience impaired liver regeneration after surgery, since sorafenib targets the same signaling pathways that are involved in normal liver regeneration [9–11]. This aspect was recently investigated by Hora *et al.*, who studied the effect of sorafenib on murine liver regeneration up to 5 d after partial hepatectomy [12]. They demonstrated an inhibitory effect on liver regeneration in animals receiving sorafenib after liver resection, while no effect was seen if the treatment was stopped 1 d before surgery. In contrast, in a recent paper by Kurniali *et al.*, no inhibiting effect on liver weight, DNA synthesis, or cellular proliferation was seen if sorafenib was given after liver resection [13]. Thus, previous results are conflicting. The aim of the present study was to elucidate to which degree treatment with sorafenib would impair liver weight gain and/or hepatocyte proliferation if it was administered during the whole regenerative process, until regeneration is complete. We used a model in which rats were subjected to a two-thirds partial hepatectomy [14]. Sorafenib was given 1 wk prior to partial hepatectomy to ensure a steady state of drug metabolism, and then for different time periods after the surgical event. Liver tissue was analyzed at different time points from 16 h up to 14 d after surgery.

2. Methods

2.1. Animals and treatment procedures

Male Fischer-344 rats weighing 150 g were housed in a 12-h light-dark cycle where temperature, humidity, and ventilation were controlled according to international standards. They were fed with standard pellet chow and water *ad libitum*. Four animals were kept in each cage that was enriched with wood chips and PVC tubes. Animals were allowed to recover from transportation and adapt to the new environment for 1 wk prior to treatment.

Sorafenib tosylate (BAY 54-9085; Bayer HealthCare Pharmaceuticals, Montville, NJ) was dissolved in a 50% Cremophor EL (Sigma Cat. No 5135) 50% ethanol mixture diluted with water (12.5% Cremophor EL/12.5% ethanol/75% water) and administered daily in a volume of 250 μL/100 g body weight (for concentrations used in the study, see below) by oral gavage. Control animals received Cremophor-ethanol/water liquid in the same volume but without sorafenib. After 1 wk treatment with sorafenib or control solvent, animals were subjected to a two-thirds partial hepatectomy (PH) under general anesthesia with isoflurane. A midline laparotomy was performed, the two largest lobes of the liver were mobilized, a ligature was adapted around the vessels of the two largest lobes, the vessels were ligated, the liver lobes were removed, and the peritoneum and the wound sutured. At the day of PH, sorafenib was given 2 h prior to surgery. The uridine analogue bromodeoxyuridine (BrdU, B-5002; Sigma-Aldrich, Stockholm, Sweden) was injected intraperitoneally (100 mg/kg body weight) 2 h before sacrifice. Animals were weighed daily and their general condition monitored.

At sacrifice, animals were anesthetized with isoflurane, the abdomen was cut open, and blood was drawn from the aorta. Serum samples were secured for analyses of aminotransferase measurements and liver tissue prepared for immunohistochemistry as described below.

2.2. Experimental design

Two sets of experiments were performed.

2.2.1. Study 1

First, a short-term experiment using three different doses of sorafenib (5, 10, and 15 mg/kg body weight/d) was conducted to investigate possible dose-response relationship and toxic effects on the liver, in order to determine the dose for the long-term study (Fig. 1A). Animals were sacrificed in conjunction with PH and then at 16, 20, and 24 h after surgery. Each control and treatment group included four animals at each time point (total $n = 64$).

2.2.2. Study 2

In the long-term experiment (1–3 wk, Fig. 1B) the dose of sorafenib 5 mg/kg/d was chosen, since it lacked toxic effects but still decreased liver weight gain in the short-term experiment. The long-term experiment included one control group and three treatment groups ($n = 160$). Animals in the control group received Cremophor-ethanol 1 wk before surgery and 2 wk post PH ("Controls"). The first treatment group received sorafenib 1 wk prior to PH, and after surgery they were shifted to Cremophor-ethanol until sacrifice ("Sorafenib pre-PH"). The second treatment group was treated with sorafenib 1 wk before PH and 3 d after PH, at which time treatment was shifted to Cremophor-ethanol until sacrifice ("Sorafenib pre- and 3 d post-PH"). The third treatment group was treated with sorafenib 1 wk before PH and continuously until sacrifice up to 2 wk after surgery ("Sorafenib pre- and post-PH"). Animals were sacrificed in each group at 16, 20, 24, 48, and 72 h and at 5, 7, 10, and 14 d post surgery ($n = 4$/time point/group). These time points were chosen because rat livers display a maximum proliferative activity 18–72 h after partial hepatectomy, and regeneration is fully complete 7–14 d after PH [14]. Animals in the "Sorafenib pre- and 3 d post-PH" group were not sacrificed at 48 and 72 h, since they received the same treatment as animals in the "Sorafenib pre- and post-PH" group at these time points.

2.3. Tissue preparations

At sacrifice the liver was removed and weighed, cut into slices, fixed in phosphate-buffered 4% formaldehyde, embedded in paraffin, and stained with hematoxylin-eosin, periodic acid–Schiff (PAS), and Sirius (collagen staining). Additional