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Hepatic regeneration in a rat model is impaired by chemotherapy agents used in metastatic colorectal cancer

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Abstract

Purpose: Administering Oxaliplatin prior to resection of colorectal liver metastases often induces a Sinusoidal Obstruction Syndrome (SOS), which can affect postoperative patient outcome. Bevacizumab (Anti-VEGF-A) can decrease the severity of SOS and the associated risk of postoperative liver failure. We investigated the impact of both Oxaliplatin (Oxali) and Bevacizumab on liver regeneration in a rat model.

Material and methods: Male Wistar rats underwent a 70% partial hepatectomy (PH) 3 days after a 2 ml intraperitoneal injection of either saline (controls, n = 17), or Oxaliplatin 10, 20 or 50 mg/kg, 5-Fluorouracil 100 mg/kg (5-FU) and Bevacizumab 5 or 10 mg/kg in various combinations (total 98 rats, 11 groups, n = 5–18/group). Liver regeneration was assessed by remnant liver weight recovery and cell proliferation by immunodetection of BrDU incorporation (days 1, 2, 3, 7). Hepatic mRNA expression levels of VEGF-A and of its 2 receptors (Flt-1 and KDR) were quantified by PCR technique.

Results: Liver regeneration was impaired for 3 days post PH by Oxali 20 alone and Oxali 10 + 5-FU, without any rescue effect by neither Bevacizumab 5 nor 10 mg/kg. Unlike in humans, there were no sinusoidal changes. VEGF-A mRNA expression and receptor 2 (KDR) expressions decreased 24 h post PH in a similar fashion in controls, Oxali 20 and Oxali 10 + 5-FU groups. All groups had recovered over 60% of their liver weight by day 7.

Conclusion: Oxaliplatin causes early hepatocyte proliferation impairment post PH, unaffected by Bevacizumab and unexplained by changes in VEGF-A signalling in a Wistar rat model.

Keywords: Chemotherapy; Liver regeneration; Bevacizumab

Introduction

Patients suffering from colorectal adenocarcinoma have or will develop liver metastases in 60% of the cases.[1] Liver resection remains the standard treatment for patients with resectable colorectal liver metastases (CLRM) and is the only single-modality therapy associated with cure. A five-year-survival rate after liver resection of CLRM as high as 58% has been reported.[2–4] Only a minority (15–30%) of patients suffering from CLRM have metastases that are resectable at the time of diagnosis.[5,6] For others, a chemotherapy neoadjuvant treatment for tumour downstaging or downsizing is necessary prior to resection.[7] A response rate of 54–56% is obtained after treatment with 5-fluorouracil (5-FU) combined either to Oxaliplatin, a platinum derivative, or to Irinotecan, a topoisomerase I inhibitor.[8–10] However, these chemotherapeutic agents may induce toxic side-effects on the non-tumoral liver parenchyma, potentially leading to liver dysfunction or defective

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hepatic regeneration post resection. Oxaliplatin is known to cause sinusoidal damage in the form of a Sinusoidal Obstruction Syndrome (SOS),[11] whilst Irinotecan can induce steatohepatitis, associated with 15% perioperative mortality following liver resection, mainly linked to liver failure.[12] In Oxaliplatin-induced SOS, hepatic sinusoids are dilated associated to extravasation into centrolobular hepatic zones, leading to portal hypertension in the most severe form, called Nodular Regenerative Hyperplasia (NRH).[13,14] Several authors have indeed reported an increased morbidity with liver failure after major hepatectomy, an increased need for transfusion and a longer hospital stay after liver resection in patients treated preoperatively with Oxaliplatin.[15–17] However, recent newly developed molecular targeted therapies such as Bevacizumab, a monoclonal antibody to vascular endothelial growth factor (VEGF), and Cetuximab, an anti-epidermal growth factor receptor (EGFR) appear promising. These drugs, usually administered in combination with cytotoxic agents, are reported to induce a cytostatic response rate reaching 70%.[18] In addition to its antitumoral effects, Bevacizumab has been shown to decrease both the severity of SOS[19–21] and the associated risk of postoperative liver failure.[22] Experimental studies have to date, however, yielded conflicting results regarding the impact of anti-VEGF therapies on liver regeneration.[23,24] In this study we investigated whether Oxaliplatin alone or combined to 5-FU had a direct impact on liver regeneration in a Wistar rat model, and whether Bevacizumab modified this effect.

Material and methods

Animals

In total, 98 male Wistar 6–7-week-old rats weighing 175–250 g (Centre d'élevage JANVIER, Le Genest-Saint Isle, France) were housed in our animal facility and were kept in a 12-h-light cycle, temperature and humidity controlled environment where they had ad libitum 24-h access to water and food. Animals were handled following the guidelines for humane care for laboratory animals established by the Université catholique de Louvain (UCL), in accordance with European regulations.

Chemotherapy

Three days prior to partial hepatectomy (day-3), following tail-blood collection, 98 rats were given an intraperitoneal (IP) injection of either chemotherapy diluted into 2 ml of saline or saline alone (controls, 2 ml) under light diethyl ether anaesthesia. There were 11 groups of rats (including 1 control group, n = 17), receiving Oxaliplatin (Oxali) 10 mg/kg alone (Oxali 10, n = 17), 20 mg/kg (Oxali 20, n = 18), or 50 mg/kg alone (n = 5), or 5-FU 100 mg/kg alone (5-FU 100, n = 6), Oxali 10 + 5-FU 100 (n = 7), Oxali 20 + 5-FU 100 (n = 5), Bevacizumab 5 mg/kg (Beva 5, n = 5) or 10 mg/kg (Beva 10, n = 6), Oxali 10 + 5-FU 100 + Beva 5 (n = 6), Oxali 10 + 5-FU 100 + Beva 10 (n = 6) (Fig. 1A).

Partial hepatectomy

After tail-blood collection, a partial hepatectomy (PH) 70% was performed (day 0) according to the method described by Higgins and Anderson.[25,26] A midline ventral abdominal incision was performed on anaesthetised animals, and, after mobilization of the liver, a ligature was tied around the pedicle (including vessels and bile ducts) of the anterior lobe (including left lateral and median lobes). This lobe was then removed and the abdomen was closed.

Animals were sacrificed 1, 2, 3 or 7 days after PH in the initial experiment. In following experiments, the analyses were performed at 24 h post PH time point. At time of sacrifice, blood was withdrawn by puncture of the inferior vena cava and the remnant posterior lobe (including right lateral and caudate lobes) was excised, weighed and sampled. Liver wedges were frozen in liquid nitrogen or fixed in paraformaldehyde for further analyses. BrdU was administrated IP at the dose of 50 mg/kg 2 h prior to sacrifice.

Clinical measurement and biological and specimen collection

Animals were weighed and blood samples from either the tail or inferior vena cava were taken at every step of the experimental procedures. Blood samples were centrifuged and stored until assayed. Aspartate aminotransferase (AST, mmol/l) and alanine aminotransferase (ALT, mmol/l) were measured in all blood samples.

Histology, immunochemistry, BrdU immunostaining

Paraffin liver 3 μm thick sections were stained with haematoxilin and eosin, using standard histological procedures. Morphological analysis included routine liver examination, mitosis count and assessment of sinusoidal dilation. In addition, sections were immunostained with 5-bromo-2-deoxyuridine (BrdU). BrdU positive cells were counted in at least five randomly selected high-power fields per slide.

Liver regeneration

Restitution of hepatic liver mass was determined as the percentage of regenerated liver mass calculated as follows:

Liver mass recovery (%) = 100 × (Weight of the posterior lobe at the time of final resection/estimated total liver weight), the estimated total liver weight being extrapolated from hepatectomized anterior lobes representing 70% of the liver mass. DNA synthesis was determined by