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Evaluation of anastomotic strength and drug safety after short-term sunitinib administration in rabbits

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ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form
7 October 2013
Accepted 9 October 2013
Available online 12 October 2013

Keywords:
Sunitinib
Sutent
Adhesions
Anastomosis
Bursting strength
Cardiotoxicity
Echocardiogram
Hepatotoxicity
Rabbit
Safety

ABSTRACT

Background: Sunitinib (Sutent) is a Food and Drug Administration–approved receptor tyrosine kinase inhibitor found to reduce postoperative adhesion formation in animal models. The objective of the present study was to evaluate anastomotic healing and potential drug-related toxicities after short-term sunitinib administration in New Zealand White rabbits.

Materials and methods: Under an approved study protocol, 40 rabbits underwent a laparotomy followed by colonic transection and anastomosis. Animals were randomly assigned to treatment with oral sunitinib (10 mg/kg/d) or placebo, received one preoperative dose followed by 10 postoperative doses, and were divided into two groups following the procedure: group I animals were euthanized on completion of drug treatment and group II animals were euthanized 30 d after completion of treatment. Prior to study completion, animals underwent an echocardiogram and laboratory test results were obtained. At necropsy, intestinal bursting strength (in mmHg) was evaluated.

Results: All animals survived until designated euthanasia. There was no evidence of intra-abdominal sepsis or intestinal obstruction. Sunitinib-treated animals were found to have lower intestinal anastomotic strength compared with placebo-treated animals, as measured by bursting pressure at euthanasia, and a greater percentage of bursting at the anastomosis. On echocardiography, all ejection and shortening fractions were within established normal reference values. There were no significant differences in liver enzymes between animals. There were no wound infections, dehiscence, or delayed wound healing in any animal.

Conclusions: These results caution against the administration of sunitinib in cases involving intestinal anastomoses because of the elevated risk of anastomotic leak. No evidence of cardiotoxicity, hepatotoxicity, or detrimental effect on wound healing was found in any animal.

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http://dx.doi.org/10.1016/j.jss.2013.10.016

1. Introduction

Sunitinib (Sutent, SU11248; Pfizer Labs, New York, NY) is a Food and Drug Administration–approved receptor tyrosine kinase inhibitor (TKI) recently found to reduce adhesion formation after surgical abrasion procedures in murine and leporine models [1–4]. Sunitinib is an orally active TKI with selectivity for vascular endothelial growth factor receptors 1, 2, and 3, platelet-derived growth factor receptors α and β, fms-like tyrosine kinase (FLT-3), colony-stimulating factor-1 receptor (CSF-1R), glial cell line-derived neurotrophic factor receptor RET, and stem cell factor receptor (KIT) [5,6]. It is currently approved for the treatment of imatinib-resistant gastrointestinal stromal tumors , metastatic renal cell carcinoma, and advanced pancreatic neuroendocrine tumors. Previous studies in our laboratory have demonstrated intra-abdominal adhesion reduction without any untoward effect after drug administration, detrimental effects on wound healing, or adverse effects on fertility or parturition in females who received sunitinib prior to pregnancy [2,4]. Nonetheless, TKIs are known to affect multiple signaling pathways including those involved in cell growth, invasion, metastasis, and angiogenesis [7]. Because of its selectivity on tyrosine kinases, distinct and potentially significant adverse events (e.g., cardiotoxic, hepatotoxic, teratogenic, and poor wound healing) can potentially occur with its use, often associated with the dose, timing, and duration of administration. The objective of the present study was to evaluate anastomotic healing and potential drug-related toxicities after short-term postoperative sunitinib administration in New Zealand White rabbits.

2. Materials and methods

The animal study protocol (#10-10-1822R) was approved by the Boston Children's Hospital Animal Care and Use Committee. Forty New Zealand White female rabbits (Millbrook Breeding Labs, Amherst, MA) weighing 2.5–3.2 kg were housed in single cages with *ad libitum* access to a standard pellet rabbit diet (Pro-Lab Hi-Fiber; LabDiet, St Louis, MO) and water. An initial weight was obtained on arrival. After 72 h of acclimatization, animals underwent a laparotomy followed by colonic transection and anastomosis. Animals were randomly assigned to treatment with either sunitinib (Sutent, SU11248) at 10 mg/kg/d ($N = 20$) or placebo ($N = 20$) with the first dose administered 1 d before the procedure. The dose of sunitinib was based on previous work [8]. Sunitinib was prepared in an oral suspension using a 1:1 ratio of Ora-plus and Ora-sweet (Paddock Laboratories, Inc, Minneapolis, MN). The placebo consisted of the 1:1 mixture of Ora-plus and Ora-sweet only.

2.1. Intestinal anastomosis procedure

Animals were anesthetized with inhaled isoflurane (1%–3% mixed with 1 L/min oxygen *via* mask) and given one preoperative intravenous dose of cefazolin (20 mg/kg). The abdomen was shaved, prepped with alcohol and betadine, and draped in the standard sterile fashion. A 3-cm lower midline incision was made and the abdomen entered. The proximal ascending colon was identified and the lumen was sharply and completely transected. An immediate end-to-end anastomosis was created using a single layer of interrupted 4-0 polydioxanone (PDS) sutures (Ethicon Inc, Somerville, NJ). After inspection for hemostasis, the colon was returned to the abdomen and the peritoneum and skin were closed using running 4-0 PDS and 4-0 Vicryl suture, respectively (Ethicon Inc, Somerville, NJ), respectively. At the completion of the operation, a 12-μg/h fentanyl patch (Mylan Pharmaceutical Inc, Morgantown, WV) was placed on the back for 72 h for postoperative analgesia. Following the procedure, animals received 10 daily doses of oral sunitinib (10 mg/kg/d) or placebo, beginning on the day of the operation. Animals were allowed *ad libitum* access to water postoperatively and standard food 24 h later. Signs and symptoms of anastomotic leak and intestinal obstruction (e.g., tachycardia, tachypnea, abdominal distension, lethargy, and decreased or absent urine or stool output) were closely monitored. Animals were randomly divided into groups postoperatively, which differed by the timing of euthanasia. Group I animals were euthanized on completion of drug treatment and group II animals were euthanized 30 d after completion of treatment, at which time necropsy was performed. Each group had 10 sunitinib-treated and 10 placebo-treated animals. Prior to study completion, animals were weighed and an echocardiogram and laboratory test results were obtained.

2.2. Echocardiogram

Animals were anesthetized with inhaled isoflurane (1%–3% mixed with 1 L/min oxygen *via* mask) and underwent an echocardiogram. Imaging was performed using a 12-MHz transducer and an ultrasound machine (model iE33, Philips). Left ventricular function was assessed by shortening fraction (SF) from short axis views, and ejection fraction (EF) using the 5/6 area $\times$ length method. Echocardiograms were performed by a cardiologist blinded to the treatment randomization.

2.3. Measurement of hepatic function

Approximately 2 mL of blood was obtained from an ear vein for the measurement of alanine transaminase (ALT), alkaline phosphatase (AP), total bilirubin (TB), and albumin (ALB) levels (Mammalian Liver Profile; VetScan, Union City, CA). Hematocrit (Hct) was additionally collected. Laboratory test results were obtained from 10 animals before treatment randomization as a baseline for comparison.

2.4. Intestinal anastomotic strength

Using a previously described and validated method [9,10], a 10-cm segment of colon with the anastomosis was isolated. An intestinal clamp was used to occlude the ends of the segment, and a 16-gauge angiocatheter was used to cannulate the proximal end through which 0.9% saline was infused. Pressure transducer tubing was inserted distal to the anastomosis and attached to a monitor (Surgivet V9212AR, Waukesha, WI). The bursting pressure (in mmHg) was defined as the maximum intraluminal pressure the segment resisted