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Anti-inflammatory liposomes have no impact on liver regeneration in rats

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HIGHLIGHTS

- Use of anti-CD163-dexamethasone is an attractive strategy for anti-inflammatory treatment.
- In the present study the impact of anti-CD163 dexamethasone on liver regeneration in rats was studied.
- We show that low dose anti-CD163 dexamethasone has no negative effect on liver regeneration after 70% hepatectomy in rats. Characters should then be down to 122.

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ABSTRACT

Introduction: Surgical resection is the gold standard in treatment of hepatic malignancies, giving the patient the best chance to be cured. The liver has a unique capacity to regenerate. However, an inflammatory response occurs during resection, in part mediated by Kupffer cells, that influences the speed of regeneration. The aim of this study was to investigate the effect of a Kupffer cell targeted anti-inflammatory treatment on liver regeneration in rats.

Methods: Two sets of animals, each including four groups of eight rats, were included. Paired groups from each set received treatment with placebo, low dose dexamethasone, high dose dexamethasone or low dose anti-CD163 dexamethasone. Subsequently, the rats underwent 70% partial hepatectomy. The two sets were evaluated on postoperative day 2 or 5, respectively. Blood was drawn for circulating markers of inflammation and liver cell damage; liver tissue was sampled for analysis of regeneration rate and proliferation index.

Results: The high dose dexamethasone group had significantly lower body and liver weight than the placebo and anti-CD163-dex groups. There were no differences in liver regeneration rates between groups. Hepatocyte proliferation was completed faster in the placebo group, although this was not significant. The anti-CD163-dex group showed increased blood levels of albumin and alanine aminotransferase and a diminished inflammatory response in terms of significantly reduced haptoglobin, α2-macroglobulin and Interleukine-6.

Conclusion: Low dose dexamethasone targeted to Kupffer cells does not affect histological liver cell regeneration after 70% hepatectomy in rats, but reduces the inflammatory response judged by circulating markers of inflammation.

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1. Introduction

The liver has a striking regenerative capacity after hepatic resection. Postoperative regeneration is mediated through an orchestrated proliferation of hepatocytes (not involving stem cells), resulting in enlargement of the residual liver lobes with restoration of liver mass [1]. This process is carried out at the same time as the liver maintains support for body homeostasis [1].

Surgical resection or transplantation, are the only potentially curative treatment options for most cases of hepatic malignancy [2]. Thus, the possible demand for therapeutic hepatectomy is enormous. For example, colorectal cancer is the third most prevalent cancer in the world [2]. Almost half of these patients will develop colorectal liver metastases during the course of their disease [3–5]. Untreated, their median survival is only 10 months [4], whilst 5-year survival is very rare [3,6]. Similarly, primary liver cancer (in most cases hepatocellular carcinoma) is globally the second most frequent cause of cancer death in men [7]. Again, surgery remains the only curative treatment for most cases.

Perioperative blood loss and transfusion requirements are factors known to be associated with the degree of morbidity and mortality following hepatic resection [8,9]. Vascular occlusion techniques such as Pringle's maneuver may be used in an attempt to limit hemorrhage [9]. However, the ischemic effect of this type of maneuver may induce a harmful inflammatory response known as ischemia-reperfusion (IR) injury [9,10].

From previous studies, Kupffer cells are known to be involved in the activation of cell division during the process of liver regeneration by the production of tumor necrosis factor (TNF)-α and interleukin (IL)-6 [1,11,12]. However, Kupffer cells are also known to be mediators of IR injuries [13–15].

Considerable research has been conducted on different approaches to enhance liver survival and viability after resection [9,12]. Focus has mainly been on surgical procedures such as ischemic conditioning [16] and intermittent clamping [17]. However more recently, attention has been directed at pharmacological strategies and positive results have been reported suggesting a protective effect of treatment with sevoflurane [18] and with prednisolone [19,20]. Against this backing, a possible new strategy could be anti-inflammatory therapy with the novel reagent anti-CD163-mAb conjugated PEGylated liposomes [21] that can be loaded with dexamethasone to form anti-CD163-dex-lipo. Anti-CD163-dex-lipo is directed against the CD163 receptor. CD163 is a hemoglobin scavenger receptor, highly expressed on macrophages in the liver, spleen and bone marrow, and at sites of inflammation [22].

Previously, it has been shown that anti-CD163-dex constructed as an antibody-drug conjugate (ADC) inhibits the inflammatory response of rat macrophages after injection with lipopolysaccharides (LPS) [22]. In addition, we showed that anti-CD163-dex ADC protects against IR injury after liver ischemia by inhibiting apoptosis [23]. The aim of the present study was to investigate the possible effect of Kupffer cell targeted anti-inflammatory treatment with anti-CD163-dex liposomes on liver regeneration in rats after partial hepatectomy (PHx).

2. Material and methods

2.1. Animals and ethics

All animal experiments were performed under the approval of Danish Animal Experiment Inspectorate, Copenhagen, Denmark (license number 2012-15-2934-00591 expansion), and in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institute of Health, USA [24]. Male Wistar rats of 200 g, corresponding to an age of app 50 days, were obtained from Taconic Biosciences (Borup, Denmark) and were acclimatized for one week prior to operation. The animals were housed in standard animal laboratories with the temperature maintained at 23 °C, an artificial 12 h light-dark cycle, and free access to food (Altromin) and water. The rats were daily monitored with regard to weight, behavior and physical appearance by veterinary nurses and the first author. All animals were daily scored by humane endpoint with 'General Distress Score' as described by Lloyd and Wolfensohn [25]. Briefly the rats were evaluated on the following endpoint: Appearance; Food and water intake; Clinical signs; Natural behavior and Provoked behavior. If a score of 2 was observed in any parameter the animals were even closer observed and attended to at minimum 8th hour intervals. If the condition was not remedied during 24 h the rat was euthanized by cervical dislocation. If a score of 3 was observed in any parameter the animals were euthanized immediately. Dead rats were autopsied to establish the cause of death. The species, sex and size/age, was chosen based on previous experience in the research group regarding studies in liver regeneration in rats [26,27].

2.2. Experimental design

2.2.1. Design

Sixty-four rats were given treatment, underwent 70% PHx, and were evaluated on postoperative day (POD) 2 or 5 (Fig. 1). The choice of evaluation days was based on a previously conducted study, which showed day 2 and 5 to be key points in rat liver regeneration [26].

2.2.2. Treatment

With the operator blinded to treatment, the animals were block randomized into four groups. They received either 1) placebo (phosphate buffered saline), 2) low dose dexamethasone-phosphate (LDD; 0.2 mg/kg), 3) high dose dexamethasone phosphate (HDD; 1.0 mg/kg), or 4) Anti-CD163-mAb conjugated liposome-encapsulated low dose dexamethasone-21-hemisuccinate (anti-CD163-dex; 0.2 mg/kg). The synthesis of anti-CD163-liposomes has been described previously [21] and remote loading with dexamethasone-21-hemisuccinate was performed as described in Ref. [28]. The conjugate was administrated by intravenous injection (2 ml/kg) to the tail vein 18 h before liver resection. For animals evaluated on POD 5, an additional dose of treatment was given at POD 2. The doses used in the present study, were based on experience from earlier studies conducted on dexamethasone and anti-CD163-dexamethasone [22,29].

2.2.3. Anesthetics and analgesia

General anesthesia with sevoflurane was used during administration of treatment, surgical procedures and at euthanisation. The animals were anesthetized in an induction chamber with a mixture of oxygen (2.0 L/min), N_2O (0.5 L/min) and 4% sevoflurane (Forene; Abbott Laboratories, Maidenhead, UK). During procedures, anesthesia was maintained with 3% sevoflurane in oxygen and N_2O as described above, which was administered through a mask covering the face of the rat. Before surgery, the animals were given a subcutaneous injection of a long-lasting non-steroid anti-inflammatory drug, 5 mg/kg Carprofen (RimadylVet; Pfizer Animal Health, Exton, USA) and 2.5 ml of isotonic saline. Injection of analgesics was repeated on POD 1, 2, and 3.

2.2.4. Surgical procedure

The animal was placed in a supine position on a temperature controlled heating pad, a midline abdominal incision was made and the liver was mobilized. PHx was performed as first described by *Higgins and Anderson* [30]. In brief, the median and left lateral lobes