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SHORT COMMUNICATION

Potential safety concerns of TLR4 antagonism with irinotecan: a preclinical observational report

Janet K. Coller[1] · Joanne M. Bowen[2] · Imogen A. Ball[2] · Hannah R. Wardill[3] · Ysabella Z. A. van Sebille[2] · Romany L. Stansborough[3] · Zenab Lightwala[3] · Anthony Wignall[2] · Joseph Shirren[2] · Kate Secombe[2] · Rachel J. Gibson[3,4]

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Abstract
Purpose Irinotecan-induced gut toxicity is mediated in part by Toll-Like receptor 4 (TLR4) signalling. The primary purpose of this preclinical study was to determine whether blocking TLR4 signalling by administering (−)-naloxone, a TLR4 antagonist, would improve irinotecan-induced gut toxicity. Our secondary aim was to determine the impact of (−)-naloxone on tumour growth.
Methods Female Dark Agouti (DA) tumour-bearing rats were randomly assigned to four treatments ($n = 6$ in each); control, (−)-naloxone (100 mg/kg oral gavage at −2, 24, 48, and 72 h), irinotecan (175 mg/kg intraperitoneal at 0 h), and (−)-naloxone and irinotecan. Body weight and tumour growth were measured daily, and diarrhoea incidence and severity were recorded 4× per day up to 72 h post-treatment.
Results At 72 h, all rats that received irinotecan lost weight compared to controls ($p = 0.03$). In addition, rats that received (−)-naloxone and irinotecan lost significantly more weight compared to controls ($p < 0.005$) than irinotecan only compared to controls ($p = 0.001$). (−)-Naloxone did not attenuate irinotecan-induced severe diarrhoea at 48 and 72 h. Finally, (−)-naloxone caused increased tumour growth compared to control at 72 h ($p < 0.05$) and significantly reduced the efficacy of irinotecan ($p = 0.001$).
Conclusions (−)-Naloxone in our preclinical model was unable to block irinotecan-induced gut toxicity and decreased the efficacy of irinotecan. As (−)-naloxone-oxycodone combination is used for cancer pain, this may present a potential safety concern for patients receiving (−)-naloxone-oxycodone and irinotecan concurrently and requires further investigation.

Keywords Irinotecan · TLR4 antagonist · Gut toxicity · Efficacy

Introduction

Chemotherapy-induced gut toxicity (CIGT) occurs in >50% of cancer patients, necessitating treatment reductions and/or treatment breaks [1]. Unfortunately, these patients have twice the infection risk leading to a 4-fold higher chance of death and 3-fold longer hospital stays, compromising survival and creating a burden on patients' quality of life [2]. Economically, CIGT also adds substantial healthcare costs with US data estimating a combined cost of $15,500 for each hospitalisation due to severe CIGT [3]. Patients with CIGT typically experience severe consequences including, but not limited to, oral mucositis [4], increased infection rates [5], and diarrhoea [6]. Currently, CIGT cannot be accurately predicted and is without an effective intervention.

TLR4 signalling is vital for the maintenance of epithelial homoeostasis within the gut [7]. Homoeostasis of the gut is carefully balanced by interactions between the resident microflora, epithelial barrier function, and the mucosal

✉ Janet K. Coller
janet.coller@adelaide.edu.au

[1] Discipline of Pharmacology, Adelaide Medical School, University of Adelaide, North Terrace, Adelaide, SA 5005, Australia

[2] Discipline of Physiology, Adelaide Medical School, University of Adelaide, Adelaide, Australia

[3] Discipline of Anatomy and Pathology, Adelaide Medical School, University of Adelaide, Adelaide, Australia

[4] Division of Health Sciences, University of South Australia, Adelaide, Australia

immune system [8]. Our previous research has clearly demonstrated CIGT disrupts the delicate balance of these three factors leading to a substantial inflammatory response [9] and changes in both epithelial turnover [10] and microflora characteristics [11]. TLR4 expression changes in response to chemotherapy [12]. It is activated in response to both the tissue damage generated as a consequence of treatment, and the invading pathogens from epithelial barrier breakdown due to gut toxicity [12].

Irinotecan is a commonly used chemotherapy agent that inhibits the topoisomerase 1 enzyme leading to irreversible DNA damage [13]. Briefly, irinotecan is administered as a pro-drug and is subsequently hydrolysed to its active metabolite SN-38 that is then detoxified in the liver and converted to the inactive SN-38-glucuronide. However, due to enterohepatic recirculation SN-38-glucuronide is converted back to the active SN-38 by β-glucuronidase, an enzyme produced by some bacteria in the gut. Importantly, we have shown irinotecan increases bacteria that produce β-glucuronidase in the gut [11].

Previously, we have shown up-regulation of TLR4, IL-1β, and TNF gene expression in the colon of animals with severe CIGT that was not evident in animals with mild CIGT [14]. More recently, we have demonstrated TLR4 knockout (KO) mice have significantly less weight loss ($p = 0.0003$) and diarrhoea ($p = 0.0001$) following irinotecan than their wild-type counterparts [15]. Taken together, this provides strong evidence that TLR4 plays a key role in the initiation and subsequent development of CIGT.

(−)-Naloxone is an opioid antagonist used clinically to treat effects of opioid overdose and is a highly effective TLR4 inhibitor [16]. (−)-Naloxone can be dosed orally, with significant concentrations achieved at the level of the gut lumen, but minimal systemic exposure owing to extensive and almost complete first-pass hepatic metabolism. (−)-Naloxone has a significant advantage over other TLR4 antagonists as it can be rapidly translated to clinical use for CIGT. Therefore, we hypothesised that TLR4 activation is the key driver of CIGT that is targetable for clinical management. The aim of this study was to determine the impact of blocking TLR4, using (−)-naloxone, on CIGT severity following irinotecan. Our secondary aim was to determine the impact of (−)-naloxone on tumour growth.

Materials and methods

Animals and ethics

Female Dark Agouti (DA) rats, weighing between 130 and 190 g, were used for this study. Rats were housed in Perspex cages at a temperature of 22 ± 1 °C and subject to a 14 h light/10 h dark cycle. Animals had ad libitum access to autoclaved chow and water. Experimental design was approved by the Animal Ethics Committee of The University of Adelaide and complied with the National Health and Medical Research Council (Australia) Code of Practice for Animal Care in Research and Teaching (2014).

Experimental design

Twenty-four rats were randomly assigned to one of the following treatment groups: control, (−)-naloxone, irinotecan, or irinotecan + (−)-naloxone ($n = 6$ each group). All rats received breast cancer inoculum subcutaneously (s.c.) as described previously [17], and tumours were allowed to grow for one week prior to administration of irinotecan. Tumour-bearing animals were used in this study to define the effect of (−)-naloxone on both the tumour and the cytotoxicity of irinotecan.

(−)-Naloxone or vehicle (water) was administered via oral gavage at a dose of 100 mg/kg 2 h prior to irinotecan or vehicle administration and every 24 h thereafter for 72 h. All rats received 0.03 mg/kg s.c. atropine (to reduce the cholinergic reaction) immediately prior to administration of either 175 mg/kg intraperitoneal (i.p.) irinotecan (kindly supplied by Pfizer), or vehicle (sorbitol/lactic acid buffer: 45 mg/mL sorbitol/0.9 mg/mL lactic acid, pH 3.4, previously shown to have no gut toxicity effects [17]) at time 0 h. Rats were killed using 3% isoflurane in 100% O_2 anaesthesia and cervical dislocation 72 h post-irinotecan treatment.

Tumour analysis

Tumour growth was measured daily using digital calipers and assessed as a percentage of body weight.

Gut toxicity assessment

Gut toxicity was assessed through weight loss and the occurrence of diarrhoea. Animals were weighed daily at the same time, and total weight loss/gain recorded. Diarrhoea occurrence and severity was recorded 4× daily according to previous grading [17]: 0, no diarrhoea; 1, mild diarrhoea (staining of anus); 2, moderate diarrhoea (staining spreading over top of legs); and 3, severe diarrhoea (staining over legs and abdomen, often with continual anal leakage). All gut toxicity assessments were conducted in a blinded fashion.

Statistics

All data for tumour growth, diarrhoea, and weight loss were compared to baseline. Comparisons of tumour growth, diarrhoea severity scores, and percentage weight loss over

72 h between treatment groups were performed using Kruskal–Wallis or one-way ANOVA tests as appropriate. GraphPad Prism version 5.01 (GraphPad Software Inc, La Jolla, USA) was used for all statistical analysis. All data are presented as either median (range) or mean ± SEM unless otherwise stated, and $p < 0.05$ was considered statistically significant.

Results

(−)-Naloxone does not improve CIGT following irinotecan

Rats receiving irinotecan had a significant weight loss at 72 h compared to controls (−4.8%, Fig. 1, $p = 0.03$). Rats that received (−)-naloxone and irinotecan lost significantly more weight compared to controls ($p < 0.005$) than irinotecan only compared to controls (−6.9%, Fig. 1, $p = 0.001$). In addition, irinotecan caused severe diarrhoea at 48 and 72 h that was not attenuated by (−)-naloxone.

(−)-Naloxone increased breast tumour growth

(−)-Naloxone caused increased tumour growth compared to control at 72 h, with relative tumour sizes (% body weight) of 6.8 and 4.5, respectively (Fig. 2a, $p < 0.05$). In addition, (−)-naloxone significantly reduced the efficacy of irinotecan, with a significant increase in relative tumour growth as % body weight (normalised to baseline), 239 versus 142% when irinotecan was given alone (Fig. 2b, $p = 0.001$).

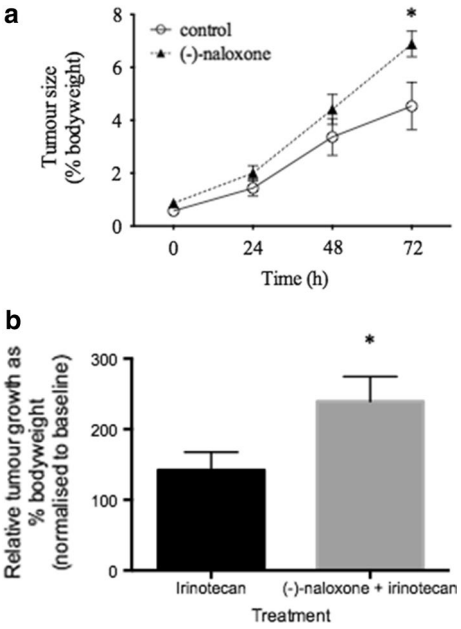

Fig. 2 **a** Tumour growth as percentage (%) body weight from baseline over the 72 h period in rats receiving control and (−)-naloxone, *$p < 0.05$; and **b** relative tumour growth as percentage (%) body weight (normalised to baseline) at 72 h in rats receiving irinotecan and (−)-naloxone and irinotecan, *$p = 0.001$

Discussion

Our study has revealed for the first time that (−)-naloxone is not effective in reducing CIGT following irinotecan in our tumour-bearing rat model. In addition, and of potential clinical significance, is the observation that (−)-naloxone when given alone increased breast cancer tumour growth, and when given in combination with irinotecan, significantly reduced irinotecan efficacy. The mechanism underlying this interaction is currently unknown; however, previous studies have implicated a role for TLR4 signalling in tumour growth. For example, TLR4 absence in a knockout mouse model was associated with increased tumour growth during treatment with oxaliplatin and doxorubicin [18]. Further, patients with a TLR4 genetic deficiency had an increased incidence of metastases 5 years after surgery for breast cancer [18]. Consequently, (−)-naloxone may be

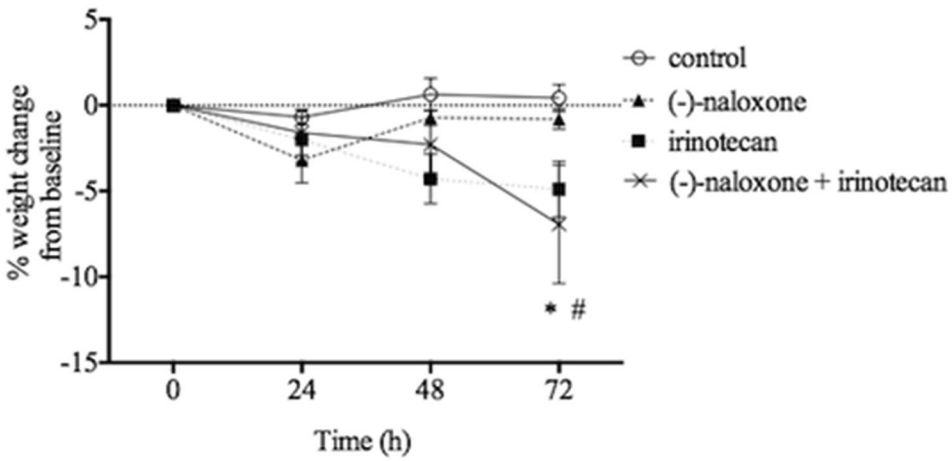

Fig. 1 Percentage (%) change in body weight from baseline over the 72 h study period in rats receiving control, (−)-naloxone, irinotecan, or (−)-naloxone and irinotecan. *$p = 0.03$ versus control and #$p = 0.001$ versus control

exerting its effect through inhibition of TLR4 signalling. Our observations indicate a need for further studies investigating the impact of (−)-naloxone on efficacy of irinotecan and other chemotherapeutics especially given the increasing clinical use of (−)-naloxone, in combination with oxycodone, for cancer pain.

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Compliance with ethical standards

Conflict of interest Professor Rachel Gibson is a current consultant/advisory board member to Mundipharma (Singapore).

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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