BJP British Journal of Pharmacology

DOI:10.1111/bph.12719
www.brjpharmacol.org

COMMENTARY

Interleukin-18 as a target for modulation of irinotecan-induced intestinal toxicity: a step towards a better therapeutic index?: Commentary on Lima-Junior *et al.*, Br J Pharmacol 171: 2335–2350

B G Spyropoulos

First Department of Propaedeutic Surgery, School of Medicine, University of Athens, Athens, Greece

Correspondence
Vasileios G Spyropoulos, National and Kapodistrian University of Athens, School of Medicine, First Department of Propaedeutic Surgery, Hippokration Hospital, 114 Vasilissis Sofias Avenue, Athens 11527, Greece. E-mail: bill.spiropoulos@hotmail.com

Keywords
chemotherapy-induced mucosal injury; mucositis; diarrhoea; irinotecan; interleukin-18

Received
26 March 2014
Accepted
3 April 2014

LINKED ARTICLE

This article is a Commentary on Lima-Júnior RCP, Freitas HC, Wong DVT, Wanderley CWS, Nunes LG, Leite LL, Miranda SP, Souza MHLP, Brito GAC, Magalhães PJC, Teixeira MM, Cunha FQ and Ribeiro RA (2014). Targeted inhibition of IL-18 attenuates irinotecan-induced intestinal mucositis in mice. Br J Pharmacol 171: 2335–2350. doi: 10.1111/bph.12584

Abbreviations

IFN-γ, interferon-gamma; IL, interleukin; NF-κB, nuclear factor kappa B; SN38, 7-ethyl-10-hydroxycamptothecin; TNF, tumour necrosis factor; UGT-1A1, uridine diphosphate glucuronosyltransferase-1A1

This is a Commentary on an article in BJP by Lima-Júnior RC *et al.*, 2014; 171: 2335–2350. Lima-Júnior *et al.* evaluate the effects of the interleukin 18 (IL-18) inhibition in the pathogenesis of irinotecan-induced intestinal mucositis. The results demonstrated that genetic deletion or neutralization of IL-18 and, to a lesser extent, caspase-1 genetic deletion could down-regulate the inflammatory responses and their consequences. These observations offer a clear impetus for further investigation into the role of IL-18 in mucositis arising on the ground of cytotoxic chemotherapy.

The elimination of systemic toxicity while attempting to maximize tumour response has been the Holy Grail of oncological research for as long as cytoablative chemotherapy has been employed. Although the advent of colony-stimulating factors has substantially improved the management of haematopoietic dysfunction and enabled dose-escalation, much less progress has been made on the prevention and the treatment of gastrointestinal toxicities. In this context, intestinal mucositis and diarrhoea are among the most common and potentially severe non-haematological complications encountered in cancer patients undergoing chemotherapy (Naidu *et al.*, 2004; Stein *et al.*, 2010). While these toxicities are closely related, and to some extent inseparable, they arise from different events having synergistic and interdependent effects on each other, and also rely on the concurrent immunological state, as well as the gut's indigenous flora.

Chemotherapy affects the integrity of the intestinal mucosa through depletion of the rapidly proliferating crypt stem cells, which impairs epithelial replacement and compromises mucosal barrier function. Once the mucosal barrier is disrupted, various luminal antigens penetrate the lamina propria and stimulate resident macrophages and other antigen-presenting cells to secrete several cytokines, thereafter determining the differentiation of $CD4^+$ T lymphocytes (Th0) into either Th1/Th17 or Th2 effector cells and the production of pro-inflammatory or anti-inflammatory cytokines. Pro-inflammatory cytokines mediate the transmural infiltration of phagocytic leukocytes and the release of an array of biologically active compounds, which coordinate the inflammatory response. Taken together, the reduced absorbent surface area, and the accompanying mucosal inflammation and intestinal motility alterations, have all been implicated as the mechanisms responsible for the manifestation of diarrhoea.

The chemotherapeutic agent irinotecan is notorious for its propensity to induce unpredictable, and often very severe, diarrhoea, having a clinical impact greater than its other major toxic effect, myelosuppression. While immediate diarrhoea is of little clinical significance as it can be readily controlled by atropine, delayed diarrhoea does not respond well to conventional management and demonstrates considerable interpatient pharmacokinetic variability. In brief, irinotecan is a prodrug that undergoes de-esterification by carboxylesterases to yield its active metabolite (SN-38), which is a potent topoisomerase-I inhibitor. Subsequently, SN-38 is detoxified in the liver by the polymorphic enzyme UGT-1A1 to SN-38-glucuronide and both metabolites are eliminated by biliary excretion. However, the reverse reaction can also take place in the intestinal lumen by bacterial-derived β-glucuronidase activity, which also represents the current focus of research efforts (Mathijssen *et al*., 2001). The development of delayed diarrhoea is generally ascribed to the direct cytotoxic effects of the intraluminal SN-38 on the mucosa resulting in villous atrophy, crypt hypoplasia, goblet-cell hyperplasia and increased apoptosis (Stein *et al*., 2010). Several lines of evidence have demonstrated that irinotecan activates NF-κB, and up-regulates the production of pro-inflammatory cytokines, TNF, IL-6 and IL-1β, which are responsible for the initiation and amplification of mucositis (Logan *et al*., 2008). Receptor nomenclature follows Alexander *et al*. (2013).

Among the cytokines, IL-18 has been identified by its ability to induce IFN-γ production, thus driving predominantly the Th1-polarization pattern. Caspase-1 converts the precursor of IL-18 to its mature protein, whereas IL-18 binding protein (IL-18bp) blocks its bioavailability and function (Dinarello *et al*., 2013). Until now, the most relevant data regarding the pro-inflammatory effects of IL-18 in mucosal inflammation have been obtained from studies of inflammatory bowel diseases (Reuter and Pizarro, 2004); however, they may also hold true in the case of chemotherapy-induced mucositis.

An experimental study by Lima-Júnior *et al*. (2014) published in the *British Journal of Pharmacology* provides interesting insights into the role of IL-18 in the pathogenesis of irinotecan-induced enteropathy. The investigators initially studied the effects of irinotecan in caspase-1 and IL-18 knockout mice comparing them with their wild-type controls, and demonstrated that both caspace-1 and IL-18 genetic deletion exerted a preventive effect on mucosal morphometry, reduced neutrophil accumulation and NO synthesis, and diminished duodenal hypercontractility. Along with these structural and functional modifications, a significantly improved survival of IL-18 gene-deleted mice was also observed, further confirming the significance of IL-18 in the inflammatory response. However, genetic deletion of caspace-1 did not prevent the occurrence of diarrhoea, and was also associated with marked epithelial vacuolation. This phenomenon was attributed to the fact that caspace-1 only partially cleaves the IL-18 precursor into its active molecule. When this is taken into account, along with the observed apoptotic cells in the mucosa, another possible explanation could be that IL-18 is released independently by stimulation mediated by an activated Fas ligand (Dinarello *et al*., 2013).

In the second part of the study, the authors evaluated whether inhibiting the activity of IL-18 by exogenous supplementation of IL-18 binding protein ameliorates the severity of irinotecan-induced mucositis. The results demonstrated that IL-18 inhibition rendered the intestinal mucosa significantly chemoresistant to irinotecan injury in almost all of the parameters examined, apart from survival. One of most intriguing findings of this study was the absence of nitrotyrosine immunoexpression despite the increased neutrophil accumulation and up-regulation of iNOS. Activated neutrophils generate and release large amounts of superoxide anion (O_2^-), which in turn react with NO to form the highly reactive peroxynitrite ($ONOO^-$), a powerful oxidative agent. Although the authors proposed that this injury mechanism cannot be excluded, as peroxynitrite also degrades proteins in other ways, it is more likely that oxidative/nitrosative stress is not a critical mediator of irinotecan-induced mucositis, at least at this stage. Although there is still no direct evidence for the mechanism of irinotecan, it is well established that most chemotherapeutics functionally impair neutrophils, as further indicated by suppressed O_2^- production (Hara *et al*., 1990). Furthermore, there is evidence that NO is involved in other aspects of irinotecan-induced enteropathy besides being a mediator of tissue damage. Indeed, it has been demonstrated that irinotecan-induced diarrhoea is also accompanied by NO-induced chloride secretion, which is mediated by thromboxane A2 (Sakai *et al*., 2002). It is also noteworthy that, despite the conspicuously improved histological, inflammatory and functional indices in irinotecan-injected IL-18 knockout mice in comparison with their respective disease control, the difference in IFN-γ expression was negligible. This finding may also imply a shift to a Th2 cytokine profile and deserves further investigation, although it should be noted that the IFN-γ levels in irinotecan-injected wild-type mice were extremely varied (Dinarello *et al*., 2013).

The paper by Lima-Júnior *et al*. (2014) raises an important as well as difficult clinical problem; the narrow therapeutic index of irinotecan, coupled with the paucity of adequate surrogate markers of toxicity, limits the therapeutic effectiveness of this important agent. Even though the originality of the study is undeniable, both from a context and methodological point of view, the ambiguity regarding the functions of IL-18 is expected to spark debate about the possible extrapolation of these findings to the cancer situation. In

particular, the decreased or abolished synthesis of IL-18 in human colon adenocarcinomas (ironically the most common application of irinotecan) has been linked with distant metastases and an unfavourable outcome (Pagès *et al.*, 1999). These antitumour effects are believed to be mediated through IFN-γ, and Fas ligand-dependent cytotoxicity Moreover, IL-18 may trigger the secretion of granulocyte-macrophage colony-stimulating factor, and contribute to the proliferation of haematopoietic cells (Nakanishi *et al.*, 2001).

From another point of view, the amelioration of the absorption and the elimination of the increased capillary permeability induced by targeting IL-18 pro-inflammatory responses would prevent irinotecan's diffusion across the mucosal layer, thereby reducing its detrimental effects at this location. Theoretically, this approach may constitute an interesting alternative strategy for preventing the intestinal toxicity induced by irinotecan, as it does not interfere with irinotecan's complex pharmacokinetics. Of course, further and more targeted research into the pleiotropic role of IL-18 in cancer and inflammation is required, and the study by Lima-Junior *et al*. will be a valuable contribution to this topic, which is of paramount interest.

Acknowledgements

The author gratefully acknowledges the assistance of Mr Quentin Mackrell during the preparation of the paper.

Conflict of interest

The author declares that he has no competing interests.

References

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, Peters JA, Harmar AJ, CGTP Collaborators (2013). The Concise Guide to Pharmacology 2013/14: Catalytic Receptors. Br J Pharmacol 170: 1676–1705.

Dinarello CA, Novick D, Kim S, Kaplanski G (2013). Interleukin-18 and IL-18 binding protein. Front Immunol 4: 289.

Hara N, Ichinose Y, Motohiro A, Kuda T, Aso H, Ohta M (1990). Influence of chemotherapeutic agents on superoxide anion production by human polymorphonuclear leukocytes. Cancer 66: 684–688.

Lima-Júnior RC, Freitas HC, Wong DV, Wanderley CW, Nunes LG, Leite LL *et al*. (2014). Targeted inhibition of interleukin-18 attenuates irinotecan-induced intestinal mucositis in mice. Br J Pharmacol 171: 2335–2350.

Logan RM, Gibson RJ, Bowen JM, Stringer AM, Sonis ST, Keefe DM (2008). Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: implications for the pathobiology of mucositis. Cancer Chemother Pharmacol 62: 33–41.

Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G *et al*. (2001). Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). Clin Cancer Res 7: 2182–2194.

Naidu MU, Ramana GV, Rani PU, Mohan IK, Suman A, Roy P (2004). Chemotherapy-induced and/or radiation therapy-induced oral mucositis – complicating the treatment of cancer. Neoplasia 6: 423–431.

Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001). Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev 12: 53–72.

Pagès F, Berger A, Henglein B, Piqueras B, Danel C, Zinzindohoue F *et al*. (1999). Modulation of interleukin-18 expression in human colon carcinoma: consequences for tumor immune surveillance. Int J Cancer 84: 326–330.

Reuter BK, Pizarro TT (2004). Commentary: the role of the IL-18 system and other members of the IL-1R/TLR superfamily in innate mucosal immunity and the pathogenesis of inflammatory bowel disease: friend or foe? Eur J Immunol 34: 2347–2355.

Sakai H, Suzuki T, Murota M, Takahashi Y, Takeguchi N (2002). Nitric oxide-induced Cl- secretion in isolated rat colon is mediated by the release of thromboxane A2. J Physiol 543: 261–271.

Stein A, Voigt W, Jordan K (2010). Chemotherapy-induced diarrhea: pathophysiology, frequency and guideline-based management. Ther Adv Med Oncol 2: 51–63.