Toxicology in Vitro 41 (2017) 75–82

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Role of Toll-like receptor 4 in drug-drug interaction between paclitaxel and irinotecan in vitro

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ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form 10 February 2017
Accepted 23 February 2017
Available online 27 February 2017

Keywords:
Paclitaxel
Irinotecan
Toll-like receptor 4
Drug-drug interactions
Drug metabolizing enzymes

ABSTRACT

The bacterial receptor, Toll-like receptor (TLR) 4 mediates inflammatory responses and has been linked to a broad array of diseases. TLR4 agonists are being explored as potential treatments for cancer and other diseases. We have previously shown that activation of TLR4 by lipopolysaccharide (LPS) leads to down-regulation of drug metabolizing enzymes/transporters (DMETs), and altered pharmacokinetics/pharmacodynamics (PK/PD) of drugs. These changes can increase the risk of drug-drug interactions (DDIs) in patients on multiple medications. Clinically, DDI was observed for combination chemotherapy of paclitaxel (TLR4 ligand) and irinotecan. To determine the role of TLR4 in DDI between paclitaxel and irinotecan in vitro, primary hepatocytes from TLR4-wild-type (WT) and mutant mice were pre-treated with paclitaxel, followed by irinotecan. Gene expression of DMETs was determined. Paclitaxel treatment increased the levels of irinotecan metabolites, SN-38 and SN-38 glucuronide (SN-38G) in TLR4-dependent manner. Paclitaxel-mediated induction of genes involved in irinotecan metabolism such as *Cyp3a11* and *Ugt1a1* was TLR4-dependent, while induction of the transporter *Mrp2* was TLR4-independent. These novel findings demonstrate that paclitaxel can affect irinotecan metabolism by a TLR4-dependent mechanism. This provides a new perspective towards evaluation of marketed drugs according to their potential to exert DDIs in TLR4-dependent manner.

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1. Introduction

Toll-like receptor 4 (TLR4) is a transmembrane receptor that detects components of microbial pathogens and plays a critical role in innate immunity (Marshak-Rothstein, 2006). Emerging genetic data also support the association of TLR4 with several diseases (Bochud et al., 2009; de Oliveira and Silva, 2012). Thus, activating or suppressing TLR4 provides access to a new generation of therapeutics (Manthey et al., 1993; Thoelen et al., 2001; Mullarkey et al., 2003; Ziakas et al., 2013).

Currently, TLR4 agonists are being explored extensively as immunomodulators and vaccine adjuvants for allergic diseases, cancers and infectious diseases (Hawkins et al., 2002; Evans et al., 2003; Lee et al., 2003; Przetak et al., 2003; Stover et al., 2004; Cluff et al., 2005; Krieg, 2006; Kanzler et al., 2007; Casella and Mitchell, 2008). Additionally, medications including opioid agonists (Hutchinson et al., 2010a; Hutchinson et al., 2010b) and widely-used chemotherapy drug, Paclitaxel (Byrd-Leifer et al., 2001; Zimmer et al., 2008) have been reported to activate TLR4 signaling in macrophages. Studies show that TLR4 signaling enhances resistance to paclitaxel therapy in breast and ovarian cancers (Rajput et al., 2013; Wang et al., 2014), and contributes to paclitaxel-induced peripheral neuropathy (Li et al., 2014).

Marked antitumor activity of paclitaxel in ovarian and breast cancer has led to its extensive evaluation as combination therapy with other cytotoxic agents for various metastatic cancers (Bellmunt et al., 2000; Kondagunta et al., 2005; Grau et al., 2009; Li et al., 2011). However, pharmacokinetics (PK) drug-drug interactions (DDIs) pose major clinical problem with such combination therapy. In a Phase-I study, DDI was observed in patients with advanced small non-small cell lung cancer (NSCLC) (Hotta et al., 2004) treated with paclitaxel and the potent anticancer drug, irinotecan. In this study, pre-treatment of paclitaxel, followed by irinotecan, increased the area under curve (AUC) of the toxic metabolite of irinotecan, 7-ethyl-10-hydroxycamptothecin (SN-38) (Kasai et al., 2002).

The plasma level of both paclitaxel and irinotecan is determined by drug metabolism and disposition. Paclitaxel is an auto inducer, and

Abbreviations: PXR, Pregnane X receptor; LPS, Lipopolysaccharide; TLR4, Toll-like receptor 4; DMETs, Drug metabolizing enzymes and transporters; CES, Carboxylesterase; CYP, Cytochrome P450; SN-38, 7-Ethyl-10-hydroxycamptothecin; SN-38G, SN-38 glucuronide; UGT1A1, Uridine diphosphate glucuronosyltransferases 1A1; APC, 7-Ethyl-10[4-N-(5-aminopentanoicacid)-1-piperidino] carbonyloxycamptothecin; NPC, 7-Ethyl-10[4-amino-1 piperidino]carbonyloxycamptothecin; DDIs, Drug-drug interactions; MRP, Multidrug resistance-associated protein; Pgp, P-glycoprotein; TNF, Tumor necrosis factor; MDR, Multidrug resistance; IL, Interleukin.

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http://dx.doi.org/10.1016/j.tiv.2017.02.019
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induces drug metabolizing enzymes (DMEs) and transporters involved in its disposition (Nallani et al., 2003). It undergoes metabolism via cytochrome P450 (CYP) 2C8 (Rahman et al., 1994; Dai et al., 2001) and 3A4 (Cresteil et al., 1994; Harris et al., 1994); with biliary excretion via MDR1B (also known as P-glycoprotein [Pgp]) (Monsarrat et al., 1993; Kang et al., 2001). Irinotecan, a topoisomerase I inhibitor, is metabolized by two pathways (Fig. 1) (i) bio activation by carboxylesterase (CES) to form active and toxic metabolite, SN-38 (Satoh et al., 1994; Slatter et al., 1997) which is detoxified to SN-38 glucuronide (SN-38G) by uridine diphosphate glucuronosyltransferases (UGT) 1A1; (ii) oxidation by CYP3A4/5 enzyme (Santos et al., 2000) to form APC (7-ethyl-10[4-N-(5-aminopentanoicacid)-1-piperidino] carbonyloxycamptothecin) and NPC (7-ethyl-10[4-amino-1-piperidino]carbonyloxycamptothecin). NPC is converted to SN-38 by CES. Irinotecan and its metabolites are predominantly eliminated in the bile. Accumulation of SN-38 in the intestine is primarily due to deconjugation of SN-38G to SN-38 by bacterial β-glucuronidase, which accounts for life-threatening diarrhea (Sparreboom et al., 1998; Buajordet et al., 2001).

We have previously shown that activation of TLR4 by lipopolysaccharide (LPS) downregulate DME/transporter (DMET) genes (Ghose et al., 2009) leading to altered PK/pharmacodynamics (PD) of drugs (Gandhi et al., 2012). We observed that downregulation of DMETs by LPS was associated with reduced expression of the xenobiotic nuclear receptor (NR), pregnane X receptor (PXR) (Ghose et al., 2004a). PXR forms a dimer with the central NR, retinoid X receptor (RXR)-alpha to regulate DMET genes. It is well-established that alterations in DMET expression/activity can cause DDIs (Kohler et al., 2000; Polasek et al., 2011). Since TLR4 is activated by paclitaxel, we hypothesized that paclitaxel will cause TLR4-mediated alterations in DMETs, leading to changes in irinotecan PK. We found that in TLR4-wild type (WT) hepatocytes, paclitaxel increased the levels of SN-38 and SN-38G, while no such induction was observed in TLR4-mutant hepatocytes. Gene expression of *Cyp3a11* and *Ugt1a1* was induced up to ~600 and ~4 fold respectively by paclitaxel in TLR4-WT hepatocytes. In the TLR4-mutant hepatocytes *Cyp3a11* was induced ~300-fold by paclitaxel, while *Ugt1a1* expression was not increased. Thus, our finding proposes a novel mechanism of regulation of DDIs by TLR4 and indicates two possible unwanted effects due to TLR4 involvement in regulation of DMEs, (i) SN-38 levels may be affected in individuals with TLR4 polymorphism, resulting in changes irinotecan efficacy and safety; and (ii) TLR4 agonists pose potential for DDIs when given as combination therapy with other drugs.

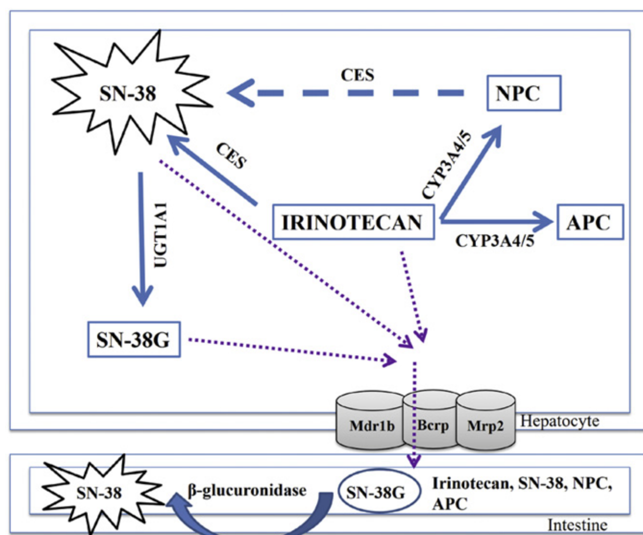

Fig. 1. Schematic representation of the metabolic pathway of irinotecan.

2. Materials and methods

2.1. Chemicals

Camptothecin (CPT; internal standard (I.S.)), Irinotecan hydrochloride (Cat # I1406), Paclitaxel (Cat # T7402) and 4-Nitrophenyl acetate (PNPA; Cat # N8130) were purchased from Sigma-Aldrich, St. Louis, MO. SN-38 and SN-38G were kind gifts from Dr. Ming Hu's laboratory at the University of Houston, Houston, TX. The sequences of the primers and probes were reported in our previous publications (Ghose et al., 2007, 2008, 2009). Bicinchoninic acid (BCA) assay kit (Cat # 23225) was purchased from Thermo Fisher Scientific Inc. All other reagents for real-time PCR were purchased from Applied Biosystems (Foster City, CA). Liquid chromatography mass spectrometry (LCMS) grade solvents were purchased from VWR international, LLC (Suwanee, GA, USA) for chromatography. Unless specified, all other materials were purchased from Sigma-Aldrich (St Louis, MO, USA.).

2.2. Animals

Adult, male, 6–8 week old C3HeB/FeJ (TLR4-wildtype; TLR4-WT) and C3H/HeJ (TLR4-mutant) mice were purchased from Jackson Labs, Bar Harbor, ME. The C3H/HeJ mice are homozygous mutants that have spontaneous mutation in TLR4 gene as a result of a missense mutation (proline → histidine) at codon 712 (Poltorak et al., 1998). TLR4-mutant mice exhibit a defective response to LPS stimulation. They are genetically similar to TLR4-WT (C3HeB/FeJ) mice but display significantly reduced expression of pro-inflammatory genes compared TLR4-WT mice (Ghose et al., 2008). All animals were maintained in a 12 h dark/light cycle and a temperature-and-humidity-controlled environment. The mice had access to rodent chow ad libitum. All the animal care and use protocols were approved by the Institutional Animal Care and Use Committee guidelines. All experiments were performed in triplicate and repeated at least three times.

2.3. Primary mouse hepatocyte culture

Primary hepatocytes were isolated from adult male mice according to the two-step perfusion procedure, as described previously (Li and Koda, 2002; Ghose et al., 2011; Shah et al., 2014) with some modifications (Ghose et al., 2016). Cell viability was measured using trypan exclusion method. Cells plated at a density of 250,000 cells/ml in six-well Primaria plates (BD, Franklin Lakes, NJ) were allowed to attach for 4 h in Williams E medium (Invitrogen) containing the following; 10,000 U/ml of Penicillin/streptomycin solution (Invitrogen), 200 mM of L-glutamine, 5 mg of gentamicin, 5 μg/ml Insulin-transferrin-sodium selenite [ITS], 4 ng/ml glucagon and 10% fetal bovine serum [FBS] (Invitrogen). Hepatocyte preparations with >85% viability were used in experiments. Cells were maintained for 48 h with a daily change of medium.

2.4. Cell treatments

After 48 h in culture, on the day of treatment, primary mouse hepatocytes were incubated with serum-free Williams E treatment media 2 h prior to treatment with drugs. For gene expression studies, cells were incubated with vehicle (<0.1% DMSO) or paclitaxel for 4 and 24 h. The assay was terminated by addition of 0.25 ml of cold TRIzol reagent to each well. Cells were harvested for RNA isolation for real-time PCR analysis. For DDI studies, cells were pretreated with either vehicle or 20 μM paclitaxel for 24 h to induce DMEs, followed by addition of irinotecan (20 and 50 μM) or SN-38 (5 μM) for additional 48 h. Supernatants were collected at different time points till 48 h. The concentrations of SN-38 and SN-38G were quantified by LCMS/MS. All experiments were performed in triplicate.

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