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Original Article

Transporter-mediated interaction of indican and methotrexate in rats

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ABSTRACT

Indican (indoxyl-β-D-glucoside) is present in several Chinese herbs e.g. *Isatis indigotica*, *Polygonum tinctorium* and *Polygonum perfoliatum*. The major metabolite of indican was indoxyl sulfate (IS), an uremic toxin which was a known substrate/inhibitor of organic anion transporter (OAT) 1, OAT 3 and multidrug resistance-associated protein (MRP) 4. Methotrexate (MTX), an important immunosuppressant with narrow therapeutic window, is a substrate of OAT 1, 2, 3, 4 and MRP 1, 2, 3, 4. We hypothesized that IS, the major metabolite of oral indican, might inhibit the renal excretion of MTX mediated by OAT 1, OAT 3 and MRP 4. Therefore, this study investigated the effect of oral indican on the pharmacokinetics of MTX. Rats were orally given MTX with and without indican (20.0 and 40.0 mg/kg) in a parallel design. The serum MTX concentration was determined by a fluorescence polarization immunoassay. For mechanism clarification, phenolsulfonphthalein (PSP, 5.0 mg/kg), a probe substrate of OAT 1, OAT 3, MRP 2 and MRP 4, was intravenously given to rats with and without a intravenous bolus of IS (10.0 mg/kg) to measure the effect of IS on the elimination of PSP. The results indicated that 20.0 and 40.0 mg/kg of oral indican significantly increased the area under concentration–time curve$_{0\text{-}t}$ ($AUC_{0\text{-}t}$) of MTX by 231% and 259%, prolonged the mean residence time (MRT) by 223% and 204%, respectively. Furthermore, intravenous IS significantly increased the $AUC_{0\text{-}t}$ of PSP by 204% and decreased the Cl by 68%. In conclusion, oral indican increased the systemic exposure and MRT of MTX through inhibition on multiple anion transporters including OAT 1, OAT 3 and MRP 4 by the major metabolite IS.

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1. Introduction

Indican (indoxyl-β-D-glucoside, chemical structure shown in Fig. 1) is a constituent of several Chinese herbs such as *Isatis indigotica*, *Polygonum tinctorium* and *Polygonum perfoliatum*, which are used to treat colds, fever and influenza in clinical Chinese medicine [1,2]. Pharmacological studies of these herbs have reported numerous beneficial effects such as anti-inflammatory [3], antipyretic [3], antiviral [2,4], antimicrobial [5] and anticancer activities [4,6].

In pharmacokinetic aspect, indican was mainly metabolized to indoxyl sulfate (IS) [7], which was a well known endogenous uremic toxin derived from tryptophan [7–9] and also associated with the progression of cardiovascular diseases [10–12]. Being a strong acid, IS is completely ionized in bloodstream and thus unable to permeate cell membrane *via* passive diffusion [13]. Recent studies reported that IS was a substrate/inhibitor of organic anion transporters (OAT) such as OAT 1, OAT 3 and multidrug resistance-associated protein (MRP) 4 [14,15].

OATs and MRPs have been well recognized as important transporters for anions, which were responsible for the uptake and efflux transports of numerous acidic compounds at various organs [16,17]. Methotrexate (MTX), a dicarboxylic acid, is a substrate of numerous anion transporters such as OAT 1, 2, 3, 4 and MRP 1, 2, 3, 4 [18,19]. In clinical practice, MTX is commonly used for the treatment of certain neoplastic diseases, rheumatic arthritis and psoriasis, but with narrow therapeutic window. More than 80% of MTX was excreted *via* urine by OATs- and MRPs-mediated transports [20]. The serum levels of MTX should be carefully monitored because of the accompanied adverse drug reactions, including gastrointestinal problems, central nervous system symptoms, pulmonary damage, hepatotoxicity and nephrotoxicity [21–23].

We herein hypothesized that IS, the major metabolite of oral indican, might inhibit the renal excretion of MTX mediated by the anion transporters including OAT 1, OAT 3 or MRP 4. Therefore, this study investigated the effect of oral indican on the pharmacokinetics of MTX, a probe substrate of OAT 1, 2, 3, 4 and MRP 1, 2, 3, 4 in rats. Furthermore, in order to verify the proposed mechanism, phenolsulfonphthalein (PSP), a substrate of OAT 1, OAT 3, MRP 2 and MRP 4, was injected intravenously to rats as a probe to measure the effect of intravenous IS, which mimicked the metabolite of oral indican, on its elimination.

2. Materials and methods

2.1. Chemicals

Indican, indoxyl sulfate (IS) and phenolsulfonphthalein (PSP) were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, U.S.A.). Methotrexate (MTX) (25.0 mg/mL) was obtained from Wyeth Pharma Gmbh (Wolfratshausen, Germany). MK 571 (purity 98%) was obtained from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). Dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS), 3-(4′,5′-dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide (MTT) and triton X-100 were supplied by Sigma (St. Louis, MO, USA). Fetal Bovine Serum (FBS) was obtained from Biological Industries Inc. (Kibbutz,

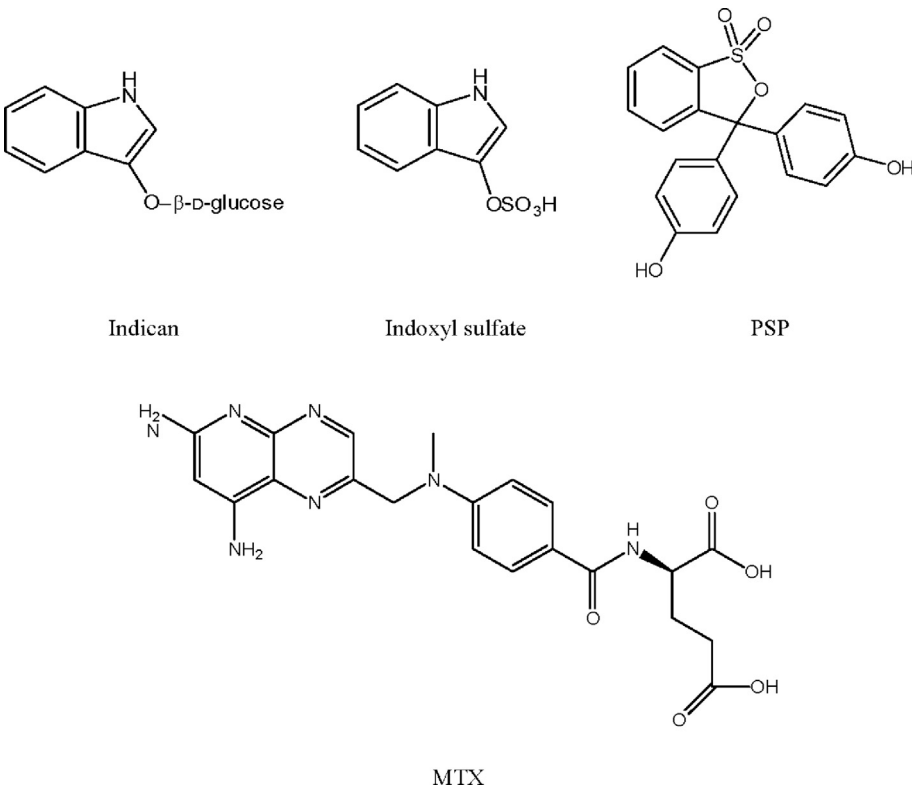

Fig. 1 – Structures of indican, indoxyl sulfate, phenolsulfonphthalein (PSP) and methotrexate (MTX).

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