Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Biochemical and Biophysical Research Communications 360 (2007) 7–13
BBRC
www.elsevier.com/locate/ybbrc

Targeted inhibition of glucuronidation markedly improves drug efficacy in mice—A model

Nikhil K. Basu [a,*], Labanyamoy Kole [a], Mousumi Basu [a], Antony F. McDonagh [b], Ida S. Owens [a,*]

[a] Heritable Disorders Branch, National Institute of Child Health and Human Development, National Institutes of Health, Building 10, Room 8D-42, Bethesda, MD 20892-1830, USA

[b] Division of Gastroenterology and the Liver Center, Department of Medicine, University of California at San Francisco, San Francisco, CA 94143, USA

Received 10 May 2007
Available online 14 June 2007

Abstract

Finding UDP-glucuronosyltransferases (UGT) require protein kinase C-mediated phosphorylation is important information that allows manipulation of this critical system. UGTs glucuronidate numerous aromatic-like chemicals derived from metabolites, diet, environment and, inadvertently, therapeutics to reduce toxicities. As UGTs are inactivated by downregulating PKCs with reversibly-acting dietary curcumin, we determined the impact of gastro-intestinal glucuronidation on free-drug uptake and efficacy using immunosuppressant, mycophenolic acid (MPA), in mice. Expressed in COS-1 cells, mouse GI-distributed Ugt1a1 glucuronidates curcumin and MPA and undergoes irreversibly and reversibly dephosphorylation by PKC-specific inhibitor calphostin-C and general-kinase inhibitor curcumin, respectively, with parallel effects on activity. Moreover, oral curcumin-administration to mice reversibly inhibited glucuronidation in GI-tissues. Finally, successive oral administration of curcumin and MPA to antigen-treated mice increased serum free MPA and immunosuppression up to 9-fold. Results indicate targeted inhibition of GI glucuronidation in mice markedly improved free-chemical uptake and efficacy using MPA as a model.
Published by Elsevier Inc.

Keywords: UDP-glucuronosyltransferase; Phosphorylation; Proof of principle; Immunosupression; Mycophenolic acid; CTL assay; Curcumin; Calphostin-C; PKC; Drug efficacy

Whereas the primary role of UDP-glucurononsyltransferases (UGT) is to detoxify numerous structurally diverse lipophilic chemicals, including metabolites, dietary constituents, environmental toxicants, carcinogens and, inadvertently, therapeutic chemicals, there is a limited capacity to manipulate glucuronidation to improve protection or reduce premature clearance of medications. As UGTs inactivate aromatic-like chemicals by attaching each to a glucuronic acid molecule(s) to promote water-solubility with high excretability, investigators have used recombinant UGTs under in-vitro conditions [1] to explore substrate suitability to gain information on the potential clearance of chemicals. While we know glucuronidation indiscriminately removes chemicals for protective purposes, glucuronidation can have a negative impact due to premature clearance of therapeutic drugs, which is inherently overcome by administering compensatorily higher levels, sometimes with side-effects. Despite the long-standing biochemical value associated with glucuronidation of chemicals, it has not been possible to establish or measure impact of this system, and it is not known whether biochemical manipulation of this system is warranted for protection against toxicants or to enhance drug efficacy. Unfortunately, it has not been possible to establish an effective, reversible biochemical inhibitor for all UGT

* Corresponding authors. Fax: +1 301 451 4288 (N.K. Basu), +1 301 480 8042 (I.S. Owens).
E-mail addresses: basun@mail.nih.gov (N.K. Basu), owensi@mail.nih.gov (I.S. Owens).

0006-291X/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.bbrc.2007.05.224

activities. Because our recent studies demonstrated all UGTs tested require protein kinase C (PKC)-mediated phosphorylation [2,3] that is subject to reversible anti-oxidant downregulation, we have developed a strategy to determine the impact of UGTs found in the mucosa layer of the gastrointestinal (GI) tract [1] on drug efficacy. We have downregulated glucuronidation by orally administering the kinase inhibitor and dietary condiment, curcumin, to block UGT phosphorylation on a transient basis [3], followed by administration of a test drug.

As we considered a UGT test substrate for in-vivo glucuronidation studies, immunosuppressant mycophenolic acid (MPA), administered to transplant patients as the mofetil-formulated prodrug MMF [4], was considered ideal for assessing the impact of GI glucuronidation on drug efficacy because it undergoes high levels of glucuronidation [5], it avoids the pharmacokinetics of in-vivo esterase-dependent cleavage of MMF and it is a potent immunosuppressant of cytotoxic T-lymphocyte (CTL) proliferation in renal transplant recipients [5] that is assessed in mice with a sensitive immunosuppression assay [6]. Potent MPA-inhibition of the rate-limiting enzyme, inosine monophosphate dehydrogenase (IMPDH) [7], is highly selective toward type II IMPDH isozyme over type I because of its specific role in de-novo synthesis of guanosine monophosphate in activated mononuclear cell proliferation. Selective inhibition of type II IMPDH by MPA prevents proliferation of activated T- and B-lymphocytes and allows the agent to exert a potent immunosuppressant effect, as well as it induces apoptosis of activated T-lymphocytes [8]. Immunosuppression is less toxic than anti-rejection therapy to alleviate acute allograft rejection of transplanted organs.

Whereas MPA readily forms ether-linked-(MPAG) or acyl-glucuronides (AcMPAG) [9–11], we studied MPA glucuronidation in-vitro with human UGTs and found 4 isozymes are avid metabolizers [12] that reach saturation kinetics between 1.6 and 2.4 mM with K_m values between 0.25 and 0.55 mM. These UGT isozymes, encoded at the *UGT1* complex locus, were found strategically and differentially expressed in the gastrointestinal (GI) mucosa [1]. In this report, we established under both in-vitro and in-vivo conditions that mouse Ugt1a1 also requires phosphorylation, which can be transiently downregulated by nontoxic kinase inhibitor, curcumin [2,3,13], and irreversibly by calphostin-C, similar to that for human UGTs [2,3]. Moreover, effects of curcumin pretreatment elicited a marked increase in free MPA levels in blood and a comparable improvement in immunosuppression in antigen-stimulated mice detected by the spleen CTL assay [6]. In short, our use of MPA demonstrates a 'proof of principle' that glucuronidation at the GI level can greatly compromise chemical uptake, including drug efficacy in mice.

Materials and methods

Materials and methods are provided as supporting online materials.

Results

Metabolism of MPA and curcumin by mouse Ugt1a1

As shown in Fig. 1A, mouse Ugt1a1 metabolized MPA, generating 64% MPA-7-0- and 36% acylMPA-glucuronides, respectively, and reaching saturation kinetics at 2.0 mM driven by K_m of 0.250 mM. Whereas the 9 mouse Ugt1a isozymes, except stomach-specific Ugt1a2, are distributed in all GI tissues [18], the level of MPA turnover by other mouse isozymes is unknown. Previously, we showed 4 out of 5 different human GI-distributed UGTs avidly metabolized MPA; human UGT1A1 [12,19] poorly metabolized MPA.

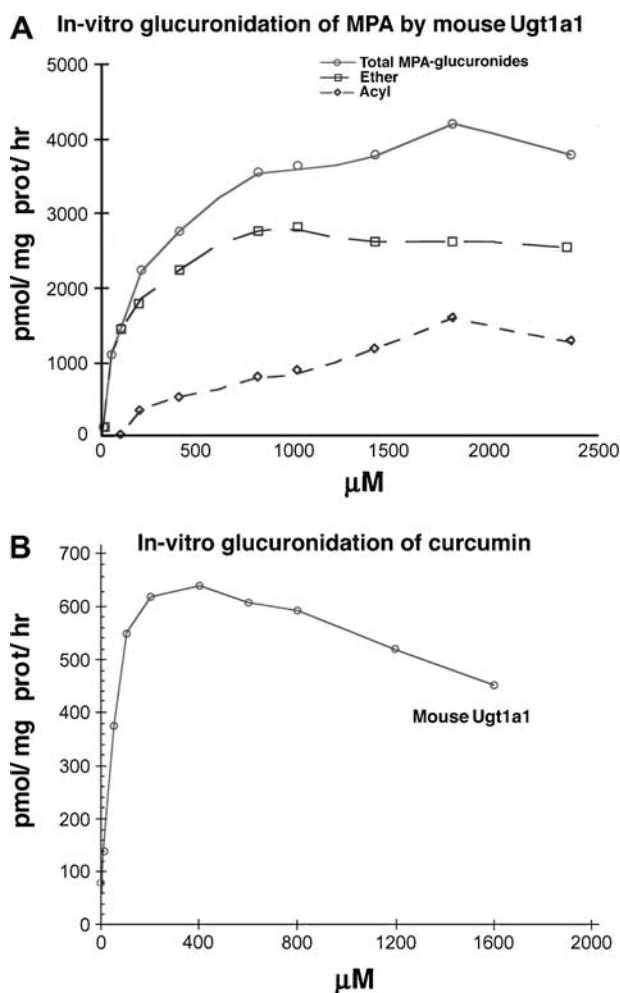

Fig. 1. (A) In-vitro glucuronidation of mycophenolic acid by mouse Ugt1a1. Analysis of MPA glucuronidation was carried out with 150 µg protein in 2 h incubation with ether (MPAG) and acyl-glucuronides (AcMPAG) distinguished as described under Methods. Total MPA-glucuronides, MPAG, and AcMPAG are pmol MPA-β-glucuronide/mg prot./h. Experiments, repeated 3 times, have SE of ±1–5%. (B) In-vitro glucuronidation of curcumin by mouse Ugt1a1. One-hundred fifty µg of mouse Ugt1a1 (pH 6.4) expressed in COS-1 cells incubated 2 h with increasing concentration of curcumin according to Methods. Activity is expressed as pmol/mg protein/hr. The experiment, repeated 3 times, has SE of ±1–4%.

Download English Version:

https://daneshyari.com/en/article/1937362

Download Persian Version:

https://daneshyari.com/article/1937362

Daneshyari.com