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Transport of the investigational anti-cancer drug 5,6-dimethylxanthenone-4-acetic acid and its acyl glucuronide by human intestinal Caco-2 cells

Shufeng Zhou [a,*], Xia Feng [b], Philip Kestell [c], James W. Paxton [b], Bruce C. Baguley [c], Eli Chan [a]

[a] *Department of Pharmacy, Faculty of Science, National University of Singapore, Block S4, 18 Science Drive 4, S 117543, Singapore*
[b] *Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*
[c] *Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

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Abstract

5,6-Dimethylxanthenone-4-acetic acid (DMXAA), a potent cytokine inducer, exhibited marked antitumor activity when given as multiple oral doses in mice. The aim of this study was to examine the transport of DMXAA and its acyl glucuronide (DMXAA-G) using the human Caco-2 cells. DMXAA was minimally metabolized by Caco-2 cells and both DMXAA and DMXAA-G were taken up to a minor extent by the cells. The permeability coefficient (P_{app}) values of DMXAA over 10–500 μM were 4×10^{-5} cm/s to 4.3×10^{-5} cm/s for both apical (AP) to basolateral (BL) and BL-AP transport, while the P_{app} values for the BL to AP flux of DMXAA-G were significantly greater than those for the AP to BL flux, with R_{net} values of 4.5–17.6 over 50–200 μM. The BL to AP active efflux of DMXAA-G followed Michaelis-Menten kinetics, with a K_m of 83.5 ± 5.5 μM, and V_{max} of 0.022 ± 0.001 nmol/min. The flux of DMXAA-G was energy and Na^+-dependent and MK-571 significantly ($P < 0.05$) inhibited its BL to AP flux, with an estimated K_i of 130 μM. These data indicate that the transport of DMXAA across Caco-2 monolayers was through a passive process, whereas the transport of DMXAA-G was mediated by MRP1/2.
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Keywords: DMXAA; Acyl glucuronide; Caco-2 cells; Intestinal absorption; MRP

1. Introduction

The investigational anti-cancer drug 5,6-dimethylxanthenone-4-acetic acid (DMXAA) was developed as an analogue of flavone-8-acetic acid by the Auckland Cancer Society Research Centre (ACSRC). DMXAA has multiple pharmacological activities including induction of cytokines (in particular tumour necrosis factor (TNF-α)) (Ching et al., 1994; Philpott et al., 1995; Philpott et al., 2001), serotonin (Baguley et al., 1993, 1997) and nitric oxide (Thomsen et al., 1990, 1991), immuno-modulating effects (Ching and Baguley, 1988, 1989), NF-κB activation (Woon et al., 2003), induction of endothelial apoptosis (Ching et al., 2004), anti-vascular (Zwi et al., 1994) and anti-angiogenic activity (Cao et al., 2001). Two recently completed Phase I clinical studies on DMXAA involving 109 cancer patients in New Zealand

Abbreviations: ACSRC, Auckland Cancer Society Research Centre; AP, apical; BL, basolateral; CYP, cytochrome P450; DMSO, dimethyl sulphoxide; DMXAA, 5,6-dimethylxanthenone-4-acetic acid; DMXAA-G, DMXAA acyl glucuronide; HBSS, Hanks' balanced salt solution; HEPES, N-[2-hydroxyethyl]piperazine-*N*′-[4-butanesulfonic acid]; IC_{50}, drug concentration producing 50% of maximum inhibition at effect site; K_i, inhibition constant; K_m, Michaelis-Menten constant; MRP, multidrug resistance protein; 6-OH-MXAA, 6-hydroxymethyl-5-methylxanthenone-4-acetic acid; P_{app}, permeability coefficient; P-gp, P-glycoprotein; R_{net}, the ratio of Papp in the basolateral to apical direction versus Papp in the apical to basolateral direction; TEER, Transepithelial electrical resistance; TNF-α, tumour necrosis factor-α; SRB, Sulforhodamine B; UGT, uridine diphosphate glucuronosyltransferase; V_{max}, maximal transport rate

* Corresponding author. Tel.: +65 6874 2931; fax: +65 6779 1554.
E-mail address: phazsf@nus.edu.sg (S. Zhou).

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and the UK indicated that DMXAA at 1100 and 1300 mg/m^2 gave unconfirmed partial response in two patients (Jameson et al., 2003; Rustin et al., 2003). This compound was well tolerated at low to moderate doses, but the patients experienced rapidly reversible dose-limiting toxicities at 4900 mg/m^2, including confusion, tremor, slurred speech, visual disturbance, anxiety, and urinary incontinence (Jameson et al., 2003; Rustin et al., 2003).

Oral anticancer chemotherapy is becoming an accepted and standard approach for the treatment of cancer, due to several advantages such as greater safety and flexibility, reduced financial cost, improved quality of life, and the potential for improved efficacy (DeMario and Ratain, 1998; Greco, 1998). To obtain maximal efficacy and minimal toxicity, appropriate intestinal absorption of oral anti-cancer agents are required (Terwogt et al., 1999). The oral bioavailability of DMXAA was 70% in the mouse, but low anti-tumor activity was observed when given as a single dose (Zhao et al., 2002). The latter may be due to low maximal plasma DMXAA concentrations achieved after oral administration (Zhao et al., 2002). However, when the mice bearing colon cancer 38 were treated with a loading dose (30 mg/kg) and supplementary doses (15 mg/kg after 4 and 8 h), it gave a 90% cure rate (Zhao et al., 2003). Thus, oral administration of DMXAA is becoming a possibly effective administration schedule for future clinical trials.

Acyl glucuronidation is the dominant hepatic metabolic pathway for DMXAA giving rise to DMXAA acyl glucuronide (DMXAA-G), with the 6-methylhydroxylation to 6-hydroxymethyl-5-methylxanthenone-4-acetic acid (6-OH-MXAA) of lesser importance (Kestell et al., 1999; Webster et al., 1995; Zhou et al., 2001b). To better understand the oral pharmacokinetic properties of DMXAA, it is important to characterize the intestinal absorption of DMXAA and its major metabolite. DMXAA (MW = 284) is a weak acid with a pK_a of 5.5. Thus, it is mainly present as ionised form at pH 7.4. DMXAA-G (MW = 459) is a highly hydrophilic conjugate with an approximate pK_a of 3.5. Based on the known physiochemical properties of DMXAA and DMXAA-G, an active mechanism may be involved in their intestinal transport. Therefore, the human intestinal cell line Caco-2, a well-accepted model of human intestinal absorption, was used to test this hypothesis (Artursson, 1990; Meunier et al., 1995). DMXAA has been combined with many drugs such as thalidomide (Ching et al., 1998), diclofenac (Zhou et al., 2001a) and cyproheptadine (Zhao et al., 2001) in the mouse, and these drugs have been shown to modulate the pharmacokinetics of DMXAA. In particular, both thalidomide and cyproheptadine appeared to inhibit the biliary excretion of DMXAA-G in the mouse (Kestell et al., 2000; Zhao et al., 2001). It is unknown whether modulation of transport of DMXAA and/or DMXAA-G is involved in these DMXAA-drug interactions. Thus, the effects of these drugs on the transport of DMXAA and DMXAA-G by Caco-2 monolayers were also investigated.

2. Materials and methods

2.1. Chemicals and reagents

DMXAA (98% purity, as determined by thin layer chromatography) and 2,5-dimethylxanthenone-4-acetic acid (SN24350, as internal standard) were synthesised in the ACSRC (Rewcastle et al., 1989). DMXAA was protected from light exposure to avoid degradation (Rewcastle et al., 1990). Authentic DMXAA-G and 6-OH-MXAA were isolated and purified from the bile and urine of rats treated with DMXAA, and their structures confirmed by mass spectrometry and [^{1}H]-nuclear magnetic resonance (Kestell et al., 1999). Sulforhodamine B (SRB), diclofenac, cyclophosphamide, ifosphamide, cimetidine, probenecid, quinidine, cyproheptadine, and 2-[*N*-morpholino]ethanesulfonic acid were purchased from Sigma–Aldrich Chemicals Co. (Auckland, New Zealand). ^{3}H-thymidine and ^{14}C-mannitol (specific activity of 5 and 351 mCi/mmol, respectively) were obtained from Amersham Pharmacia (Auckland, New Zealand). MK-571 was a gift of Dr. Ford-Hutchinson (Merck Frosst Canada Inc.) (Jones et al., 1989). Thalidomide (purity > 99%, determined by HPLC) was provided by Celgene Co. (Warren, NJ). All other reagents were of analytical or HPLC grade as appropriate.

2.2. Cell culture

The Caco-2 cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained by serial passage in T-75 plastic culture flasks (Life Technologies). The cells were cultured in complete Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 1% nonessential amino acids, and 100 U/ml penicillin and gentamycin (all from Life Technologies). The cells were grown in an atmosphere of 5% CO_2/95% air at 37 °C and given fresh medium every 3 or 4 days. Viable cells were counted using the trypan blue exclusion method. For transport studies, the cells were seeded in 12 mm i.d. Transwell polycarbonate inserts (Corning Costar Corp.) in 12-well plates at a density of 10^5 cells/insert. Cells were used for transport experiments at passage 27–36 at 20–31 days after seeding. The transmembrane specific resistance, expressed in Ω cm^2, was measured using a Millicell-ERS apparatus (purchased from Millipore Corporation) at room temperature. The integrity of Caco-2 monolayers was confirmed when the transepithelial electrical resistance (TEER) exceeded 300 Ω cm^2, and the paracellular flux of ^{14}C-mannitol was <1%/h.

2.3. Stability of DMXAA and DMXAA-G in HBSS at various pH

The method for the study of DMXAA and DMXAA-G stability has been previously described (Zhou et al., 2001b). Briefly, DMXAA or DMXAA-G (50 μM) was

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