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The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated efflux

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ABSTRACT

The potential inhibitory effect on P-glycoprotein (Pgp) by antiparasitic drugs and natural compounds was investigated. Compounds were screened for Pgp interaction based on inhibition of Pgp mediated [^{3}H]-taxol transport in Caco-2 cells. Bidirectional transport of selected inhibitors was further evaluated to identify potential Pgp substrates using the Caco-2 cells. Of 21 antiparasitics tested, 14 were found to inhibit Pgp mediated [^{3}H]-taxol with K_{iapp} values in the range 4–2000 μM. The antimalarial quinine was the most potent inhibitor with a K_{iapp} of 4 μM. Of the 12 natural compounds tested, 3 inhibited [^{3}H]-taxol transport with K_{iapp} values in the range 50–400 μM. Quinine, amodiaquine, chloroquine, flavone, genistein, praziquantel, quercetin and thiabendazole were further investigated in bidirectional transport assays to determine whether they were substrates for Pgp. Transport of quinine in the secretory direction exceeded that in the absorptive direction and was saturable, suggesting quinine being a Pgp substrate. The rest of the compounds inhibiting Pgp showed no evidence of being Pgp substrates. In conclusion, we have demonstrated that a substantial number of antiparasitic and natural compounds, in a range of concentrations, are capable of inhibiting Pgp mediated [^{3}H]-taxol efflux in Caco-2 cells, without being substrates and this may have implications for drug interactions with Pgp.

1. Introduction

Most antiparasitic drugs were introduced more than 30 years ago; suramin, used for treatment of African trypanosomiasis was introduced in the 1920s and pentamidine and melarsoprol, used for the same disease, were introduced in the 1940s (Fairlamb, 2003). Very few new drugs have been, or indeed, are being introduced for treatment of antiparasitic diseases and with the emergence of resistance to the existing drugs, communities may resort to traditional natural product-based therapies. In addition, the communities most affected by infectious diseases often may not afford health care and may use traditional remedies concurrently with any available drugs. For instance, in certain communities, extracts from plants may be used, together with chloroquine, for the treatment of malaria (Mueller et al., 2004). Also, efficacy of anti-infective agents depends on the immune system of the patient which in turn is governed by the nutritional status (Pereira, 2003). In light of this, a patient on antiparasitic therapy may feel that consumption of food-derived extracts, which are deemed to be healthy, may aid in recovery. The potential of these natural products to bring about interactions with therapeutics

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(food/herb–drug interactions) is now being discovered and discussed both on the level of the CYP 450 system and on the P-glycoprotein transporter level. (Butterweck et al., 2004) Drug interactions have meanwhile been presumed for at least 150 herbal medicines (Butterweck et al., 2004).

P-glycoprotein (Pgp; MDR1 gene product) is an efflux protein that plays a major role in drug absorption and distribution and has been reported to play a role in drug–drug and food/herb–drug interactions. Pgp limits permeability across the gastrointestinal tract (Ayrton and Morgan, 2001; Hunter and Hirst, 1997) by actively pumping potentially toxic substances back into the intestinal lumen. The transporter is also expressed in other tissues such as the liver and blood–brain barrier where it also plays an important excretory role. Consequently, inhibition of Pgp may lead to adverse drug interactions at these sites due to elevated intracellular concentrations or altered pharmacokinetics. Drug–drug and food/herb–drug interactions could be mediated via either competition between substrates for Pgp or through non-competitive inhibition. Studies have shown increased oral exposure of the Pgp substrates digoxin, and talinolol by Pgp inhibitors such as quinidine (Hager et al., 1979), and verapamil (Grammate and Oertel, 1999). Loperamide, an opiate that does not affect the CNS at normal doses, brought about respiratory depression when co-administered with quinidine (Sadeque et al., 2000), probably because of Pgp inhibition at the blood–brain barrier by quinidine. Recently, interactions of herbal medicines and food ingredients such as St. John's wort, grapefruit juice and Seville orange juice have also been shown to increase the oral bioavailability of certain drugs, presumably by inducing or inhibiting Pgp mediated transport or cytochrome P450 3A4 or both (Dresser and Bailey, 2003; Harris et al., 2003). For instance, when quercetin was orally co-administered with digoxin in pigs, there was a fatal interaction (Wang et al., 2004). Quercetin presumably inhibited intestinal Pgp leading to enhanced digoxin absorption and hence toxic levels of the drug. The potential side effects or interactions of the concomitant use of herbs, food and drugs can be even further potentiated since it is obvious that patients use more than one of these treatments and in most cases unknowing of the consequences (Kaufman et al., 2002; Klepser et al., 2000). Recently, strategies have also been presented for the use of natural products to specifically enhance intestinal absorption in humans of low permeable compounds by standardised modulation of Pgp by apricot extracts (Deferme et al., 2002), thus making positive use of food–drug interactions.

The pharmacokinetic properties of antiparasitic agents have been extensively studied, but their effect on Pgp and hence their potential to be involved in drug interactions has not been widely investigated. A few studies with chloroquine have shown potential for interactions with plant derived beverages but there is no further data and the cause for interaction was not explored. It was shown that Sudanese beverages may reduce bioavailability of chloroquine (Mahmoud et al., 1994) and only recently has this been followed up in a study showing that grapefruit juice affects the pharmacokinetics of chloroquine in chickens as well as in mice (Ali et al., 2001, 2002). *In vitro* assessments of potential interactions using anti-infective agents and plant derived extracts are needed in view of any potential adverse interactions that may result.

Hence, in the present study we aim to investigate the potential inhibition of Pgp by several anti-infective agents and a set of natural compounds. Most of the antiparasitics are used in malaria treatment, but antihelminthic, antischistosomal and antitrypanosomal agents are also included. The natural compounds are mostly flavonoids and other phenolic compounds that are ubiquitous in plants. Also included are diospyrin, a plant derived bisnaphthoquinone that has antitumour as well as antiparasitic activities (Ray et al., 1998), and geshoidin, a naphthalenic glucoside found to be the bittering agent in a popular traditionally brewed beverage in Ethiopia (Abegaz and Kebede, 1995). We have studied the interactions of the above compounds with Pgp using human intestinal epithelial Caco-2 cells since it has been reported that this transporter is expressed in the apical membranes of Caco-2 cells at a similar expression level as in the human jejunum (Seithel et al., 2006; Taipalensuu et al., 2001). A taxol transport inhibition assay was used to identify compounds that are potential inhibitors to Pgp (Gao et al., 2001). In addition, compounds that are substrates for Pgp exhibit net secretory transport in the basolateral to apical direction and this asymmetry, calculated as efflux ratio (ER) was generally used as an indication of Pgp mediated transport of the test compounds.

2. Materials

[^{3}H]-taxol was purchased from NEN^{TM} Life Science Products Inc. (Boston, MA, USA). [^{3}H]-quinine was purchase from American Radiolabeled Chemicals (St. Louis, MO, USA). OptiPhase 'Highsafe' 3 was purchased from Wallac (Loughborough, UK). Amodiaquine, 4-chlorophenylbiguanide, proguanil, and cycloguanil were generous gifts from Prof. Anders Björkman and Prof. Lars Gustafsson (Karolinska Institute, Stockholm, Sweden). Dr. Michael Ashton (Gothenburg University, Gothenburg, Sweden) kindly provided dihydroartemisinin and artesunate. Diospyrin was kindly provided by Dr. Banasri Hazra (Jadavpur University, Calcutta, India). The compound was purified meticulously as described (Hazra et al., 1984). Geshoidin was kindly provided by Dr. Berhanu Abegaz (University of Botswana, Gaborone, Botswana). The purity of the compound was checked by comparing the physical and spectroscopic data (Abegaz and Kebede, 1995). All other chemicals were obtained from Sigma–Aldrich Co. (Stockholm, Sweden).

3. Methods

3.1. *Cell cultures*

The Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD, USA). The cells were used in the same conditions as those used for permeability screening at AstraZeneca (Mölndal, Sweden). That is, the cells were seeded on 12 mm polycarbonate membranes with a pore size of 0.4 μM and an area of 1.13 cm^2 (Transwell® Corning Costar® Corporation, Cambridge, MA, USA) at a seeding density of 225 000 cells/cm^2, and maintained at 37 °C in a humidified atmosphere with 5% CO_2 as previously described (Neuhoff et