British Journal of Pharmacology (2007) 152, 815–824
 0007–1188/07 $30.00
www.brjpharmacol.org

RESEARCH PAPER

The multidrug transporter ABCG2 (BCRP) is inhibited by plant-derived cannabinoids

ML Holland[1], DTT Lau[2], JD Allen[2] and JC Arnold[1]

[1]*The Discipline of Pharmacology, The University of Sydney, Sydney, New South Wales, Australia and* [2]*The Centenary Institute of Cancer Medicine and Cell Biology, Sydney, New South Wales, Australia*

Background and purpose: Cannabinoids are used therapeutically for the palliation of the adverse side effects associated with cancer chemotherapy. However, cannabinoids also inhibit both the activity and expression of the multidrug transporter, P-glycoprotein *in vitro*. Here we address the interaction of cannabinol (CBN), cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) with the related multidrug transporter, ABCG2.
Experimental approach: Cannabinoid inhibition of Abcg2/ABCG2 was assessed using flow cytometric analysis of substrate accumulation and ATPase activity assays. The cytotoxicity and chemosensitization by cannabinoids was determined with cell viability assays. Expression of cannabinoid and vanilloid receptors was assessed using reverse transcriptase polymerase chain reaction, and cannabinoid modulation of ABCG2 expression was examined using immunoblotting.
Key results: CBN, CBD and THC increased the intracellular accumulation of the Abcg2/ABCG2 substrate, mitoxantrone, in an over-expressing cell line. The THC metabolite, (−)-11-nor-9-carboxy-Δ^9-THC was much less potent. The plant cannabinoids inhibited both basal and substrate stimulated ATPase activity of human ABCG2. Cannabinoid cytotoxicity occurred in the absence of known cannabinoid cell surface receptors, and only at concentrations higher than those required for Abcg2/ABCG2 inhibition. Sub-toxic concentrations of the cannabinoids resensitized the overexpressing cell line to the cytotoxic effect of Abcg2/ABCG2 substrates, mitoxantrone and topotecan. This occurred in the absence of any effect on ABCG2 expression.
Conclusions and implications: Cannabinoids are novel Abcg2/ABCG2 inhibitors, reversing the Abcg2-mediated multidrug-resistant phenotype *in vitro*. This finding may have implications for the co-administration of cannabinoids with pharmaceuticals that are ABCG2 substrates.
British Journal of Pharmacology (2007) **152,** 815–824; doi:10.1038/sj.bjp.0707467; published online 1 October 2007

Keywords: cannabinoids; multidrug transporters; ABCG2; BCRP; chemotherapy; multidrug resistance; breast cancer resistance protein; Δ^9-tetrahydrocannabinol; cannabis; cannabidiol

Abbreviations: BCRP, breast cancer resistance protein; CBD, cannabidiol; CBN, cannabinol; Dnase, deoxyribonuclease; MDR, multiple drug resistance; MEF, mouse embryonic fibroblasts; P-gp, p-glycoprotein; THC, Δ^9-tetrahydrocannabinol; THC-A, (−)-11-nor-9-carboxy-Δ^9-THC

Introduction

Multiple drug resistance (MDR) is one of the principal causes of chemotherapeutic treatment failure in malignant disease. Several ATP-binding cassette transporters contribute to this MDR phenotype. The first multidrug transporter identified was P-glycoprotein (P-gp) (ABCB1/MDR1) (Juliano and Ling, 1976). Other multidrug transporters believed to contribute to clinical resistance have since been discovered, including the multidrug resistance-related protein 1 (ABCC1/MRP1) (Cole *et al*., 1992) and more recently, breast cancer resistance protein (ABCG2/BCRP) (Doyle *et al*., 1998). Expression of these proteins in cancer cell lines results in reduced intracellular substrate accumulation and consequently resistance to cytotoxicity as a result of ATP-dependent substrate extrusion.

ABCG2 substrates include a range of clinically employed anticancer agents such as mitoxantrone, topotecan and related drugs (Doyle *et al*., 1998; Maliepaard *et al*., 1999; Kawabata *et al*., 2001; Robey *et al*., 2001). ABCG2 is expressed in the liver, placental syncytiotrophoblasts and at the apical surface of epithelial cells in the small intestine, colon and kidney (Jonker *et al*., 2000; Maliepaard *et al*., 2001), where it limits the bioavailability and foetal penetration of substrate xenobiotics (Jonker *et al*., 2000). ABCG2 has also been found in haemopoietic stem cells (Zhou *et al*., 2001) and in brain microvessel endothelial cells (Cooray *et al*., 2002), where it may serve a protective function as part of the blood–brain barrier.

Correspondence: Dr JC Arnold, The Discipline of Pharmacology, Bosch Institute of Medical Sciences, Bosch Building 1B D05, The University of Sydney, Sydney, New South Wales 2006, Australia.
E-mail: arnie@med.usyd.edu.au
Received 13 August 2007; accepted 23 August 2007; published online 1 October 2007

ABCG2 has been found to have a significant impact on the pharmacokinetics of substrate drugs. The oral availability of topotecan is predominantly limited by ABCG2 in both mice (Jonker *et al*., 2000) and humans (Kruijtzer *et al*., 2002). Adverse drug interactions due to competition for ABCG2 have also been reported, with co-administration of benzimidazoles with methotrexate causing elevated serum concentrations of methotrexate and its metabolites (Breedveld *et al*., 2004). ABCG2 expression in malignant cells may also contribute directly to tumour drug resistance. Induction of ABCG2 mRNA expression following anticancer drug treatment has been reported in both adult (van den Heuvel-Eibrink *et al*., 2002) and childhood (Steinbach *et al*., 2002) acute myeloid leukaemia. Furthermore, ABCG2 is expressed in an array of distinct solid tumours (Diestra *et al*., 2002). However, the correlation between tumoural ABCG2 expression and clinical drug resistance requires further investigation.

Cannabis and cannabinoid preparations are used as therapeutic agents. One of the many applications of cannabinoids is in the palliation of cancer chemotherapy-induced nausea, vomiting and anorexia (Tramer *et al*., 2001). Indeed, the commercial preparations, Marinol and Cesamet, containing the synthetic Δ^9-tetrahydrocannabinol (THC) analogue, dronabinol (or nabilone), are approved in some countries for this use. Interestingly, in the future, cannabinoids might be co-administered with conventional cancer chemotherapies not only in a palliative capacity but also as primary anticancer medications. Accordingly, cannabinoids have demonstrated antiproliferative actions on cancer cells *in vitro* and *in vivo* (Guzman, 2003). In addition, the first Phase I clinical trial assessing the direct antitumour activity of THC on glioblastoma was recently published yielding promising preliminary results (Guzman *et al*., 2006).

The use of cannabinoid preparations by chemotherapy patients raises the issue of whether cannabinoid compounds modulate the effectiveness of concurrently administered anticancer agents. We have already assessed the interaction of plant-derived cannabinoids with the multidrug transporter, P-gp, finding that both cannabidiol (CBD) and THC reduced the expression of this efflux pump in a drug-selected human T-lymphoblastoid cell line. However, these drugs did not inhibit the efflux activity of P-gp at the subcytotoxic concentrations assayed (0.1–10 μM) (Holland *et al*., 2006). Two independent reports addressing cannabinoid modulation of P-gp have since been published. The first showed that CBD, but not THC, inhibits P-gp efflux activity at concentrations greater than 10 μM (Zhu *et al*., 2006). The second study demonstrated that the endocannabinoid anandamide and several synthetic cannabinoids also inhibit P-gp function (Nieri *et al*., 2006).

The recent findings that cannabinoids can inhibit both the activity and expression of P-gp led us to investigate whether these compounds affect the related multidrug transporter, ABCG2. We conclude that the plant-derived compounds, cannabinol (CBN), CBD and THC inhibit the activity of both the human and mouse orthologues of wild-type ABCG2, although they do not affect ABCG2 protein expression.

Materials and methods

Cell culture

Mdr1a/b$^{-/-}$*Mrp1*$^{-/-}$ mouse embryonic fibroblasts (MEF3.8) and the *Abcg2 (Bcrp1)* transduced subline MEF3.8/*Bcrp1* A2 have been described (Allen *et al*., 2002). The *ABCG2 (BCRP)* transduced subline MEF3.8/BCRP (unpublished data, T Murry, Centenary Institute of Cancer Medicine and Cell Biology, Sydney, NSW, Australia) is similar to MEF3.8/*Bcrp1* A2 but uses human ABCG2 cDNA provided by Dr Douglas Ross (Greenebaum Cancer Centre, University of Maryland, USA). The human colon cancer cell line, WiDr, was obtained from the American Type Tissue Culture Collection (Manassas, VA, USA). These cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum and 50 IU ml^{-1} penicillin, 50 mg ml^{-1} streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. Cell cultures were maintained within the exponential phase of growth and limited to five or less successive passages. All experiments were performed in 10% foetal calf serum. Cells were regularly screened for *Mycoplasma* contamination using the Mycoprobe Mycoplasma detection assay (Bio Scientific Pty. Ltd, Gymea, NSW, Australia).

Cell viability assays

MEF3.8 and MEF3.8/*Bcrp1* A2 cells were plated at 1000 cells per well into 96-well plates and allowed to attach overnight. Mitoxantrone (0.1 nM–1 μM), topotecan (1 nM–10 μM), vinblastine (0.01–100 nM), CBN, CBD or THC (all 0.1–100 μM) were added to the plates and incubated for 72 h. Cell viability was then measured using the [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) (MTS) assay as described previously (Holland *et al*., 2006). Maximum vehicle concentrations were 4×10^{-5}% (v v^{-1}) dimethylsulphoxide (DMSO), 1×10^{-3}% (v v^{-1}) tartaric acid and 0.7% (v v^{-1}) ethanol.

To assess the ability of CBN, CBD and THC to act as chemosensitizing agents, cells were plated at 2000 cells well^{-1} and allowed to attach overnight. Vehicle or CBN, CBD and THC (at subcytotoxic concentrations of 2, 5 and 10 μM for mitoxantrone and 10 μM only for topotecan) were then added to the plates, and incubated for 1 h prior to the addition of mitoxantrone (0.01 nM–1 μM), or topotecan (1 nM–10 μM). Maximum vehicle concentrations were 4×10^{-5}% (v v^{-1}) DMSO with 1.5×10^{-4}% (v v^{-1}) ethanol when mitoxantrone was the substrate, and 1×10^{-3}% (v v^{-1}) tartaric acid with 1.5×10^{-4}% (v v^{-1}) ethanol when topotecan was the substrate. The plates were then incubated for 48 h. Medium was aspirated and the plates were stored at −20°C for 12 h prior to the addition of SYBR Green I nucleic acid gel stain diluted 1:4000 in 10 mM Tris–HCl (pH 8.3), 5 mM EDTA, 0.1% (v v^{-1}) Triton X-100. The plates were then incubated at 4 °C for 7 days. The fluorescence at 485/535 nm was measured using a Wallac VICTOR3V plate reader (PerkinElmer Pty Ltd, Melbourne, VIC, Australia).

Substrate accumulation assays

MEF3.8 and MEF3.8/*Bcrp1* A2 cells were seeded at 5×10^4 cells well^{-1} into 24-well plates 18 h prior to

commencing the experiment. The medium was aspirated and replaced with medium containing either vehicle, Ko143 (1 μM), or CBN, CBD, THC or (−)-11-nor-9-carboxy-Δ^9-THC (THC-A) (80 nM–50 μM) for 30 min prior to the addition of the ABCG2 substrate, mitoxantrone (20 μM). Maximum vehicle concentrations were 0.5% ($v\,v^{-1}$) for Ko143 samples; 0.4% ($v\,v^{-1}$) DMSO with 1.7% ($v\,v^{-1}$) ethanol for CBN and CBD; 0.4% ($v\,v^{-1}$) DMSO with 0.6% ($v\,v^{-1}$) ethanol for THC; and 0.4% ($v\,v^{-1}$) DMSO with 17.9% ($v\,v^{-1}$) methanol for THC-A. This was followed by a further 60-min incubation in the dark at 37 °C. Cells were transferred to ice and maintained at 0 °C while they were harvested by treatment with trypsin-EDTA for 15 min, dislodged by pipetting and washed in phosphate-buffered saline containing 1% ($v\,v^{-1}$) foetal calf serum. Cells were analyzed on a FACScan flow cytometer with a 633 nm helium-neon laser using CellQuest software (BD, North Ryde, NSW, Australia). Mitoxantrone fluorescence (FLH-4) was measured by a 661 nm band pass filter, and gates were set so as to exclude clumps and debris on the basis of forward and side scatter.

ATPase assays

The ATPase activity of wild-type human ABCG2 was measured according to previously described methods (Sarkadi *et al.*, 1992) using the SB-MXR-HAM-Sf9-ATPase Assay purchased from SOLVO Biotechnology (Budapest, Hungary). This kit uses a cholesterol-loaded Sf9 insect cell membrane preparation, which demonstrates similar sensitivity to activation by known ABCG2 substrates as membrane preparations derived from mammalian cells (Pal *et al.*, 2007). The effect of increasing concentrations of CBN, CBD and THC (80 nM–50 μM) on both the basal ATPase activity and the activity stimulated by the reference substrate sulphasalazine (10 μM) was determined as the difference in inorganic phosphate liberated (mg membrane protein) per min with and without the presence of 1.2 mM sodium orthovanadate using a KH_2PO_4 calibration curve. Vehicle concentration was kept at 2% ($v\,v^{-1}$) ethanol across all cannabinoid concentrations assessed. Results are represented as a percentage of maximum activity, which is defined as the ATPase activity in the presence of 10 μM sulphasalazine. The activity in the presence of the cannabinoids was compared at each concentration to the respective control. The effect on basal ATPase activity was compared to that determined in the presence of vehicle alone. The effect on substrate-stimulated activity was compared to the ATPase activity determined in the presence of 10 μM sulphasalazine with inhibitor vehicle present.

RNA isolation and reverse transcriptase polymerase chain reaction

Total RNA was isolated using TRIzol Reagent according to the manufacturer's instructions, and treated with DNase I to remove any contaminating genomic DNA. Two micrograms of RNA was then reverse transcribed with Moloney-murine leukaemia virus reverse transcriptase using random hexamers. The cytoplasmic protein, β-actin was amplified as a positive control for cDNA integrity using forward (5′-GCG CAAGTACTCTGTGTGGA-3′) and reverse (5′-AGGGAGACC AAAGCCTTCAT-3′) primers designed to amplify a 723 bp region of the mRNA sequence (NM_007393). Thermal cycling conditions were 94 °C for 15 min, followed by 35 cycles of 94 °C for 45 s, 55 °C for 20 s, 72 °C for 30 s, with a final cycle of 72 °C for 5 min. Expression of the cannabinoid receptor CB_1 was assayed using forward (5′-ACAGGGCAGT ACCCCTTCTT-3′) and reverse (5′-AGCCCCTGGTGGTATTCT CT-3′) primers designed to amplify a 175 bp region of the mRNA sequence (NM_007726). Thermal cycling conditions were as above, except with an annealing temperature of 57 °C. Expression of the cannabinoid receptor CB_2 was measured using forward (5′-TCATTGCCATCCTCTTTTCC-3′) and reverse (5′-GAACCAGCATATGAGCAGCA-3′) primers to amplify a 188 bp sequence from the mRNA sequence (NM_009924), under the same thermal cycling conditions as for CB_1. Expression of the transient receptor potential vanilloid receptor (subtype 1) (TRPV1) was analysed using forward (5′-AAGGCTCTATGATCGCAGGA-3′) and reverse (5′-CAGATTGAGCATGGCTTTGA-3′) primers to amplify a 169 bp product from the mRNA sequence (NM_001001445) or a 245 bp product from genomic DNA using the same thermal cycling program, however, with an annealing temperature of 58 °C. 5 × Orange G Loading dye was then added to the PCR reaction to a final concentration of 1 ×, and 12 μl was loaded into each lane of a 2% (w/v) agarose gel with GeneRuler 50 bp DNA Ladder (Quantum Scientific Pty Ltd, Murarrie, QLD, Australia) for electrophoresis.

Western blot analysis

Whole-cell lysates were prepared from MEF3.8/BCRP and WiDr cells treated with CBN, CBD or THC (0.4, 2 or 5 μM) for 72 h or at 5 μM for 24, 48 or 72 h. Both of these cell lines express wild-type human ABCG2. Cells were trypsinized and washed twice in phosphate-buffered saline before being resuspended in lysis buffer (10 mM Tris–HCl (pH 7.4), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5% ($v\,v^{-1}$) sodium dodecyl sulphate with mini-protease inhibitor cocktail (Roche Diagnostics, Castle Hill, NSW, Australia)) at 4×10^7 cells ml^{-1} and sonicated on ice. Protein concentration was determined using the BCA Protein Assay Kit (Progen Biosciences, Archerfield, QLD, Australia).

Protein (20 μg per lane) was separated using sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Membranes were cut according to the molecular weight marker. α-tubulin was detected on the low molecular weight portion by sequential incubation with Abcam rat monoclonal YL1/2 α-tubulin antibody (Sapphire Biosciences Pty Ltd, Redfern, NSW, Australia), and anti-rat IgG (H + L) horseradish peroxidase conjugate (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). ABCG2 was detected on the high-molecular-weight portion with the monoclonal antibody BXP-21 (Maliepaard *et al.*, 2001), and the anti-mouse IgG (H + L) horseradish peroxidase conjugate (Promega Corporation, Annandale, NSW, Australia).

Statistical analysis

The cell viability data were fitted with a sigmoidal concentration–response curve using GraphPad Prism v.4. (GraphPad

Software, San Diego, CA, USA), and the IC_{50} values were determined as the concentration at which cell viability was reduced by 50%. The subtoxic threshold is determined as the concentration at which cell viability first decreased below 100% according to the 95% CIs (confidence intervals) of the curve fit.

All experimental results were determined in at least duplicate measures. The number of independent experiments performed is described in each figure or table legend. Statistical analysis is described in the individual figure legends.

Chemicals and reagents

Dulbecco's modified Eagle's medium, foetal calf serum, SYBR Green I nucleic acid gel stain, deoxyribonuclease (DNase) I, Moloney-murine leukaemia virus reverse transcriptase and TRIzol Reagent were purchased from Invitrogen (Mount Waverley, VIC, Australia). Mitoxantrone, vinblastine and THC were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). CBN was purchased from Australian Government Analytical Laboratories (Pymble, NSW, Australia) and CBD from Australian Laboratory Services Pty Ltd (Sydney, NSW, Australia). THC-A was purchased from Novachem Pty Ltd (Collingwood, VIC, Australia). Topotecan was the clinical formulation (hycamptin) provided by Glaxo Smith Kline, (Boronia, VIC, Australia). BXP-21 was provided by Dr George Scheffer (Free University Medical Centre, Amsterdam, The Netherlands).

Drugs were prepared as stock solutions prior to use in experiments. Mitoxantrone (50 mM) and Ko143 (1 mM) were dissolved in DMSO. Vinblastine (22 mM), CBD (30 mM), CBN (30 mM) and THC (86 mM) were dissolved in ethanol. Topotecan (10 mM) was dissolved in tartaric acid. THC-A was purchased as a 2.8 mM stock in methanol.

Results

Cell viability assays

Cell viability assays were conducted to confirm the resistant phenotype in the Abcg2 overexpressing MEF3.8/*Bcrp1* A2 cell line. The MEF3.8/*Bcrp1* A2 cells were found to have increased resistance to the cytotoxic action of the BCRP substrates, mitoxantrone (11 ×) and topotecan (64 ×) when compared to the parental MEF3.8 cell line (Table 1). This is consistent with increased expression of Abcg2 in the transduced subline (Allen *et al*., 1999). The MEF3.8/*Bcrp1* A2 cells were not resistant to vinblastine, as it is not an Abcg2 substrate (Litman *et al*., 2000). Taken together, this confirms that the resistant phenotype is due to Abcg2 overexpression. Interestingly, Abcg2 overexpression did not confer resistance to the cytotoxic effects of CBN, CBD or THC in the MEF3.8/*Bcrp1* A2 cell line (Table 1).

The effect of cannabinoids on BCRP substrate accumulation

The intracellular accumulation of the ABCG2 substrate, mitoxantrone, was assayed in MEF3.8 and MEF3.8/*Bcrp1* A2 cells using flow cytometry. The potent Abcg2 inhibitor Ko143 (Allen *et al*., 2002) was used as a positive control and comparison. CBN, CBD and THC significantly increased the accumulation of mitoxantrone (FLH-4) in the MEF3.8/*Bcrp1* A2 cell line in a dose-dependent manner (Figure 1).

None of these plant-derived compounds significantly affected the accumulation of mitoxantrone in the parental MEF3.8 cell line within the concentration range assayed, indicating that the effect observed is unique to the Abcg2 overexpressing cell line, as endogenous expression of Abcg2 in the MEF3.8 cell line is extremely low (Allen *et al*., 2000). A principal, non-psychotropic metabolite of THC was also assayed (THC-A). This compound reduced the intracellular accumulation of mitoxantrone in the parental cell line, while only marginally increasing the accumulation within the MEF3.8/*Bcrp1* A2 cell line. Thus, it was not further investigated.

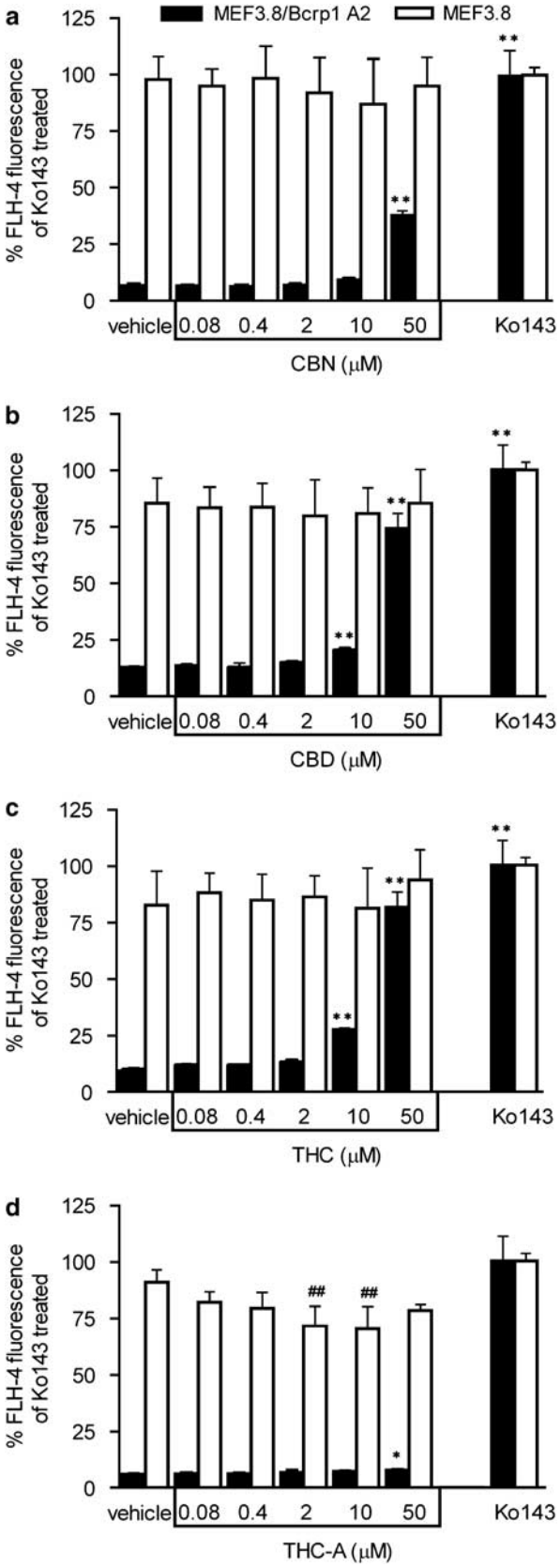
The effect of cannabinoids on ABCG2 ATPase activity

Using the SB-MXR-HAM-Sf9-ATPase assay kit, cholesterol-loaded Sf9 membranes overexpressing human wild-type ABCG2 were tested for ATPase activation and inhibition by the cannabinoids CBN, CBD and THC (Figure 2). All three compounds were found to significantly inhibit both the basal ATPase activity of ABCG2 and that stimulated by the known substrate, sulphasalazine (10 μM). Fitting the data

Table 1 Toxicity of cannabinoids in *Bcrp1*-overexpressing cells compared to known cytotoxic substrates

Drug	*Mean IC_{50} ± s.d.*		*Fold resistance*	*Subtoxic Threshold (MEF3.8, MEF3.8/Bcrp1 A2)*
	MEF3.8	*MEF3.8/Bcrp1 A2*		
Mitoxantrone (nM)	5.4 ± 1.7	59.3 ± 10.1**	10.94	ND
Topotecan (μM)	0.05 ± 0.01	3.04 ± 0.63*	63.52	ND
Vinblastine (nM)	0.58 ± 0.18	0.64 ± 0.23	1.10	ND
CBN (μM)	23.6 ± 0.8	22.3 ± 5.1	0.95	12.5, 11.03
CBD (μM)	23.3 ± 0.9	20.9 ± 3.2	0.90	12.5, 8.91
THC (μM)	18.6 ± 4.1	16.3 ± 3.9	0.88	12.5, 14.15

Abbreviations: CBD, cannabidiol; CBN, cannabinol; MEF, mouse embryonic fibroblasts; ND, not determined; THC, Δ^9-tetrahydrocannabinol.
Values shown are derived from 3 to 8 independent experiments, each conducted in quadruplicate. The statistical significance of the difference between the mean IC_{50} value for the MEF3.8 and MEF3.8/*Bcrp1* A2 cell lines was determined for each drug using an unpaired Student's *t*-test, with Welch's correction being applied where the assumption of equal variance had been violated. Fold resistance is determined as the IC_{50} for the MEF3.8/*Bcrp1* A2 cells/the IC_{50} for the parental MEF3.8 cell line.
**$P<0.01$, *$P<0.05$.



with a sigmoidal concentration–response curve revealed the 50% inhibitory concentration (IC_{50}) for basal activity to be 3.6 μM for CBD and 1.7 μM for THC. The IC_{50} values for the activity stimulated by 10 μM sulphasalazine were determined to be 4.5 μM for CBN, 7.3 μM for CBD and 4.4 μM for THC. The effect of cannabinoids on the basal and substrate stimulated activity of ABCG2 mimics that previously reported for the known ABCG2 inhibitor Ko143 (Allen *et al*., 2002; Pal *et al*., 2007). In contrast to other known wild-type ABCG2 substrates, no significant stimulation of basal ATPase activity was observed for any of the cannabinoids within the concentration range tested (80 nM–50 μM) (Pal *et al*., 2007).

Cannabinoid and vanilloid receptor expression

The antiproliferative effects of cannabinoids are mediated by the cannabinoid receptors CB_1 (Portella *et al*., 2003), CB_2 (McKallip *et al*., 2006) or TRPV1 (Contassot *et al*., 2004), depending on the receptor agonist or the cancer cell type investigated. In addition, receptor-independent mechanisms (Ruiz *et al*., 1999) may be involved. To investigate the potential role of these receptors in the cannabinoid-induced reduction in cell viability of MEF3.8 and MEF3.8/*Bcrp1* A2 cells (Table 1), the expression of the murine orthologues of CB_1, CB_2 and TRPV1 was assayed using reverse transcriptase PCR. Figure 3 shows that no mRNA transcripts for these receptors could be detected, indicating that the cytotoxic effects of cannabinoids in these mouse embryonic fibroblast cell lines occurs independently of these receptors. The quality of the template was confirmed by the amplification of a 723 bp fragment from the ubiquitously expressed β-actin (Actb) transcript. Genomic DNA was used as a positive control for the reaction and amplification conditions. The results are representative of three independent experiments.

Cannabinoids as chemosensitizers

To confirm that cannabinoid inhibition of Abcg2 activity will reverse the Abcg2-mediated multidrug-resistant phenotype, we assessed the ability of subtoxic concentrations of the cannabinoids (see Table 1) to sensitize the Abcg2 overexpressing cell line to the cytotoxic effects of the Abcg2 substrates, mitoxantrone (Table 2) and topotecan (Figure 4).

Subtoxic concentrations of CBN, CBD and THC significantly reduced the IC_{50} of mitoxantrone in the MEF3.8/*Bcrp1* A2 cell line in a dose-dependent manner. No significant change in the IC_{50} of mitoxantrone was observed in the parental MEF3.8 cell line. While the Abcg2-mediated resistance to mitoxantrone was not completely abolished

Figure 1 Mean ± s.d. mitoxantrone fluorescence (FLH-4) in MEF3.8 and MEF3.8/*Bcrp1* A2 cells. CBN (**a**), CBD (**b**), THC (**c**) and THC-A (**d**). The ABCG2/Abcg2 inhibitor Ko143 (1 μM) was used as a positive control and comparison. Fluorescence values were compared for each cell line to the value for vehicle in that cell line using one-way analysis of variance followed by Dunnett's post-test. $^{*}P<0.05$, $^{**}P<0.01$, $^{\#\#}P<0.01$, $n=4$. CBD, cannabidiol; CBN, cannabinol; MEF, mouse embryonic fibroblasts; THC, Δ^9-tetrahydrocannabinol; THC-A, (−)-11-nor-9-carboxy-Δ^9-THC.

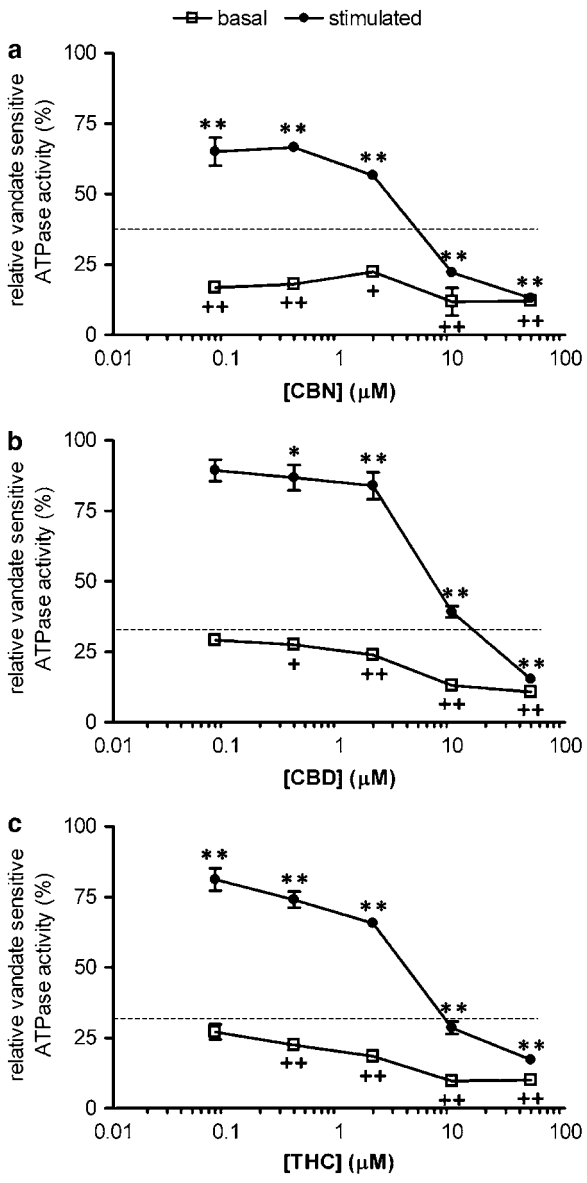


Figure 2 The mean (±s.e.m.) relative vanadate-sensitive ATPase activity of Sf9 insect cell membranes overexpressing wild-type ABCG2. Activity is represented as a percentage of that observed in the presence of the reference substrate sulphasalazine (10 μM). The basal ATPase activity of the membranes (–□–) in the presence of increasing concentrations of the cannabinoids CBN (**a**), CBD (**b**) and THC (**c**) was compared to the basal activity observed in the presence of vehicle (ethanol, dashed line), using one-way analysis of variance with Dunnett's post-test. $^{+}P<0.05$, $^{++}P<0.01$. The effect of CBN (**a**), CBD (**b**) and THC (**c**) on the vanadate-sensitive ATPase activity of ABCG2 when stimulated by the known substrate sulphasalazine (–●–) was compared with the activity in the presence of sulphasalazine and vehicle (ethanol), defined here as 100% activity $^{*}P<0.05$, $^{**}P<0.01$ ($n=2$–7). CBD, cannabidiol; CBN, cannabinol; MEF, mouse embryonic fibroblasts; THC, Δ^9-tetrahydrocannabinol; THC-A, (–)-11-nor-9-carboxy-Δ^9-THC.

in the MEF3.8/*Bcrp1* A2 cell line at the highest concentration of the cannabinoids (10 μM), it was reduced by approximately 4- to 6-fold, an effect of comparable magnitude to the positive control Ko143 (20 nM).

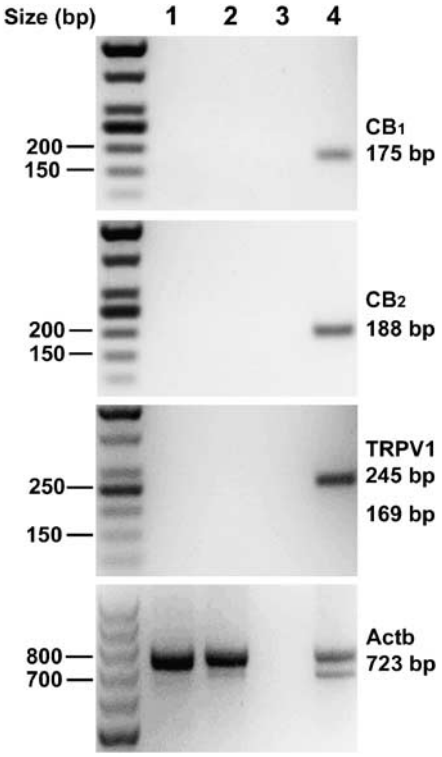


Figure 3 The expression of the cannabinoid receptors CB_1, CB_2 and the transient receptor potential vanilloid receptor subtype 1 (TRPV1) in MEF3.8 (CB_2 lane 1) and MEF3.8/*Bcrp1* A2 (lane 2) cells as determined using reverse transcriptase PCR. β-Actin (Actb) is a control for cDNA quality. Water was used as a negative control (lane 3), and genomic DNA was used as a positive control (lane 4). $n=3$; results from a single representative experiment are shown here. MEF, mouse embryonic fibroblasts.

A reduction in the IC_{50} of topotecan was also observed with 10 μM of each of the cannabinoids in the MEF3.8/*Bcrp1* A2 cell line (Figure 4), with no significant effect on the potency of topotecan in the MEF3.8 parental cell line. The effect of CBN was similar to that observed for Ko143 (20 nM) with the fold resistance (IC_{50} MEF3.8/*Bcrp1* A2 with modulator/IC_{50} MEF3.8 vehicle) being reduced from 60-fold for vehicle to 11-fold for Ko143, and to 8-, 41- and 42-fold for CBN, CBD and THC, respectively.

The effect of cannabinoids on ABCG2 expression

Previously we have reported that cannabinoids reduce the expression of the multidrug transporter P-gp (Holland *et al.*, 2006) in a drug-selected human cell line. Here we examined the effect of cannabinoids on the levels of ABCG2 in MEF3.8/*BCRP* and WiDr cells. The MEF3.8/*BCRP* cell line expresses human ABCG2 from an moloney murine leukemia virus longterminal repeat (MMLV LTR), as integrated provirus, as does the MEF3.8/*Bcrp1* A2 cell line used in the other assays described here. The lack of an antibody targeted to mouse Abcg2 meant that it was not possible to directly assess the levels of protein expression in the MEF3.8/*Bcrp1* A2 cells; therefore, the MEF3.8/*BCRP* cell line was employed here as an equivalent expression system. We wished to determine the effect of chronic (up to 72 h) cannabinoid exposure on

Table 2 Cannabinoid mediated chemosensitization to the cytotoxic effect of the BCRP substrate mitoxantrone

Substrate	*Cannabinoid concentration*	*2 μM*	*5 μM*	*10 μM*	*2 μM*	*5 μM*	*10 μM*
		MEF3.8 IC_{50} ± s.d. and (resistance factor)			*MEF3.8/Bcrp1 A2 IC_{50} ± s.d. and (resistance factor)*		
Mitoxantrone (nM)	Control		4.5 ± 2.4			139 ± 66.0 (31)	
	CBN	2.3 ± 0.4 (0.5)	3.2 ± 1.5 (0.7)	2.4 ± 2.7 (0.5)	94 ± 39.6 (21)	85 ± 21.5* (19)	29 ± 18.3** (6)
	CBD	2.8 ± 1.6 (0.6)	2.2 ± 2.1 (0.5)	3.1 ± 2.3 (0.7)	114 ± 17.5 (25)	41 ± 25.8** (9)	21 ± 13.3** (5)
	THC	3.2 ± 1.9 (0.7)	3.8 ± 3.5 (0.8)	4.3 ± 2.5 (1.0)	89 ± 43.4** (20)	65 ± 21.0** (14)	32 ± 5.7** (7)
	Ko143 (20 nM)		4.5 ± 3.0 (1.0)			21 ± 14.8** (5)	

ANOVA, analysis of variance; BCRP, breast cancer resistance protein; CBD, cannabidiol; CBN, cannabinol; MEF, mouse embryonic fibroblasts; THC, Δ^9-tetrahydrocannabinol.
Values shown are derived from 4 to 16 independent experiments. The resistance factor is calculated relative to the parental cell line in the presence of substrate alone. The statistical significance between the mean IC_{50} values for substrate alone and in the presence of a test compound were compared for each cell line using one-way ANOVA with Dunnett's post-test.
*$P<0.05$, **$P<0.01$.

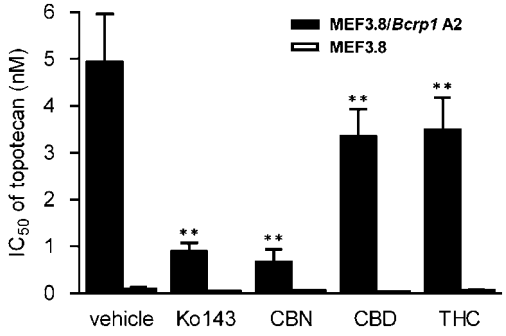


Figure 4 The mean ± s.d. IC_{50} of topotecan in the presence of Ko143 (20 nM) or CBN, CBD and THC (10 μM) for the MEF3.8 and MEF3.8/*Bcrp1* A2 cell lines. The statistical significance between the mean IC_{50} values for substrate alone and in the presence of a test compound were compared for each cell line using one-way analysis of variance with Dunnett's post-test. **$P<0.01$. $n=3$–9. CBD, cannabidiol; CBN, cannabinol; MEF, mouse embryonic fibroblasts; THC, Δ^9-tetrahydrocannabinol.

ABCG2 expression to determine if changes in expression levels were contributing to the effect of the cannabinoids on the cytotoxicity of ABCG2 substrates. Furthermore, by comparing expression in a transduced (MEF3.8/*BCRP*) and an endogenously expressing cell line (WiDr), we would obtain preliminary evidence as to whether any observed effects were transcriptional or post-transcriptional in origin. Neither a concentration nor a time-dependent effect (Figure 5) was seen for CBN, CBD or THC on the levels of ABCG2 when expression was driven from either the retroviral LTR or endogenous human promoter.

Discussion and conclusions

Recent research shows that cannabinoids inhibit the activity and expression of the ABC transporter P-gp (Holland *et al*., 2006; Nieri *et al*., 2006; Zhu *et al*., 2006). Here we present the

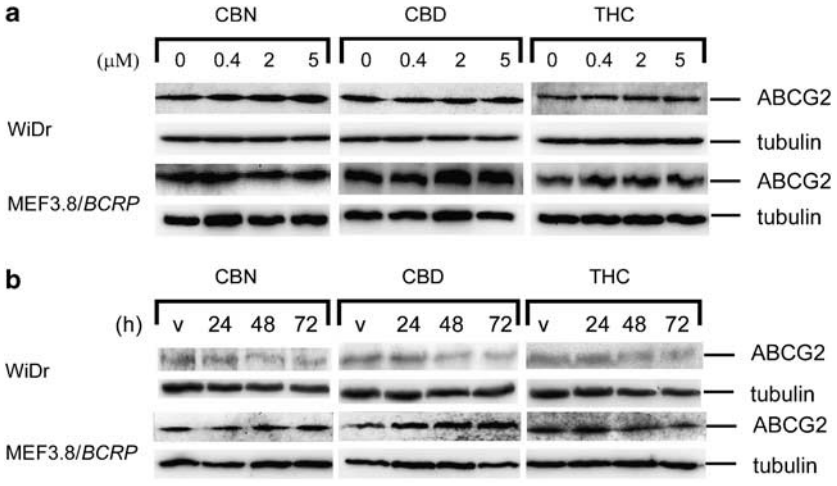


Figure 5 Western blot analysis of ABCG2 expression in WiDr human colon cancer cells and MEF3.8/*BCRP* (human wild-type ABCG2 transduced) cells. Cells were treated with increasing doses of the cannabinoids, cannabidiol (CBD), cannabinol (CBN), and Δ^9-tetrahydrocannabinol (THC) for 72 h (**a**), or with 5 μM of each cannabinoid for increasing periods of time (**b**). α-tubulin is used to demonstrate equal loading among samples. $n=2$, single experiment shown.

first *in vitro* evidence that plant-derived cannabinoids also inhibit activity of the related transporter ABCG2/Abcg2. CBN, CBD and THC increased the intracellular accumulation of the ABCG2 substrate mitoxantrone in an Abcg2 overexpressing cell line (MEF3.8/*Bcrp1* A2) with the THC metabolite (−)-11-nor-9-carboxy-Δ^9-THC demonstrating much weaker activity. In addition, CBN, CBD and THC inhibited both the basal ATPase activity of wild-type human ABCG2 and the activity stimulated by the known substrate sulphasalazine. Such results support the notion that plant cannabinoids directly interact with the transporter and inhibit activation by substrates. Although the cannabinoids were cytotoxic to MEF3.8 cells, this occurred at higher concentrations than those required for ABCG2 inhibition. Importantly, subcytotoxic concentrations of CBN, CBD and THC reversed the MDR phenotype of MEF3.8/*Bcrp1* A2 cells, resensitizing these cells to the cytotoxic actions of the Abcg2 substrates mitoxantrone and topotecan.

The MEF3.8 cell line and its *Bcrp1*- and *BCRP*-transduced sublines proved appropriate models, as seen in the large resistance factors for mitoxantrone and topotecan. These cell lines are derived from a double knockout of the mouse orthologues of both P-gp and multidrug resistance-related protein 1. Moreover, the expression of Mrp2 and Mrp3 is undetectable in the MEF3.8 cells (Allen *et al*., 2002). This eliminates the possibility that these transporters may contribute to any of the observed effects in this cell line. Interestingly, overexpression of Abcg2 did not confer resistance to the cytotoxic effect of the cannabinoids CBN, CBD or THC. The antiproliferative actions of cannabinoids have been attributed to activation of cell surface receptors such as CB_1, CB_2 and/or TRPV1 dependent on which cells are examined and the cannabinoid receptor agonist used (Portella *et al*., 2003; Contassot *et al*., 2004; McKallip *et al*., 2006). However, mRNA transcripts for the mouse orthologues of the CB_1, CB_2 or TRPV1 receptors could not be detected in the MEF3.8 cell line, ruling out their involvement in the cannabinoid-induced cytotoxicity observed here. Given the lipophilicity of these compounds and the high concentrations required for cytotoxicity, it is likely that the antiproliferative actions of cannabinoids in the MEF3.8 cell line are mediated by other intracellular actions. The lack of difference in sensitivity to cannabinoid-induced cytotoxicity in the parental and Abcg2 overexpressing cell lines implies that these agents are not Abcg2 substrates. Future studies are required to determine unambiguously whether cannabinoids are substrates for ABCG2.

CBN, CBD and THC selectively increased the intracellular accumulation of mitoxantrone in Abcg2 overexpressing cells, with no effect observed on the parental cells. This implies that the cannabinoids either inhibit the activity of Abcg2 and/or compete with mitoxantrone as a substrate for Abcg2. Unlike the plant-derived cannabinoids, the metabolite THC-A had a negligible, non-selective effect. Notably, THC-A is significantly less lipophilic than its parent compound THC and the other plant-derived cannabinoids CBD and CBN (Thomas *et al*., 1990; Skopp *et al*., 2002). The weaker modulation of Abcg2 by THC-A implies that relative hydrophobicity may be an important requirement for Abcg2 interaction, which provides evidence that Abcg2 may act in a similar manner to P-gp and recognizes substrates after partitioning within the inner leaflet of the membrane (Sharom, 2006). Consistent with this, Abcg2 has been found to redistribute the lipid phosphatidylserine from the inner to outer membrane leaflet (Woehlecke *et al*., 2003) and to transport the lipophilic substrate Hoechst 33342 from within the phospholipid bilayer into the aqueous lumen when expressed in inside-out vesicles (Janvilisri *et al*., 2003).

The modulatory actions of cannabinoids on Abcg2 activity appears to be due to a direct interaction with the transporter, as the cannabinoids THC, CBD and CBN inhibit wild-type human ABCG2-mediated ATPase activity in isolated cholesterol-loaded Sf9 insect membrane preparations. All compounds inhibited the ATPase activity of ABCG2 when stimulated by the substrate sulphasalazine confirming that inhibition of transport is not limited to a single substrate, with mitoxantrone transport also affected as demonstrated by the substrate accumulation assays. The inhibitory effects of CBN, CBD and THC on the substrate-stimulated ATPase activity of ABCG2 were concentration dependent, and more potent (IC_{50}: 4.4–7.3 μM) than that previously reported for CBD inhibition of P-gp (IC_{50}: 39.6 μM) (Zhu *et al*., 2006). In contrast to the stimulation of basal ATPase activity by CBN, CBD, THC and THC-A reported for P-gp (Zhu *et al*., 2006), we also observed a clear inhibition of basal ABCG2 ATPase activity by all three plant-derived compounds (IC_{50}: 1.7–4.4 μM). Indeed, the ATPase activation/inhibition profiles for the cannabinoids obtained here resemble those previously reported for the known ABCG2 inhibitor Ko143 (Allen *et al*., 2002; Pal *et al*., 2007). This contrasts with previous findings for the ABCG2 substrates, topotecan and prazosin, where stimulation of basal ATPase activity was observed in this system (Pal *et al*., 2007). These data together with the lack of reduced sensitivity to cannabinoid cytotoxicity in the Abcg2-overexpressing cell line suggests that these compounds are not effectively transported by ABCG2/Abcg2. However, this evidence is not conclusive, since the lipophilic ABCG2 substrate Hoechst 33342 has also previously been found to inhibit the basal activity of ABCG2 in this system (Pal *et al*., 2007). As cannabinoids are strongly lipophilic themselves (Thomas *et al*., 1990), it cannot be ruled out that they interact with ABCG2 in a similar manner to Hoechst 33342. Specific drug-binding sites in wild-type ABCG2 have not, to the best of our knowledge, yet been identified, so further speculation on the mechanism of cannabinoid inhibition of drug transport by ABCG2 is not possible.

Reinforcing our conclusion that cannabinoids inhibit ABCG2, we report here that cannabinoids act as chemosensitizers. Subtoxic cannabinoid concentrations reversed the resistance of the Abcg2 overexpressing cell line (MEF3.8/*Bcrp1* A2) to the cytotoxic effects of mitoxantrone and topotecan. Sensitization by cannabinoids was limited in the parental line suggesting the effect observed is Abcg2 specific. The resistance-reversing actions of the cannabinoids were concentration dependent, with a 4- to 6-fold reduction in the IC_{50} of mitoxantrone observed with the highest concentration tested (10 μM). This reduction in the IC_{50} was comparable to that observed with the known inhibitor of Abcg2/ABCG2 Ko143 (20 nM). The clinically employed Abcg2/ABCG2 substrate topotecan was also assessed, and

reversal in Abcg2-mediated resistance was observed in the presence of each of the cannabinoids (10 μM). *In vitro*, ABCG2 confers resistance to several clinically employed anticancer drugs, including mitoxantrone, bisantrene, topotecan, SN-38, 9-aminocamptothecin and flavopiridol (Doyle *et al*., 1998; Maliepaard *et al*., 1999; Kawabata *et al*., 2001; Robey *et al*., 2001). As such, the finding that cannabinoids can act as chemosensitizers *in vitro* suggests that cannabinoids may improve the efficacy of chemotherapeutic treatment where MDR is mediated by ABCG2.

Previously we reported that CBD and THC reduced the expression of P-gp in a human drug-selected cell line and that this conferred a degree of resensitization to the cytotoxic effects of P-gp substrates (Holland *et al*., 2006). To establish whether chronic cannabinoid exposure has any effect on ABCG2 expression, we examined ABCG2 protein levels by immunoblot after exposure to CBN, CBD or THC for 24–72 h. We found no change in ABCG2 expression levels relative to the cytoskeletal protein, α-tubulin in either a transduced or endogenously expressing cell line, indicating no transcriptional or post-transcriptional effects on protein expression. Therefore, we conclude that the resensitization by cannabinoids to the cytotoxic effects of ABCG2 substrates reported here is not due to a cannabinoid-induced reduction in ABCG2 expression.

In addition to its potential role in mediating multidrug resistance, the expression of ABCG2 at pharmacological barriers such as the intestinal epithelium has demonstrated *in vivo* importance in the disposition and pharmacokinetics of substrate drugs (Jonker *et al*., 2000; Kruijtzer *et al*., 2002). Thus, cannabinoid-mediated ABCG2 inhibition may have important implications for clinical effectiveness and/or toxicity of ABCG2 substrate drugs that are co-administered with cannabis or commercially available cannabinoid preparations. Despite the inhibitory action of cannabinoids on ABCG2 being more potent than previous reports for P-gp (Zhu *et al*., 2006), relatively high concentrations (greater than 2 μM) of these compounds were required to produce a significant resensitization to the effects of ABCG2 substrates. *In vivo*, cannabinoid blood levels are unlikely to reach this concentration (Goodwin *et al*., 2006). However, considerably higher concentrations at the intestinal epithelium might be achieved during oral dosing, as THC has a relatively low oral bioavailability of 10–20% (Wall *et al*., 1983). Further *in vivo* studies are required to assess the ability of cannabinoids to influence the disposition and pharmacokinetics of ABCG2 and P-gp substrate drugs.

To conclude, this is the first study to address the interaction of cannabinoids with the multidrug transporter ABCG2/Abcg2. The results presented here indicate that plant-derived cannabinoids are a novel class of ABCG2/Abcg2 inhibitors. Our results may have important implications for the use of cannabinoid compounds with therapeutic drugs that are substrates for ABCG2.

Acknowledgements

This work was supported by a Cancer Institute of NSW Research Scholar Award, and an Australian Postgraduate Award to ML Holland, and a National Health and Medical Research Council Project Grant and a Cancer Institute of NSW Senior Research Fellowship to JD Allen.

Conflict of interest

The authors state no conflict of interest.

References

Allen JD, Brinkhuis RF, van Deemter L, Wijnholds J, Schinkel AH (2000). Extensive contribution of the multidrug transporters P-glycoprotein and Mrp1 to basal drug resistance. *Cancer Res* **60**: 5761–5766.

Allen JD, Brinkhuis RF, Wijnholds J, Schinkel AH (1999). The mouse Bcrp1/Mxr/Abcp gene: amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone, or doxorubicin. *Cancer Res* **59**: 4237–4241.

Allen JD, van Loevezijn A, Lakhai JM, van der Valk M, van Tellingen O, Reid G *et al*. (2002). Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. *Mol Cancer Ther* **1**: 417–425.

Breedveld P, Zelcer N, Pluim D, Sonmezer O, Tibben MM, Beijnen JH *et al*. (2004). Mechanism of the pharmacokinetic interaction between methotrexate and benzimidazoles: potential role for breast cancer resistance protein in clinical drug–drug interactions. *Cancer Res* **64**: 5804–5811.

Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC *et al*. (1992). Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258**: 1650–1654.

Contassot E, Wilmotte R, Tenan M, Belkouch MC, Schnuriger V, de Tribolet N *et al*. (2004). Arachidonylethanolamide induces apoptosis of human glioma cells through vanilloid receptor-1. *J Neuropathol Exp Neurol* **63**: 956–963.

Cooray HC, Blackmore CG, Maskell L, Barrand MA (2002). Localisation of breast cancer resistance protein in microvessel endothelium of human brain. *NeuroReport* **13**: 2059–2063.

Diestra JE, Scheffer GL, Catala I, Maliepaard M, Schellens JH, Scheper RJ *et al*. (2002). Frequent expression of the multi-drug resistance-associated protein BCRP/MXR/ABCP/ABCG2 in human tumours detected by the BXP-21 monoclonal antibody in paraffin-embedded material. *J Pathol* **198**: 213–219.

Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK *et al*. (1998). A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* **95**: 15665–15670.

Goodwin RS, Gustafson RA, Barnes A, Nebro W, Moolchan ET, Huestis MA (2006). Delta(9)-tetrahydrocannabinol, 11-hydroxy-delta(9)-tetrahydrocannabinol and 11-nor-9-carboxy-delta(9)-tetrahydrocannabinol in human plasma after controlled oral administration of cannabinoids. *Ther Drug Monit* **28**: 545–551.

Guzman M (2003). Cannabinoids: potential anticancer agents. *Nat Rev Cancer* **3**: 745–755.

Guzman M, Duarte MJ, Blazquez C, Ravina J, Rosa MC, Galve-Roperh I *et al*. (2006). A pilot clinical study of delta9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. *Br J Cancer* **95**: 197–203.

Holland ML, Panetta JA, Hoskins JM, Bebawy M, Roufogalis BD, Allen JD *et al*. (2006). The effects of cannabinoids on P-glycoprotein transport and expression in multidrug resistant cells. *Biochem Pharmacol* **71**: 1146–1154.

Janvilisri T, Venter H, Shahi S, Reuter G, Balakrishnan L, van Veen HW (2003). Sterol transport by the human breast cancer resistance protein (ABCG2) expressed in *Lactococcus lactis*. *J Biol Chem* **278**: 20645–20651.

Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JH *et al*. (2000). Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J Natl Cancer Inst* **92**: 1651–1656.

Juliano RL, Ling V (1976). A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* **455**: 152–162.

Kawabata S, Oka M, Shiozawa K, Tsukamoto K, Nakatomi K, Soda H *et al.* (2001). Breast cancer resistance protein directly confers SN-38 resistance of lung cancer cells. *Biochem Biophys Res Commun* **280**: 1216–1223.

Kruijtzer CM, Beijnen JH, Rosing H, ten Bokkel Huinink WW, Schot M, Jewell RC *et al.* (2002). Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. *J Clin Oncol* **20**: 2943–2950.

Litman T, Brangi M, Hudson E, Fetsch P, Abati A, Ross DD *et al.* (2000). The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). *J Cell Sci* **113** (Part 11): 2011–2021.

Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH *et al.* (2001). Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res* **61**: 3458–3464.

Maliepaard M, van Gastelen MA, de Jong LA, Pluim D, van Waardenburg RC, Ruevekamp-Helmers MC *et al.* (1999). Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. *Cancer Res* **59**: 4559–4563.

McKallip RJ, Jia W, Schlomer J, Warren JW, Nagarkatti PS, Nagarkatti M (2006). Cannabidiol-induced apoptosis in human leukemia cells: a novel role of cannabidiol in the regulation of p22phox and Nox4 expression. *Mol Pharmacol* **70**: 897–908.

Nieri P, Romiti N, Adinolfi B, Chicca A, Massarelli I, Chieli E (2006). Modulation of P-glycoprotein activity by cannabinoid molecules in HK-2 renal cells. *Br J Pharmacol* **148**: 682–687.

Pal A, Mehn D, Molnar E, Gedey S, Meszaros P, Nagy T *et al.* (2007). Cholesterol potentiates ABCG2 activity in a heterologous expression system: improved in vitro model to study function of human ABCG2. *J Pharmacol Exp Ther* **321**: 1085–1094.

Portella G, Laezza C, Laccetti P, De Petrocellis L, Di Marzo V, Bifulco M (2003). Inhibitory effects of cannabinoid CB1 receptor stimulation on tumor growth and metastatic spreading: actions on signals involved in angiogenesis and metastasis. *FASEB J* **17**: 1771–1773.

Robey RW, Medina-Perez WY, Nishiyama K, Lahusen T, Miyake K, Litman T *et al.* (2001). Overexpression of the ATP-binding cassette half-transporter, ABCG2 (Mxr/BCrp/ABCP1), in flavopiridol-resistant human breast cancer cells. *Clin Cancer Res* **7**: 145–152.

Ruiz L, Miguel A, Diaz-Laviada I (1999). Delta9-tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells via a receptor-independent mechanism. *FEBS Lett* **458**: 400–404.

Sarkadi B, Price EM, Boucher RC, Germann UA, Scarborough GA (1992). Expression of the human multidrug resistance cDNA in insect cells generates a high activity drug-stimulated membrane ATPase. *J Biol Chem* **267**: 4854–4858.

Sharom FJ (2006). Shedding light on drug transport: structure and function of the P-glycoprotein multidrug transporter (ABCB1). *Biochem Cell Biol* **84**: 979–992.

Skopp G, Potsch L, Mauden M, Richter B (2002). Partition coefficient, blood to plasma ratio, protein binding and short-term stability of 11-nor-delta(9)-carboxy tetrahydrocannabinol glucuronide. *Forensic Sci Int* **126**: 17–23.

Steinbach D, Sell W, Voigt A, Hermann J, Zintl F, Sauerbrey A (2002). BCRP gene expression is associated with a poor response to remission induction therapy in childhood acute myeloid leukemia. *Leukemia* **16**: 1443–1447.

Thomas BF, Compton DR, Martin BR (1990). Characterization of the lipophilicity of natural and synthetic analogs of delta 9-tetrahydrocannabinol and its relationship to pharmacological potency. *J Pharmacol Exp Ther* **255**: 624–630.

Tramer MR, Carroll D, Campbell FA, Reynolds DJ, Moore RA, McQuay HJ (2001). Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. *BMJ* **323**: 16–21.

van den Heuvel-Eibrink MM, Wiemer EA, Prins A, Meijerink JP, Vossebeld PJ, van der Holt B *et al.* (2002). Increased expression of the breast cancer resistance protein (BCRP) in relapsed or refractory acute myeloid leukemia (AML). *Leukemia* **16**: 833–839.

Wall ME, Sadler BM, Brine D, Taylor H, Perez-Reyes M (1983). Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. *Clin Pharmacol Ther* **34**: 352–363.

Woehlecke H, Pohl A, Alder-Baerens N, Lage H, Herrmann A (2003). Enhanced exposure of phosphatidylserine in human gastric carcinoma cells overexpressing the half-size ABC transporter BCRP (ABCG2). *Biochem J* **376**: 489–495.

Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ *et al.* (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* **7**: 1028–1034.

Zhu HJ, Wang JS, Markowitz JS, Donovan JL, Gibson BB, Gefroh HA *et al.* (2006). Characterization of P-glycoprotein inhibition by major cannabinoids from marijuana. *J Pharmacol Exp Ther* **317**: 850–857.