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The effects of cannabinoids on P-glycoprotein transport and expression in multidrug resistant cells

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ARTICLE INFO

Article history:
Received 8 October 2005
Accepted 27 December 2005

JEL classification:
Antibiotics and chemotherapeutics

Keywords:
P-glycoprotein
Multiple drug resistance
Cannabinoids
Cannabidiol
Δ^9-Tetrahydrocannabinol
Palliative care

Abbreviations:
MDR, multiple drug resistance
P-gp, P-glycoprotein
THC, Δ^9-tetrahydrocannabinol
CBD, cannabidiol
CBN, cannabinol
Rh123, Rhodamine 123
WIN, WIN 55, 212-2
Et-743, Ecteinascidin-743

ABSTRACT

Cannabis is the most widely used illicit drug in the world. Cannabinoids are used therapeutically by some patients as they have analgesic, anti-emetic and appetite stimulant properties which palliate adverse symptoms. Use of these agents in an oncology setting raises the question of whether they act to modulate the effectiveness of concurrently administered anti-cancer drugs. The transporter, P-glycoprotein (P-gp) confers multiple drug resistance (MDR) by effluxing a diverse array of anti-cancer agents. This study was undertaken to examine the effect of cannabinoids on P-gp. Unlike the known P-gp inhibitor, PSC833, short 1 h exposure to three plant-derived cannabinoids, cannabinol (CBN), cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) and the synthetic cannabinoid receptor agonist, WIN55, 212-2 (WIN) did not inhibit the efflux of the P-gp substrate Rhodamine 123 (Rh123) in either a drug-selected human T lymphoblastoid leukaemia cell line (CEM/VLB_{100}) or in a mouse fibroblast *MDR1* transfected cell line (77.1). However, in CEM/VLB_{100} cells, prolonged 72 h exposure to the cannabinoids, THC and CBD, decreased P-gp expression to a similar extent as the flavonoid, curcumin (turmeric). This correlated with an increase in intracellular accumulation of Rh123 and enhanced sensitivity of the cells to the cytotoxic actions of the P-gp substrate, vinblastine. Taken together, these results provide preliminary evidence that cannabinoids do not exacerbate P-gp mediated MDR. Further, plant-derived cannabinoids are moderately effective in reversing MDR in CEM/VLB_{100} cells by decreasing P-gp expression.

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2005.12.033

1. Introduction

Cannabinoids are plant-derived, synthetic or endogenous compounds that produce their pharmacological actions by interacting with the endocannabinoid system [1]. In recent times there has been a resurgence of interest in the therapeutic application of cannabinoids with many governments decriminalizing the use of these compounds for medicinal purposes. Indeed, clinical studies have provided evidence for the effectiveness of cannabinoids in treating conditions such as multiple sclerosis [2] and chronic neuropathic pain [3]. In the treatment of cancer, the analgesic, anti-emetic and mood enhancing qualities of cannabinoids have justified their use as palliative care agents [4]. The emerging use of cannabinoids in this context raises the important question of whether they act to modulate the effectiveness of concurrently administered anti-cancer treatments.

One of the major issues influencing the outcome of anticancer treatment is the ability of tumour cells to develop resistance to chemotherapeutic agents [5]. Of particular concern is the phenomenon of multiple drug resistance (MDR). This form of resistance results from a mechanism that simultaneously confers resistance to many xenobiotics despite an unrelated chemical structure or target of action [6]. The most extensively characterised mechanism of MDR results from the over-expression of the ATP-binding cassette transporter, P-glycoprotein (P-gp). P-gp acts in an energy dependent manner to efflux a diverse range of large, hydrophobic, clinically employed anticancer agents such as anthracyclines, vinca alkaloids, taxol and podophyllotoxin derivatives [7]. Efflux prevents intracellular accumulation of the anticancer agents and subsequent interaction with their respective drug targets.

Clinical studies have demonstrated that human tumours arising from tissues which constitutively express P-gp are inherently multidrug resistant [8]. P-gp expression is also found de novo in a variety of lymphomas and leukaemias, or on relapse following chemotherapy induction [9]. The expression of P-gp is considered an adverse prognostic factor in adult acute myeloid leukaemia as it is strongly correlated with a reduced remission rate and a higher incidence of refractory disease [10,11]. Given the clinical relevance of P-gp mediated MDR, considerable effort has been expended in developing compounds capable of modulating P-gp activity as a means of reversing the MDR phenotype. Such compounds have two potential mechanisms through which they may act to increase the accumulation and efficacy of chemotherapeutic agents: (1) functional inhibition of P-gp mediated transport and/or (2) a reduction in P-gp expression.

There have been many functional inhibitors of P-gp identified, including verapamil [12] and PSC833 [13]. However, the results of clinical trials with these agents were disappointing due to dose limiting toxic effects of the modulator [14] or increased toxicity of the co-administered chemotherapies [15]. Considerably fewer compounds that down-regulate P-gp expression have been identified. Two such compounds are curcumin [16,17] and Ecteinascidin-743 (Et-743) [18]. However, neither of these compounds are yet to reach the clinical trial stage of development for their MDR modulating properties. Accordingly, the search for clinically applicable P-gp modulators continues.

Over 60 different cannabinoid compounds are produced by the cannabis plant, *Cannabis sativa*. Three well-characterized plant-derived cannabinoids are the main psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). It has been previously reported that CBN (6.44 μM) and CBD (6.34 μM) functionally inhibit P-gp mediated efflux of the fluorescent substrate Rhodamine 123 (Rh123) in a *MDR1* transfected mouse T lymphoma cell line (L5178) [19]. Given the need to find a clinically applicable MDR reversal agent this preliminary finding warrants further investigation. Furthermore, the increasing use of cannabinoids in the palliative care of cancer patients reinforces the need for studies assessing the interaction of these drugs with proteins responsible for MDR, such as P-gp.

The present study was undertaken to further characterize the ability of cannabinoids to act as P-gp mediated MDR reversal agents. Three plant-derived cannabinoids, CBN, CBD and THC and the synthetic cannabinoid, WIN55, 212-2 (WIN) were assayed for their ability to act as functional inhibitors of P-gp. In addition, the possibility that CBD and THC may down-regulate P-gp expression in a drug selected human T lymphoblastoid cell line was investigated.

2. Materials and methods

2.1. Cell lines and culture conditions

The acute T lymphoblastoid leukaemia cell line (CCRF-CEM) and the P-gp over expressing, multidrug resistant sub line (CEM/VLB_{100}), were kindly donated by Prof. M. Haber (Children's Cancer Institute Australia, Sydney, NSW, Australia). The CEM/VLB_{100} cell line expresses high levels of P-gp as a result of *MDR1* gene amplification [20,21]. Both cell lines were cultured in RPMI-1640 (Invitrogen Australia, Mount Waverly, VIC) supplemented with 10% (v/v) foetal calf serum (FCS) (Invitrogen Australia, Mount Waverly, VIC). Cell density was maintained between 10^5 and 10^6 cells/ml, and cultures were limited to 12 consecutive passages. Mouse 77.1 fibroblast cells lacking functional *Mdr1a* and *Mdr1b* genes and transfected with human *MDR1* cDNA [22] were kindly provided by Prof. A. Schinkel (Netherlands Cancer Institute, Amsterdam). These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen Australia, Mount Waverly, VIC) supplemented with 10% (v/v) FCS. Cell cultures were maintained within the exponential phase of growth. Cell cultures were maintained within the exponential phase of growth in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C.

2.2. Cell viability assays

The MTS assay is a colorimetric assay for mitochondrial oxidative metabolism that is used as a measure of cell viability. In order to determine the maximum sub-lethal concentrations of CBN, CBD (Australian Government Analytical Laboratories, Pymble, NSW), THC (Sigma–Aldrich, Sydney, NSW) and WIN (kindly donated by A/Prof. I. McGregor, Department of Psychology, University of Sydney, NSW,