New hydantoin derivatives: promising candidates for anticancer therapy

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ABSTRACT

Objectives
Hydantoin derivatives have various biochemical and pharmacological properties such as anticonvulsant, fungicidal, herbicidal, antitumor, anti-inflammatory, anti-HIV, hypolipidemic, antiarrhythmic and antihypertensive activities. Although hydantoin compounds are studied extensively, there are not many studies that investigate their multidrug resistance reversing effect, such as bacterial efflux pump modulating activity and anticancer properties. The aim of this study was to investigate the efflux pump modulating and apoptosis inducing activity of new hydantoin derivatives on mouse T-lymphoma cells overexpressing the P-glycoprotein (ABCB1) and the apoptosis inducing activity of these derivatives on colon carcinoma model using Colo 205/S sensitive and Colo 320/R resistant colon carcinoma cells having an over-expressed ABCB1 system.

Methods
The hydantoin derivatives were evaluated for their efflux modulating effects in cancer cells using fluorescence activated cell sorting measuring the accumulation of rhodamine 123 and their apoptosis inducing effect using Annexin V-FITC labeling in fluorescence activated cell sorting.

Results
In cancer cells, the compounds investigated were not cytotoxic and some of them significantly increased the retention of the P-glycoprotein substrate rhodamine 123 in L5178Y mouse T-lymphoma cells, furthermore eight compounds showed synergistic effect with the anticancer drug doxorubicin. The hydantoin derivatives could increase the intracellular accumulation of rhodamine 123 in multidrug resistant Colo 320 cells, however they could not induce the apoptosis of Colo 320 cells.

Conclusion
In cancer cells, some derivatives can be promising candidates to treat the P-glycoprotein related resistance. The most active structures contained aromatic substituents as well as some tertiary amine fragments. Surprisingly, the derivatives did not induce apoptosis on Colo 320/R resistant colon carcinoma cells, indicating that these hydantoin compounds are potent efflux pump inhibitors (EPI) without affecting the signaling pathways that regulate apoptosis.

INTRODUCTION and OBJECTIVES

Hydantoin derivatives possess a variety of biochemical and pharmacological properties. Although hydantoins have been in use for a long time, the anticancer activity of these derivatives has received scant attention in the last decades.

Aims of the study:

Reversal of effluf pump related multidrug resistance by hydantoin derivatives

1. Characterization of the the ABCB1 efflux pump modulating effect of previously selected hydantoin derivatives on multidrug resistant human T-lymphoma cells and multidrug resistant colon adenocarcinoma cells (Colo 320)

2. Characterization of the apoptosis inducing effect of the selected hydantoin derivatives on multidrug resistant human T-lymphoma cells and multidrug resistant colon adenocarcinoma cells (Colo 320)

3. Determination of the structure-activity relationships (QSAR)

MATERIALS AND METHODS

Cell lines: L5178 (parental, PAR) mouse T-cell lymphoma cells and the human *ABCB1* (*MDR1*)-transfected subline (MDR); human colon adenocarcinoma cell lines (Colo 205 doxorubicin sensitive parent and Colo 320/MDR-LRP resistant to anti-cancer agents expressing ABCB1 (MDR1)-LRP), ATCC-CCL-220.1 (Colo 320) and CCL-222 (Colo 205), were purchased from LGC Promochem, Teddington, England.

Media: McCoy's 5A medium, supplemented with 10% heat-inactivated horse serum, L-glutamine and antibiotics; RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM Na-pyruvate and 100 mM Hepes.

Compounds: Ethidium bromide (Sigma), Verapamil (Sigma), thirty hydantoin derivatives **(SZ-2, SZ-7, LL-9, BS-1, JH-63, MN-3, TD-7k, GG-5k, P3, P7, P10, P11, RW-15b, AD-26, RW-13, AD-29, KF-2, PDPH-3, Mor-1, KK-XV, Tioam-1, JHF-1, JHC-2, JHP-1, Fur-2, GL-1, GL-7, GL-14, GL-16, GL-18)** were tested, kindly provided by dr. Jadwiga Handzlik and Prof. Dr. Katarzyna Kieć-Kononowicz, Cracow, Poland). The compounds were dissolved in DMSO, the structures are confidential.

Assay for antiproliferative and cytotoxic effect. The effects of increasing concentrations of the drugs alone on cell growth were tested in 96-well flat-bottomed microtitre plates using MTT (thiazolyl blue tetrazolium bromide, Sigma, St. Louis, MO, USA) The cell growth was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Inhibition of the cell growth was determined by calculation of ID_{50} values, where ID_{50} is defined as the inhibitory dose that reduces the growth of the compound-exposed cells by 50%.

Real-time fluorometry was performed using our previously developed semi-automated method (1).

Assay for apoptosis induction was carried out using Annexin V-FITC Apoptosis Detection Kit Cat. No. PF 032 from Calbiochem according to the manufacturer's instructions.

Flow cytometry assay for evaluation of a compound on the retention of rhodamine 123 by MDR in tumour cells. The fluorescence uptake of the cell population was measured with FACStar Plus flow cytometer (Beckton, Dickinson and Company, Franklin Lakes, NJ, USA). Verapamil was used as a positive control in the rhodamine 123 exclusion experiments. The percentage mean fluorescence intensity was calculated for the treated MDR and parental cell lines as compared to untreated cells (2,3).

Checkerboard microplate method was applied to study the drug interactions between resistance modifiers and anticancer drugs on cancer cells. The interaction of the anticancer drug doxorubicin and the resistance modifiers hydantoins was studied in combination on MDR mouse T-lymphoma cells. The cell growth rate was determined after MTT staining and the intensity of the blue colour was measured with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Drug interactions were evaluated according to the following system (ID=inhibitory dose, FIC=fractional inhibitory concentration, FIX=fractional inhibitory index, $FIC_A = ID_{50A}$ in combination / ID_{50A} alone, $FIC_B = ID_{50B}$ in combination / ID_{50B} alone, $FIX = FIC_A + FIC_B$,

FIX = 0.51-1	Additive effect
FIX < 0.5	Synergism
1 < FIX < 2	Indifferent effect
FIX > 2	Antagonism

RESULTS

Figure 1. Accumulation of EB (1 mg/L) by human *MDR1 (ABCB1)* gene-transfected mouse T-lymphoma cells in the presence of 4 and 40 mg/L of **AD-29**

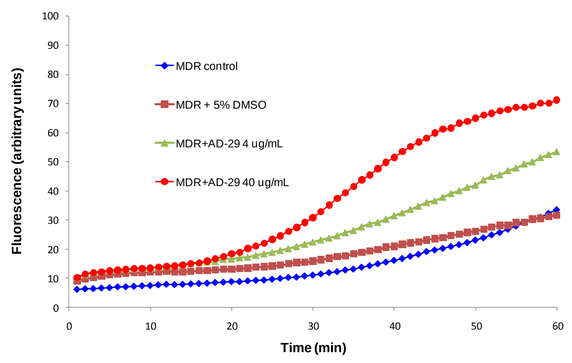

Figure 2. Schematic representation of the function of P-glycoprotein. (Red objects represent substrates, e. g. EB, rhodamine 123)

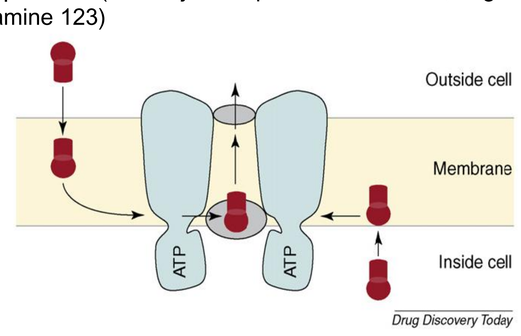

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Table 1. Effect of hydantoin derivatives on inhibition of ABCB1 transporter (P-glycoprotein) on human *MDR1 (ABCB1)* gene-transfected **mouse T-lymphoma cells**: the most effective derivatives

Samples	mg/L	FAR
Verapamil	10	13.19
BS-1	0.4	46.71
BS-1	4	77.68
BS-1	40	174.35
JH-63	0.4	9.60
JH-63	4	57.05
JH-63	40	107.62
MN-3	0.4	4.64
MN-3	4	59.95
MN-3	40	196.42
AD-26	0.4	1.33
AD-26	4	28.12
AD-26	40	144.86
AD-29	0.4	2.01
AD-29	4	8.65
AD-29	40	180.77
DMSO	4%	0.77

Figure 3. Interaction between hydantoin derivatives (**SZ-7, BS-1, MN-3** and **JH-63**) and doxorubicin on *MDR1*-gene transfected mouse lymphoma cell line by checkerboard microplate method

Compound	FIX values	Interaction
SZ-7	1.252	Indifferent
BS-1	0.217	Synergism
MN-3	0.311	Synergism
JH-63	0.493	Synergism

Interaction between doxorubicin and BS-1
MC: medium control, CC: cell control

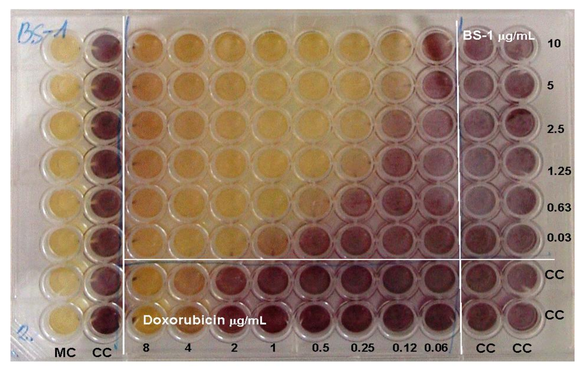

Table 2. Effect of selected hydantoin derivatives on rhodamine 123 retention by multidrug resistant **Colo 320/R colon adenocarcinoma cells**: the most effective derivatives

Samples	mg/L	FAR
Verapamil	10	3.71
BS-1	4	13.36
BS-1	40	13.44
SZ-7	4	3.07
SZ-7	40	7.04
JH-63	4	10.59
JH-63	40	9.86
MN-3	4	8.41
MN-3	40	12.59
AD-26	4	2.98
AD-26	40	8.83
AD-29	4	1.81
AD-29	40	5.06
RW-13	4	3.93
RW-13	40	7.01
DMSO	4%	0.82

FAR: fluorescence activity ratio (values were calculated by using the equation given in the experimental section). Verapamil was used as a positive control.

BUT NO APOPTOSIS INDUCTION!

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CONCLUSIONS

- The studied hydantoin derivatives are not toxic, they can **inhibit the ABCB1 multidrug transporter** system of multidrug resistant mouse T-lymphoma cells **without inducing apoptosis**
- Some derivatives showed **synergistic effect with the anticancer drug doxorubicin** on multidrug resistant mouse T-lymphoma cells
- The investigated hydantoin compounds are potent ABCB1 inhibitors in multidrug resistant Colo 320 colon adenocarcinoma cells **without affecting the signalling pathways that regulate apoptosis**
- The chemical **modifications within the hydantoin ring** can influence the inhibition of the ABCB1 transporter
- The most active structures contained **aromatic substituents as well as some tertiary amine fragments**

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