Abstract

Overexpression of some ATP-binding cassettes (ABC) family protein such as P-glycoprotein and MRPI may confer multidrug resistance to cancer cells. A new ABC protein, the breast cancer resistance protein (BCRP, also called MXR or ABC-P) was described independently by three groups. BCRP/MXR/ABCP was first identified from non-P-glycoprotein non-MRP1 overexpressing MCF7/Advp cells. It is a half ATP binding protein. BCRP/MXR/ABCP was overexpressed in several mitoxantrone selected multidrug resistant cells. These cell lines were often cross-resistant to some camptothecin analogues such as topotecan. BCRP/MXR/ABCP was overexpressed in a topotecan selected breast carcinoma cells MCF7/TPT300. Topotecan efflux was markedly enhanced. However, multiple mechanisms including down-regulation of topoisomerase I were often found in these multidrug resistant cancer cells. Thus, the role and strength of BCRP/MXR/ABCP in topotecan resistance remains unclear. In order to elucidate this, BCRP/MXR/ABCP transfected breast carcinoma cell may be useful. In this study, cytotoxicity test showed that BCRP/MXR/ABCP transfected MCF7 breast carcinoma cells is 15-fold resistant to empty vector control MCF7/pCDNA3 cells. The mechanism of topotecan resistance may be due to the increase efflux shown by flowcytometry in MCF7/pCDNA-BCRP
cells. Thus, BCRP does play important role in topotecan resistance in cancer cells.

二、緣由與目的

Drug resistance and ABC family protein in cancer chemotherapy

Drug resistance is the major obstacle to successful cancer chemotherapy. A large number of membrane, cytosolic and nuclear protein changes may be present in cells resistant to chemotherapeutic agents. Among them, p-glycoprotein, multidrug resistance-associated protein 1 overexpression was found to be associated with decreased drug accumulation, increased drug efflux and multidrug resistance in cancer cells. Several mdr1 or MRP1 homologues have been described in the literature. These proteins contain similar backbone, i.e. proteins 170-200KD in size, 2 ATP-binding regions, and 12 transmembrane domains. They are called ATP-binding cassette family proteins.

BCRP/MXR/ABCP in cancer chemotherapy

Recently, the breast cancer resistance protein (BCRP, also called MXR or ABC-P) was described independently by three groups. BCRP/MXR/ABCP was primarily found from a non-P-glycoprotein, non-MRP1 over-expressing multidrug resistant MCF7/Advp cells. It is a half size ATP-binding cassette protein that confers resistance to mitoxantrone and anthracyclines.

MCF7/TPT cells and topotecan resistance

We have developed a topotecan resistant cancer cell line from wild type MCF7 cells. MCF7/TPT300 cells were 68.9-fold resistant to topotecan, 68.3-fold to SN-38, 116-fold to mitoxantrone but only 4.1-fold resistant to camptothecin. Topotecan efflux was increased in MCF7/TPT300 cells compared to MCF7/WT cells. Topotecan efflux in MCF7/TPT300 cells was decreased when ATP was depleted by sodium azide. MCF7/TPT cells did not overexpress P-glycoprotein or the multidrug resistance-associated protein (MRP1). In contrast, overexpression of BCRP/MXR/ABCP was observed in MCF7/TPT300 cells. Our data suggest that enhanced topotecan efflux contributed to topotecan resistance in MCF7/TPT300 cells, possibly mediated by BCRP/MXR/ABCP. However, our preliminary study also revealed that topoisomerase I was down-regulated in MCF7/TPT300 cells. Despite the similarity of the cross-resistance phenotype between MCF7/TPT300 cells and other mitoxantrone selected cells, MCF7/TPT300 cells only weakly overexpressed BCRP/MXR/ABCP and MCF7/TPT50 cells did not express it at all. Therefore, the role of BCRP/MXR/ABCP in topotecan resistance is not completely clear in MCF7/TPT300 cells.

BCRP/MXR/ABCP overexpression in other multidrug resistant cancer cells

BCRP was overexpressed in some mitoxantrone resistant cell lines that were cross-resistant to topotecan (MCF7/MX³, MCF7/AdVp and EPG85-257RNOV). BCRP overexpression was found in topotecan- or mitoxantrone-selected cell lines (T8 and MX3, respectively), derived from the human IGROV1 ovarian cancer cell line. However, resistance to topotecan in BCRP/MXR/ABCP transfected cells has not yet been demonstrated.

BCRP/MXR/ABCP transfected breast carcinoma cells

Most of the multidrug resistant cancer
cells developed from escalating concentrations of chemotherapeutic agents contain multiple mechanism to evade from xenobiotics’ insult. For example, down regulation of topoisomerase I and overexpression of BCRP/MXR/ABCP were both noted in MCF7/TPT300 cells. Confounded by many other contributory factors, the role of BCRP in topotecan resistance can not be completely determined in these multidrug resistant cells. It is better to test topotecan toxicity, accumulation and efflux in BCRP/MXR/ABCP transfected cells.

Objectives of this project:
To test the contribution of BCRP/MXR/ABCP to topotecan resistance. IC50s of topotecan will be measured in BCRP/MXR/ABCP transfected MCF7 and their empty plasmid counterpart. Topotecan accumulation and efflux will be determined in transfected cells and their control as well.

Importance of this study:
Characterization of MX/TPT resistance may improve topotecan, CPT-11 and mitoxantrone treatment in cancer patients. Elucidation of the role of BCRP/MXR/ABCP in topotecan resistance may help us to explore topotecan and/or mitoxantrone resistance in tumor samples.

Specific aim
To investigate topotecan cytotoxicity and topotecan accumulation and efflux in MCF7/pcDNA3 and MCF7/BCRP cells.

Lane 1: MCF7/pcDNA3-BCRP
Lane 2: MCF7/pcDNA3
Lane 3: MCF7/TPT300
Lane 4: MCF7/WT

BCRP/MXR/ABCP is overexpressed in MCF7/pcDNA3-BCRP and MCF7/TPT300 cells.

3. Cytotoxicity test measured by SRB method. MCF7/pcDNA3 and MCF7/BCRP cells were distributed in 96-well culture plates. Various concentrations of topotecan were added to the cells growing at 37°C in D-MEM containing 10% fetal calf serum in triplicate. After 96 hours, the survival fraction was measured by the sulforhodamine B method.

Figure 2
Figure 2 showed represented cytotoxicity curve of MCF7/pcDNA3 (◆) and MCF7/pcDNA3-BCRP(■) in topotecan. IC50 of MCF7/pcDNA3 was 12uM and of MCF7/pcDNA3-BCRP was 180uM. BCRP transfected cells conferred 15-fold topotecan resistance to MCF7 cells.

4. Topotecan accumulation measured by flowcytometry. MCF7/pcDNA3 and MCF7/BCRP are trypsinized, centrifuged, washed with serum free DMEM and resuspended in PBS or Hank’s Balanced Salt Solution at 10^5 cells/ml. Various concentrations of topotecan are then added to the cells. After incubation in 37°C for 15 min, each sample is subjected to flowcytometry on a FACSscan. A 15nW argon laser will be used to deliver a 488-nm excitation to the cells. Fluorescence in the cells is detected using a 585-nm filter with a band width of 42nm. The efflux of topotecan was determined after the steady state of accumulation of topotecan is achieved. The topotecan-accumulated cells were centrifuged and re-suspended in HBSS to measure topotecan efflux. After re-suspension in HBSS for different periods of time, each sample is subjected to flowcytometric measurement. Approximately 1000 events will be recorded at each time point.

Figure 3

Figure 3 showed a typical topotecan efflux curve of MCF7/pcDNA3 and MCF7/pcDNA3-BCRP cells. X-axis denotes efflux time in minutes, Y-axis denote fold-of-increase of fluorescence in background. The efflux curve shows that MCF7/pcDNA3-BCRP cells retain less topotecan than MCF7/pcDNA3 cells. However, compare to MCF7/TPT300 cells, MCF7/pcDNA-BCRP cells retained more topotecan in cells, especially during first 5 minutes.

四、計劃成果自評

Our results demonstrate clearly that BCRP confer resistance to topotecan in BCRP/MXR/ABCP transfected MCF7/pcDNA3-BCRP cells. The mechanism of topotecan resistance is most likely due to increased efflux in BCRP transfected cells. BCRP/MXR/ABCP contribute to topotecan resistance. This model may be used to test inhibitors of BCRP resistance.

五、參考文獻