

## Diatrizoate, Iopromide and Iotrolan Enhanced Cytotoxicity of Daunorubicin in Multidrug Resistant K562/adr Cells: Impaired the Mitochondrial and Inhibited the P-Glycoprotein Function

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**Abstract:** Multidrug resistance was an obstacle in cancer chemotherapy because the cells decreased their intracellular drug accumulation by energy-dependent compounds efflux pumps such as P-glycoprotein (P-gp). This study observed some iodinated radiographic contrast media, diatrizoate, iopromide and iotrolan affected the cellular energetic state and the kinetics of P-gp in drug-sensitive K562 and drug resistant K562/adr cell lines using spectrophotometer and spectrofluorometer. By colorimetric MTT assay, it was found that contrast media (0-3500  $\mu$ M) had no effect on both K562 and K562/adr cell viabilities, but in co-treatment with daunorubicin (DNR), diatrizoate decreased cell viability in K562/adr cells by decreasing  $IC_{50}$  of DNR from  $610.7 \pm 74.5$  nM to  $360 \pm 108.9$  nM. The change in cellular energetic state was studied using rhodamine B as a probe to estimate mitochondrial membrane potential ( $\Delta\Psi_m$ ). The results showed that 3500  $\mu$ M diatrizoate decreased  $\Delta\Psi_m$  from  $162.2 \pm 0.3$  mV to  $86.9 \pm 9.9$  mV in K562/adr cells. The kinetics of P-gp-mediated efflux of DNR could be reduced by diatrizoate from 0 (no inhibition) to  $0.65 \pm 0.11$ . This inhibition could be partially prevented in co-incubation with 20 nM concanamycin A or 10  $\mu$ M cytochalasin B. Among the three molecules, diatrizoate showed the best efficiency. It could be proposed for further studies that diatrizoate could be used as MDR identification or MDR imaging and also acted as MDR sensitizing agent in cancer treatments.

**Key words:** Contrast media, Multi Drug-Resistance (MDR), mitochondrial membrane potential ( $\Delta\Psi_m$ ), P-glycoprotein-mediated efflux

### INTRODUCTION

Iodinated radiographic contrast media are used in clinical practice as diagnostic pharmaceuticals to indicate the site and size of pathology, including cancer staging. The most widely used contrast media are water soluble, fully substituted, triiodinated benzoic acid derivatives. They are commonly classified according to two characteristics, ionic versus non ionic and monomer versus dimer<sup>[1]</sup>. Tolerability of modern contrast media is very high that the huge amount (up to 100-200 g in total number) of drug can be injected as a bolus with a side effect coincidence of less than 5%<sup>[2]</sup>. However, the administered contrast media into blood vessels (about 1-2 mL  $kg^{-1}$ ,  $\sim 300$  mg of iodine per milliliter, 600-1500 mOsm) generated a concentration-

dependent effect on neutrophils. In particular, ionic contrast media dramatically decreased in the mitochondrial membrane potential ( $\Delta\Psi_m$ ) of neutrophils, affecting the membrane permeability that would allow the anion portion to actually penetrate into the cells<sup>[3]</sup>. The same effects of contrast media on mitochondrial activity and on reduction of ATP level in LLC-PK1 and HRPTE cells were reported<sup>[4]</sup>. Moreover, some radiographic contrast media such as iohexol, a monomer-non ionic contrast medium affect MRP2- and P-gp-mediated transport of Fluorescein-Methotrexate (FL-MTX) and a fluorescent cyclosporine derivative (NBD-CSA) in intact killifish renal proximal tubules<sup>[5]</sup>. However, the direct effects of contrast media on the MDR transporters such as P-glycoprotein and MRP1 did not study yet. Such activities of these hyperdensity

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molecules could be clearly demonstrated by using multidrug-resistant cancer cell lines.

In this study, the erythromylogenous leukemic K562 and its P-glycoprotein overexpression K562/adr cells were used for investigating the effects of contrast media on the function of P-glycoprotein and the mitochondrial function. All the contrast media used affected the cellular energetic state only in MDR cells by decreasing in  $\Delta\Psi_m$  and in the P-glycoprotein function leading to the restoration of intracellular daunorubicin concentration consequently the cytotoxicity was increased in the drug-resistant cells. The results provide a new possible application of contrast media other than usual utilization. The combination with appropriate mitochondrial probe that accumulated in the cells depending on  $\Delta\Psi_m$ , the contrast media could be used as a mitochondrial energetic state modulator that was specific to MDR cells.

## MATERIALS AND METHODS

**Drugs and Chemicals:** Adriamycin (Dox) and daunorubicin (DNR) were kindly provided by Professor Arlette Garnier-Suillerot, Laboratoire de Chimie Physique Biomoléculaire et Cellulaire, UFR Sante Medicine et Biologie Humaine, Bobigny, Université de Paris Nord, France. Iodinated radiographic contrast media, diatrizoate (Angiografine, Schering AG, Germany), iopromide (Ultravist, Schering AG, Germany) and iotrolan (Isovist, Schering AG, Germany) were kindly provided by Department of Radiology, Sappasitthiprasong Hospital, Ubonratchatani, Thailand. Rhodamine B and tetrazolium salt (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were from Amresco. Concanamycin A and cytochalasin B were from Sigma Chemical Co.

Deionized double distilled water was used throughout the experiments for solutions and buffers. Stock solutions of adriamycin (Dox) and daunorubicin (DNR) were prepared in water just before use and their concentrations were determined by UV-Visible Spectrophotometer, using a molar extinction coefficient of  $11500 \text{ M}^{-1} \text{ cm}^{-1}$  at  $480 \text{ nm}$ <sup>[6]</sup>. The dilutions of contrast media (diatrizoate, iopromide and iotrolan) were freshly prepared before used in water from known concentrations of these compounds in the commercially available solutions, stated in the manufacturer's information. Stock solution of MTT was prepared by dissolving  $5 \text{ mg MTT mL}^{-1}$  ( $12 \text{ mM}$ ) in HEPES- $\text{Na}^+$  buffer and filtered through a  $0.22 \mu\text{m}$  filter then stored at  $4^\circ\text{C}$ . A stock solution of cytochalasin B was prepared

in DMSO (dimethyl sulphoxide) and stored at less than ( $-20^\circ\text{C}$ ). The absorption spectra were recorded on a Hewlett Packard G1103A spectrophotometer and the fluorescence spectra were recorded on a Perkin Elmer LS 50 B spectrofluorometer.

**Cell lines and cell culture conditions:** The adriamycin-sensitive erythromylogenous leukemic cell line, K562 and its P-glycoprotein-overexpressing K562/adr cells<sup>[6,7]</sup> were kindly provided by Professor Arlette Garnier-Suillerot, Laboratoire de Chimie Physique Biomoléculaire et Cellulaire, UFR Sante Medicine et Biologie Humaine, Bobigny, Université de Paris Nord, France. These cells were routinely cultured in RPMI 1640 medium supplemented with 1% penicillin-streptomycin (Sigma Chemical Co.) and 10% fetal bovine serum (Gibco Biocult Ltd.) at  $37^\circ\text{C}$  in a humidified incubator at 95% air and 5%  $\text{CO}_2$ . The resistant K562/adr cells were cultured with  $100 \text{ nM}$  doxorubicin two weeks before the experiments. The cultures were initiated at a density of  $1 \times 10^5 \text{ cells mL}^{-1}$ , grew exponentially to about  $8 - 10 \times 10^5 \text{ cells mL}^{-1}$  in 3 days. For the assays, the cultures were initiated at  $5 \times 10^5 \text{ cells mL}^{-1}$  to have cells in the exponential growth phase and were used 24 h later after reaching a density of approximately  $8 - 10 \times 10^5 \text{ cells mL}^{-1}$ . Cell viability was assessed by Trypan blue exclusion. The number of cells was determined by a haemocytometer.

**Cytotoxicity assay:** The viability of cells was determined using colorimetric MTT- reduction assay with some modification<sup>[7,8]</sup>. Briefly, cells ( $5 \times 10^4 \text{ cells mL}^{-1}$ ) were incubated in RPMI-1640 medium supplemented with 10% fetal bovine serum in the presence of various concentrations of contrast media (diatrizoate, iopromide, or iotrolan) at  $37^\circ\text{C}$  in a humidified incubator with 95% air and 5%  $\text{CO}_2$ . After 72 h,  $2 \text{ mM}$  final concentration MTT was added to the cell suspension and then further incubated for 4 h. The concentration of daunorubicin and pirarubicin required to inhibit cell proliferation by 50% when measured at 72 h ( $\text{IC}_{50}$ ) was determined by plotting the percentage of cell growth inhibition versus drug concentration. The Resistance Factor (RF) was defined as the  $\text{IC}_{50}$  of resistant cells divided by the  $\text{IC}_{50}$  of sensitive cells and was equal to 40.

**Co-treatment of daunorubicin and contrast media:** Cells ( $5 \times 10^4 \text{ cells mL}^{-1}$ ) were incubated in RPMI-1640 medium supplemented with 10% fetal bovine serum in the presence of various concentrations of daunorubicin and a fixed concentration of 0.5, 2.0 and 3.5 mM contrast media (diatrizoate, iopromide, or iotrolan). The

experiments were performed and calculated using colorimetric MTT- reduction assay as described previously. The efficacy of contrast media ( $\delta$ ) in increasing the efficacy of daunorubicin was obtained by the following equation:

$$\delta = [\text{IC}_{50(\text{R})} - \text{IC}_{50(\text{RCM})}] / [\text{IC}_{50(\text{R})} - \text{IC}_{50(\text{S})}]$$

where  $\text{IC}_{50(\text{R})}$  was the concentration of daunorubicin that inhibited 50% of MDR cell growth,  $\text{IC}_{50(\text{RCM})}$  was the concentration of drug that inhibited 50% of MDR cell growth in the presence of contrast media and  $\text{IC}_{50(\text{S})}$  is the concentration of drug that inhibited 50% of drug-sensitive cell growth<sup>[9]</sup>.

The  $\delta$  was equal to 0 when the MDR cells were treated with daunorubicin alone and  $\delta$  was equal to 1 when the contrast media reverse 100% of the MDR phenotype resulting in the same  $\text{IC}_{50(\text{RCM})}$  as that of drug sensitive cell line.

**The effect of contrast media on mitochondrial membrane potential ( $\Delta\Psi_m$ ):** The mitochondrial membrane potential ( $\Delta\Psi_m$ ) was measured using a non-invasive functional study<sup>[10]</sup>, which can be used to determine and to monitor a spontaneous change in mitochondrial functions in drug-sensitive and drug-resistant cells. Cells ( $2 \times 10^6$  cells) were incubated with various concentrations of contrast media, diatrizoate, iopromide and iotrolan in 2 mL of HEPES-  $\text{Na}^+$  buffer with 40 nM rhodamine B in 1 cm quartz cuvette and vigorously stirred at 37°C. The rhodamine B fluorescence intensity (F) at 582 nm (excited at 553 nm) was monitored as a function of time. The addition of 200  $\mu\text{M}$  MTT were performed within 10 min of incubation, yielding a progressive decrease in rhodamine B fluorescence intensity. The estimation of  $\Delta\Psi_m$  was done using Nernst equation:  $C_m^o/C_i^o = 10^{(\Delta\Psi_m F / 2.303 RT)}$  and  $\Delta\Psi_m = -61.51 \log V_i - 258.46$ , where  $\Delta\Psi_m$  is the mitochondrial membrane potential in mV, RT/F is 26mV at 37°C,  $C_i^o$  is 40 nM and  $V_i$  is in  $\text{nM.s}^{-1}$ .

**Modulation of kinetics of the p-glycoprotein-mediated efflux of daunorubicin by contrast media:** Cells ( $2 \times 10^6$  cells) were incubated in 2 mL HEPES- $\text{K}^+$  buffer in the absence of glucose under continuous stirring at 37 °C in 1-cm quartz cuvette. The extracellular pH ( $\text{pH}_e$ ) of 7.25 was chosen all the experiments to be equal to the pH of cytoplasm since the cytosolic pH in K562/adr cells was shown to be within the range of 7.2-7.3<sup>[11]</sup>. Initially, the cells were

depleted energy by adding 10 mM  $\text{NaN}_3$ , an inhibitor of cytochrome oxidase in mitochondrial electron transport chain for 30 min, leading to depletion of ATP in these cells about 90%. The cells remained viable throughout the experiment as checked by trypan blue exclusion. Daunorubicin (DNR) was added to cell suspension at concentration of 1  $\mu\text{M}$ . The fluorescence intensity of DNR at 590 nm (excited at 480 nm) was spectrofluorometrically followed as a function of time. A decrease in fluorescence intensity was observed during incubation with cells, due to quenching of fluorescence after the intercalation of DNR between the base pairs of DNA. At the first steady state, the intracellular free drug concentration  $C'_i$  was equal to the free drug concentration in the extracellular medium ( $C_e$ ) when  $\text{pH}_i$  equals  $\text{pH}_e$ . Then 5mM glucose was added at  $t = t_{\text{glu}}$ , after the sequential addition of various concentrations of contrast media resulting in an increasing the fluorescence intensity at 590 nm. The slope of the tangent to the curve was  $(dF/dt)_{\text{tglu}}$  and the kinetics of release of DNR ( $V_a$ ) was:  $V_a = (dF/dt)_{\text{tglu}} \cdot (C_T/F_0)$  in  $\mu\text{M.s}^{-1}$ . The mean rate constant ( $k_a$ ) of P-glycoprotein-mediated can be written as:  $k_a = V_a/n(C_T - C'_i)$  in  $\text{s}^{-1}$ . The ability of contrast media to inhibit the P-glycoprotein active efflux of a drug can be determined using the ratio of  $k_a^i / k_a^o$  where  $k_a^i$  and  $k_a^o$  were stand for the P-glycoprotein -mediated efflux coefficient in the presence and in the absence of contrast media, respectively. When  $k_a^i / k_a^o$  is equal to 1 that means there is no inhibition of active efflux and to 0 means the P-glycoprotein active efflux is completely blocked.

## RESULTS AND DISCUSSION

**Enhancement of daunorubicin cytotoxicity:** All contrast media studied at the concentrations up to 3.5 mM did not inhibit the cell growth of both K562 and K562/adr cell line. Contrary to daunorubicin, an anticancer drug belongs to anthracycline family,  $\text{IC}_{50}$  value is  $17.5 \pm 0.1$  nM for K562 and  $610 \pm 74$  nM for K562/adr cells. It was clear that the co-treatment using daunorubicin and various concentration of diatrizoate, iopromide or iotrolan (0.5, 2.0 and 3.5 mM) did not affect the  $\text{IC}_{50}$  of daunorubicin in drug-sensitive K562 cells. However, these compounds increased the efficacy of daunorubicin when similar conditions of series of experiments were performed with drug-resistant K562/adr cells (Fig. 1). The capabilities of 0.5, 2.0 and 3.5 mM diatrizoate in increasing the efficacy of DNR ( $\delta$ ) were 0.11, 0.17 and 0.45, respectively.

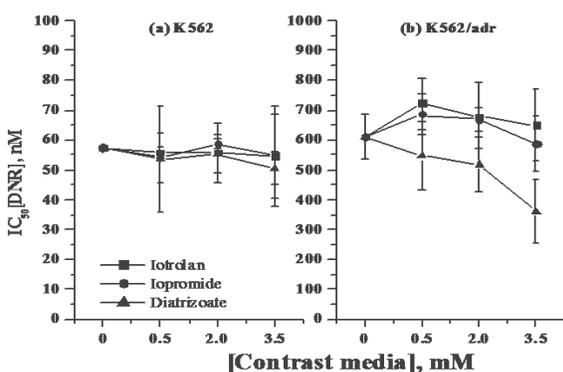


Fig. 1: Concentration of daunorubicin required to inhibit cell growth by 50% (IC<sub>50</sub>) in the presence of contrast media; iotrolan (■), iopromide (●), and diatrizoate (▲) in (a) K562 cell line and (b) K562/adr cell line. Cells ( $5 \times 10^4$  cells mL<sup>-1</sup>) were incubated with various concentrations of daunorubicin and a fixed concentration of contrast media (0.5, 2.0 or 3.5 mM) in 24-well plates at 37°C for 72 h. The cells were harvested after 4-hour of 2 mM MTT incubation. The formazan was spectrophotometrically measured at 560 nm. Each value is the mean±SD of three independent experiments

**Inhibition of the P-glycoprotein-mediated efflux of daunorubicin by contrast media:** The effects of contrast media on the P-glycoprotein function could be determined by monitoring the efficiency of protein mediated efflux of daunorubicin in the absence and presence of contrast media. The  $k_a$  of daunorubicin in the absence of contrast media was equal to  $0.67 \pm 0.06$  pL · cell<sup>-1</sup> · sec<sup>-1</sup>. The efficacy of contrast media that can inhibit the function of P-glycoprotein in the presence of 0.5, 2.0 and 3.5 mM of diatrizoate, iopromide or iotrolan were indicated in Fig. 2.

In fact, the contrast media are hyperdensity molecules that affected the cellular morphology, which could be easily observed by light microscope. Cell shrinkage was immediately occurred after contrast media addition to the cells and then they returned to normal morphology in a short time. This phenomenon could affect various cellular physiologies of cells such as acidification of the pH<sub>i</sub>, then followed by a correction of volume then pH<sub>i</sub>, leading to modifications in protein synthesis<sup>[12]</sup> and particularly, the change in membrane conformation could influence the function of P-gp.

In order to verify the direct interaction of contrast media on P-gp, two series of experiments were performed. The first set of experiments, the P-

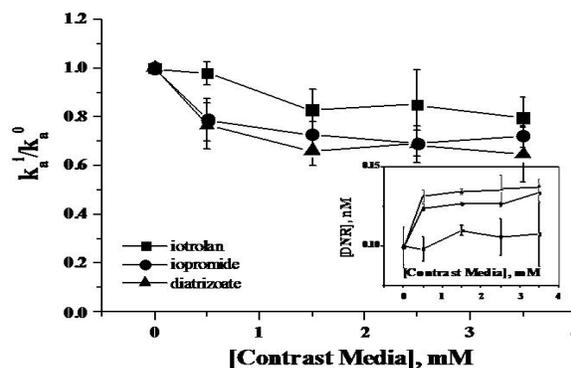


Fig. 2: Ratio of the mean P-gp-mediated efflux of daunorubicin in the presence to the absence of iotrolan (■), iopromide (●) or diatrizoate (▲) in K562/adr cell line. The experiments were performed by following the fluorescence intensity of 1 μM DNR ( $\lambda_{ex} = 480$  nm,  $\lambda_{em} = 590$  nm) in  $2 \times 10^6$  cells in 2 mL HEPES K<sup>+</sup>buffer, pH 7.25 at 37°C, which were depleted energy by adding 10 mM NaN<sub>3</sub> 30 min before daunorubicin addition. After the first steady state, 5 mM glucose was added before addition of contrast media then the active efflux coefficient of P-gp ( $k_a$ ) was measured. Each value represents the mean±SD of three independent experiments. Inset The overall cellular accumulation of daunorubicin in K562/adr cells in the absence or presence of iotrolan (■), iopromide (●) and diatrizoate (▲) at various concentrations

glycoprotein-mediated efflux of daunorubicin was measured in the MDR cells after treated using 10 μM cytochalasin B for 24h in the presence or absence of contrast media. Furthermore, using cytochalasin B, a chemical that inhibits polymerization of F-actin, that is one of important mechanisms of endocytosis or fixing the membrane movements<sup>[13,14]</sup>. The second set of experiments, the P-glycoprotein-mediated efflux of daunorubicin was measured in the MDR cells after treated using 20 nM concanamycin A for 10 minutes. Concanamycin A was used to inhibit the action of vacular H<sup>+</sup>-translocating ATPases that presence at the lysosomal membrane but did not inhibit the P-glycoprotein mediated-efflux of drugs<sup>[15,16]</sup>. Indeed, these conditions of experiments would protect the contrast media uptake by MDR cells. Figure 3a clearly showed that there was an increase in the P-glycoprotein-mediated efflux of daunorubicin in the presence of 3.5 mM diazoate. Similar results were obtained when the series of experiments with similar

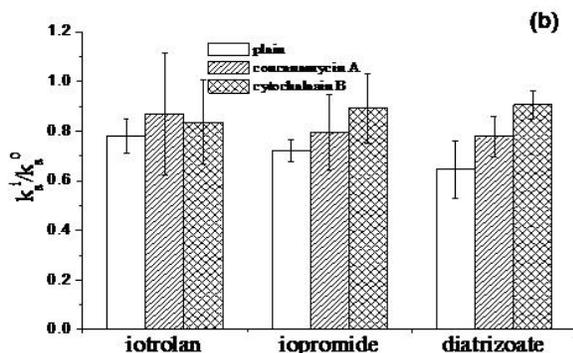
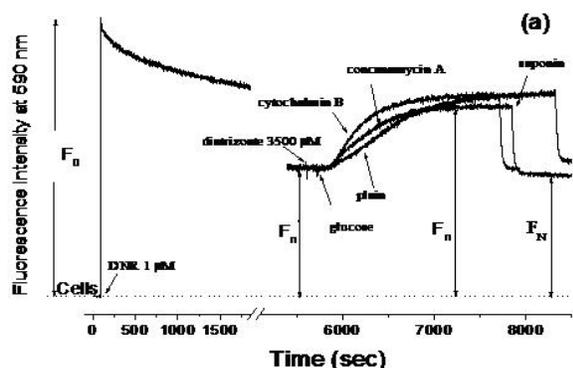


Fig. 3: Effects of 20 nM concanamycin A, or 10 µM cytochalasin B on P-gp-mediated efflux in K562/adr cells, which was modulated by 3.5 mM contrast media, (a) the kinetic studies in the presence of diatrizoate without and with 20 nM concanamycin A or 10 µM cytochalasin B, and (b)  $k_a^i/k_a^0$  in the presence of CM without (□) and with 20 nM concanamycin A (▨) or 10 µM cytochalasin B (▩). Each value is the mean±SD of three independent experiments

conditions were performed using iopromide or iotrolan. Figure 3b demonstrates that an increase in the ratio of  $k_a^i/k_a^0$  of the series of experiments with treatment with non-treatment ( $p < 0.05$ ).

**The effect of contrast media on mitochondrial membrane potential ( $\Delta\Psi_m$ ):** The uptake of contrast media by cells would be caused an additional consumption of ATP which can be resulted in a depletion of cellular ATP contents which is insufficient to assure the P-glycoprotein function. These impaired cellular energetic state can directly studied by measuring the mitochondrial membrane potential ( $\Delta\Psi_m$ ). The  $\Delta\Psi_m$  values determined for K562 and drug-resistant K562/adr cells were  $166.2 \pm 5.7$  mV and  $162.2 \pm 0.3$  mV, respectively. It was found that among

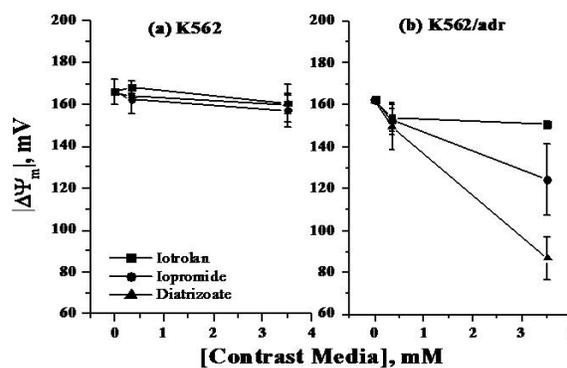


Fig. 4: Variation of the absolute values of the mitochondrial membrane potential ( $\Delta\Psi_m$ ) in intact cells (a) K562 and (b) K562/adr in the presence of iotrolan (■), iopromide (●) and diatrizoate (▲) at concentration of 0, 350 and 3500 µM. Each value is the mean±SD of three independent experiments

the contrast media used, diatrizoate (0.35, 3.5 mM) that apparently decreased  $\Delta\Psi_m$  in K562/adr cells, whereas iopromide could do with less efficiency and iotrolan was the least to do. Otherwise, in K562 cell line, there were no differences between treated cells and control (Fig. 4).

The iodinated radiographic contrast media used in this study included diatrizoate (an ionic monomer), iopromide (a non-ionic monomer) and iotrolan (a non-ionic dimer). These molecules are intravenously administered in patients undergo Intravenous Pyelography (IVP) or computed tomography, which are the standard imaging modalities used to radiologically stage some cancers<sup>[17]</sup>. The cytotoxicity of contrast media was determined by the cell growth inhibition dose ( $IC_{50}$ ). At the concentrations used in the experiments, they did not affect the cell proliferation of K562 and its corresponding MDR cell line. These concentrations were comparable to and did not over-exceed the concentration administered in clinical uses (the average clinical concentration in blood circulation after IV. injection is about  $2.5 \text{ mgI mL}^{-1}$ , dose recommended by Schering AG,  $1 \text{ mL of } 300 \text{ mgI mL}^{-1}$  of contrast media per 1 kg body weight, whereas concentrations in this study are  $0.15 - 2.66 \text{ mgI mL}^{-1}$ ). Although the contrast media themselves at the concentrations used in this study were not toxic to the cells, they (especially diatrizoate, an ionic monomer) were found, in co-treatment with daunorubicin, to enhance the efficiency of this anti-cancer drug to inhibit cell proliferation in the resistant K562/adr cell line. It

means that contrast media affected the function of P-glycoprotein, which was an active drug efflux transporter in this MDR cell line, therefore increasing the cytotoxicity of daunorubicin.

The study on the change of cellular energetic state was done by following the change in mitochondrial membrane potential ( $\Delta\Psi_m$ ) of drug-sensitive and drug-resistant cells using rhodamine B. The mitochondrial membrane potential is essential for the production of ATP via oxidative phosphorylation. In situ, it is a sensitive indicator for the energetic state of the mitochondria and cells. It should be noted that even a slight decrease in the  $\Delta\Psi_m$  could affect the cellular energetic state<sup>[9,10]</sup>. Indeed, the three molecules of contrast media were able to decrease  $\Delta\Psi_m$  in drug-resistant K562/adr cells, but not in drug sensitive K562 cells, with different degree. The aptitude to induce a decrease in  $\Delta\Psi_m$  could be obviously seen in diatrizoate at high concentration, thus this reduced intracellular ATP. This was reasonably supported by the findings in literatures<sup>[18,19]</sup> that intracellular ATP could be reduced following the exposure of diatrizoate in animal kidney cells, which were also expressed some drug transporters such as MRP2 and P-gp<sup>[5]</sup>. However, the co-incubation with contrast media did not affect the cytotoxicity of daunorubicin in drug-sensitive K562 cells, in comparison with drug-resistant K562/adr cells, which need more ATP to assure their ATPase activity of protein membrane transporter, i.e. P-glycoprotein. The function of P-glycoprotein was lessened resulting in an increase in intracellular cytotoxic agent, leading to MDR cell death<sup>[20]</sup>.

*In vitro* and *in vivo* MDR cell imaging is of uppermost importance for successful cancer chemotherapy. In this work, it was demonstrated that diatrizoate could probably be used as modulator of  $\Delta\Psi_m$  in combination with some mitochondrial probes such as, for *in vitro* studies, DiOC<sub>6</sub>, JC-1<sup>[21,22]</sup> and rhodamine derivatives<sup>[10]</sup> and for *in vivo* studies, <sup>99m</sup>Tc-sestamibi<sup>[23]</sup>. With an appropriate choice of probe, this modulation could be employed to distinguish the MDR cells or even to make the image of MDR cells, because only the MDR cells, herein K562/adr, would be affected by diatrizoate. This is the case, for example, of <sup>99m</sup>Tc-sestamibi, the  $\gamma$ -emitting probe that was administered in patients for localizing and imaging cancer in diagnostic nuclear medicine. Upon addition of diatrizoate, the chemo-resistant cancer cells would be recognized in low profile compared to the responsive cells.

The efficacies of diatrizoate, iopromide and iotrolan to inhibit the P-glycoprotein-mediated

daunorubicin efflux in MDR cells were as well observed. This work had investigated the aptitude of these contrast media to inhibit the P-glycoprotein-mediated efflux by following the uptake of daunorubicin by K562/adr cells, demonstrated with the ratio  $k_a^i/k_a^0$  as a function of contrast medium concentration added. Among the three molecules, diatrizoate had the highest capacity to inhibit the P-glycoprotein-mediated efflux. Furthermore, this was confirmed by treating experimental MDR cells with concanamycin A and cytochalasin B. Since the active binding sites of protein membrane transporter, P-glycoprotein, were located intracellularly, any molecules or compounds that regulated P-glycoprotein had to do from inside of the cell. Considerately, the physicochemical properties of contrast media determined (unpublished data) and referred to in literatures<sup>[24,25]</sup>, diatrizoate, iopromide and iotrolan could uptake into cells by endocytosis where molecules were engulfed and retained in vacuoles, which represented a part of lysosomal compartment<sup>[24]</sup>. Concanamycin A was used to inhibit the action of vacuolar H<sup>+</sup>-translocating ATPases that presented on membrane of lysosome, but did not inhibit the P-glycoprotein-mediated efflux of drug<sup>[15]</sup>. In addition, cytochalasin B was used to inhibit a wide variety of cellular movements that is one of important mechanisms of endocytosis by interacting with microfilaments in the cells<sup>[13]</sup>. These two compounds prevented contrast media to get inside the cell, leading to higher capability of P-glycoprotein-mediated efflux, when compared with the conditions that contrast media were added alone. The efficacy of contrast media, especially diatrizoate, to impair the function of P-gp seemed very apparent but the mechanism(s) of this modulation still was not clear whether they were substrates of P-gp or they affected the energy consumption of P-gp as mentioned above.

In general, most studies have focused on the effects of contrast media on kidney cells since they were excreted by nephron units after intravenous administration into patients. This is the first study to show that they also affected cellular energetic state and the function of P-glycoprotein in MDR cancer cells.

## CONCLUSION

The iodinated radiographic contrast media in diagnostic radiography, diatrizoate (an ionic monomer), iopromide (a nonionic monomer) and iotrolan (a non ionic dimer) were relatively low cytotoxicity at the concentrations used that was comparable to the dosage administered in clinics. However, in the co-treatment

with daunorubicin, an anticancer drug that was a substrate of P-glycoprotein, diatrizoate could enhance the efficacy of this anticancer drug in K562/adr cells. All the contrast media used affected the cellular energetic state only in MDR cells by decreasing  $\Delta\Psi_m$  and inhibit P-glycoprotein function, leading to the restoration of intracellular drug concentration, consequently, the cytotoxicity was increased in the drug-resistant cells. The results provide a new possible application of contrast media other than usual utilization. In combination with appropriate mitochondrial probe that accumulated in the cells depending on  $\Delta\Psi_m$ , the contrast media could be used as a mitochondrial energetic state modulator that was specific to MDR cells (K562/adr cells). This study had led to some points of view that diatrizoate might be raised for further study as MDR identification in MDR imaging *in vitro* or *in vivo* with the help of some probes that their main target were in mitochondria and, eventually, a MDR chemo-sensitizing agent in cancer treatment.

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