R 96 P R 978.1

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397-402

氟伏沙明抑制中国青年人体内米帕明去甲代谢

许振华，黄松林，周宏灏[1]
(湖南医科大学遗传药理学研究所，长沙 410078，中国)

关键词 米帕明；地昔帕明；氟伏沙明；药物相互作用；药物动力学

目的：研究氟伏沙明(Flu)对健康中国志愿者体内米帕明(Imi)去甲代谢的影响。方法：7名青年志愿者连续口服Flu (100 mg/天) 2周，在服用Flu的第8天受试者口服Imi (100 mg)，将此时Imi及其去甲代谢产物地昔帕明(Des)的药动学参数与Imi单独口服时进行比较。结果：Flu使口服单剂量Imi的体内消除过程发生显著改变，服用Flu导致Imi C_{max}和AUC约增高1倍，$T_{1/2}$显著延长，Cl_0降低60 %。同时，Imi的主要代谢产物Des的生成减少，表现在C_{max}和AUC均明显降低。结论：Flu能明显抑制Imi的去甲基代谢，CYP1A2被抑制可能是Flu和Imi发生相互作用的生化基础。

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 402-406

Doxorubicin cellular pharmacokinetics plays no role in chemosensitizing effect of verapamil on Swiss-3T3 cells

WANG Nan, TANG Zhong-Ming, ZHANG Yang-Pei, DING Qing-Ming
(*Institute of Radiation Medicine, Beijing* 100850, *China*)

KEY WORDS doxorubicin; verapamil; drug resistance; pharmacokinetics; fibroblasts; cultured tumor cells

AIM: To find whether or not the doxorubicin (Dox) cellular pharmacokinetics plays a role in chemosensitizing effect of verapamil (Ver) on drug sensitive cells. **METHODS**: Cytotoxicity and cellular Dox contents (during accumulation and retention periods) were measured in the absence and presence of verapamil in Swiss-3T3 cells and compared with those in multidrug resistant (MDR) MCF-7^{Adr} cells and drug sensitive MCF-7^{WT} cells. *mdr*-1 mRNA expression in Swiss-3T3 cells was analyzed. **RESULT**: Dox cytotoxicity was enhanced 2.0-fold in Swiss-3T3 cells by Ver (3 μmol·L^{-1}) and 3.6-fold in MCF-7^{Adr} cells by Ver (6 μmol·L^{-1}), but not in MCF-7^{WT} cells (Ver 6 μmol·L^{-1}). Cellular accumulation of equi-effective concentrations of Dox increased at 6-h incubation in the presence of Ver in Swiss-3T3 (1.5-fold) and MCF-7^{WT} cells (2.1-fold) but decreased rapidly in MCF-7^{Adr} cells by 20 % to 50 % compared to that in the absence of Ver. Cellular retention of Dox decreased after 10-min increase in the presence of Ver in Swiss-3T3 cells compared to that in the absence of Ver, that was similar to that in MCF-7^{WT} cells, while the retention was augmented by Ver in MCF-7^{Adr} cells. Slot blot analysis of RNA revealed no *mdr*-1 gene expression in Swiss-3T3 cells. **CONCLUSION**: Changes in cellular accumulation and retention of Dox did not account for the chemosensitizing effect of Ver on Swiss-3T3 cells.

Received 1995-07-03 Accepted 1996-04-24

Multidrug resistance (MDR) was associated with the expression of multidrug resistance genes encoding a family of integral membrane glycoproteins known as P-glycoproteins (Pgp)[1]. These transporting proteins bound various chemotherapeutic agents and pumped them out of the cells. Verapamil (Ver), a well-known resistant modifier or chemosensitizer, competitively bound to Pgp and increased the cellular uptake and cytotoxicity of drugs in a calcium-independent manner[2]. Sensitizing effect of Ver was occasionally noted in drug sensitive cells[3] or non-

Pgp expressed MDR cells[4-7]. To find if any role of cellular pharmacokinetic changes in Ver sensitization, we observed the cellular doxorubicin (Dox) accumulation and retention in the presence of Ver in Swiss-3T3 cells, and compared with that in MDR and drug sensitive MCF-7 cells.

MATERIALS AND METHODS

Drugs and chemicals Dox, Ver and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma (USA). Me_2SO was from E Merck (Germany). Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco/BRL (USA). [α-P^{32}]dATP was from YaHui Biomed Co (Beijing, China).

Cell culture The human breast cancer cell line MCF-7^{WT} and mouse fibroblast cell line Swiss-3T3 were cultured in DMEM supplemented with 10 % calf serum in a 5 % CO_2 humidified incubator at 37 ℃. The MCF-7^{Adr}[8] (a gift from Dr KH Cowan, NCI, NIH, USA) was maintained in the medium containing Dox (10 μmol·L^{-1}) which was removed 20 d before the experiments.

Chemosensitivity testing Cytotoxicity was determined by MTT assay[9]. Cells were plated into 96-well tissue culture plates and exposed to drugs continuously. After incubation at 37 ℃ for 4 d, 50 μL of MTT dye (2 g·L^{-1} in Hanks' solution) was added to each well and incubated for 4 h. The medium was then aspirated and 100 μL of Me_2SO was added. The plates were agitated for 5 min and absorbance at 540 nm was read on a Titertek Multiscan MCC/340 plate reader (Flow Laboratories, UK). In all experiments 4 replicate wells were used for each drug concentration. IC_{50} values of Dox were obtained from dose-response curves fitted by logistic model[9].

Intracellular Dox assay and protein assay Fluorescent measurement of Dox was performed as described[11] with slight modification. Drug-treated cells cultured in 100 or 90 mm dishes were washed 4 times rapidly with 5 mL ice-cold PBS and scraped into Eppendorf tubes. After centrifugation (3000 × g, 4 ℃, 5 min), the cell pellet was resuspended in distilled water and left overnight in the dark to lyse the cells. Aliquots of 0.1 mL were suspended in 0.9 mL methanol and centrifuged 12 000 × g at 4 ℃ for 10 min, the supernatant was read on a MPF-4 fluorescence spectrophotometer (Hitachi, Japan) (λ_{ex} = 468 nm, λ_{em} = 585 nm, slit 10 nm, and sensitivity at 3.5). The concentration of Dox was determined in comparison with a standard Dox curve. Cell lysates 5 – 10 μL were used for protein assay according to dye-binding method[12], modified to 96-well microplate scale.

Drug accumulation and retention Cell monolayer cultures were incubated with Dox at the concentrations of IC_{50} values in the absence or presence of Ver. At appropriate times the cells were harvested and intracellular accumulations of Dox were determined. For drug retention tests, the cell monolayers incubated with Dox and Ver for 6 h was further incubated in the absence or presence of Ver after withdrawal of Dox, cells were harvested at various time points for Dox measurements. The Dox concentration was corrected to the protein content in the cell sample (as nmol of Dox/g protein).

RNA analysis RNA was isolated from Swiss-3T3 cells by single-step method[13]. Slot blot analysis of total cellular RNA was carried out as described[14] using a full length cDNA probe of *mdr*-1 (a gift from Dr MM Gottesman, NCI, NIH, USA). *mdr*-1 Gene-transfected Swiss-3T3 cells were included as positive control. The blots were reprobed with mouse β-actin cDNA probe for comparison of sample loading.

Statistics The differences of Dox accumulations or retentions between Ver treatment were compared by t test.

RESULTS

Cytotoxicity The cytotoxicity of Dox to Swiss-3T3 cells was enhanced after treatment with Ver. The IC_{50} for Swiss-3T3 cells was 141 (95 % confidence limits 14 – 268) nmol·L^{-1} and 72 (95 % confidence limits 41 – 103) nmol·L^{-1} in the absence and presence of Ver 3 μmol·L^{-1}, respectively. MCF-7^{Adr} showed a high level of resistance to Dox (IC_{50} = 25.50 μmol·L^{-1}, 95 % confidence limits 14.11 – 36.89), compared with its parent cell line MCF-7^{WT} (IC_{50} = 73 nmol·L^{-1}, 95 % confidence limits 46 – 100). Ver partially reversed the resistance of Dox in MCF-7^{Adr} (IC_{50} = 7049 nmol·L^{-1} in the presence of Ver 6 μmol·L^{-1}, 95 % confidence limits 5387 – 8711). While Ver 6 μmol·L^{-1} did not enhance Dox cytotoxicity against MCF-7^{WT} cells (IC_{50} = 76 nmol·L^{-1}, 95 % confidence limits 43 – 109). At concentrations of 3 μmol·L^{-1} and 6 μmol·L^{-1} for Ver alone, no effect on cell viability or growth was observed for Swiss-3T3 and MCF-7 cells (Fig 1).

Drug accumulation When incubated with equi-effective (IC_{50}) concentration of Dox in the presence of Ver (3 μmol·L^{-1}), intracellular accumulation of Dox in Swiss-3T3 cells did not change significantly at 1 h and 3 h, until at 6 h ($P<0.01$), compared with that without Ver. Dox accumulation in MCF-7^{WT} was influenced by Ver (6 μmol·L^{-1}) in a similar manner to that in Swiss-3T3 cells (Fig 2). While in MCF-7^{Adr} accumulation of Dox in the presence of Ver

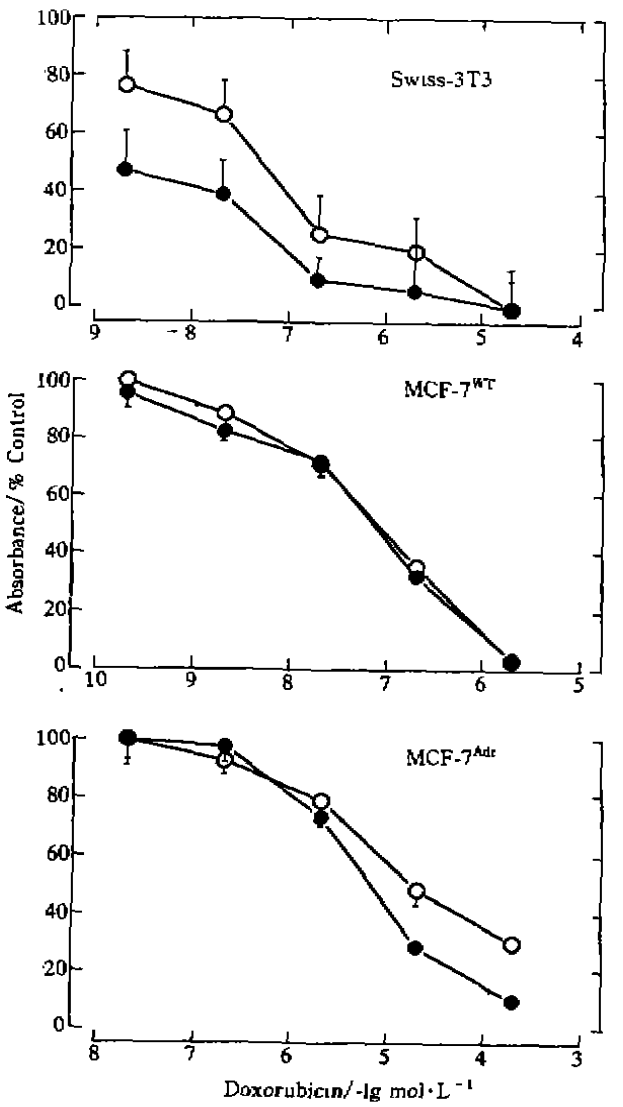

Fig 1. **Response of cells to Dox in the absence (○) and presence (●) of Ver (3 μmol · L⁻¹ for Swiss-3T3, and 6 μmol·L⁻¹ for the other two).. $n = 4$. $\bar{x} \pm s$.**

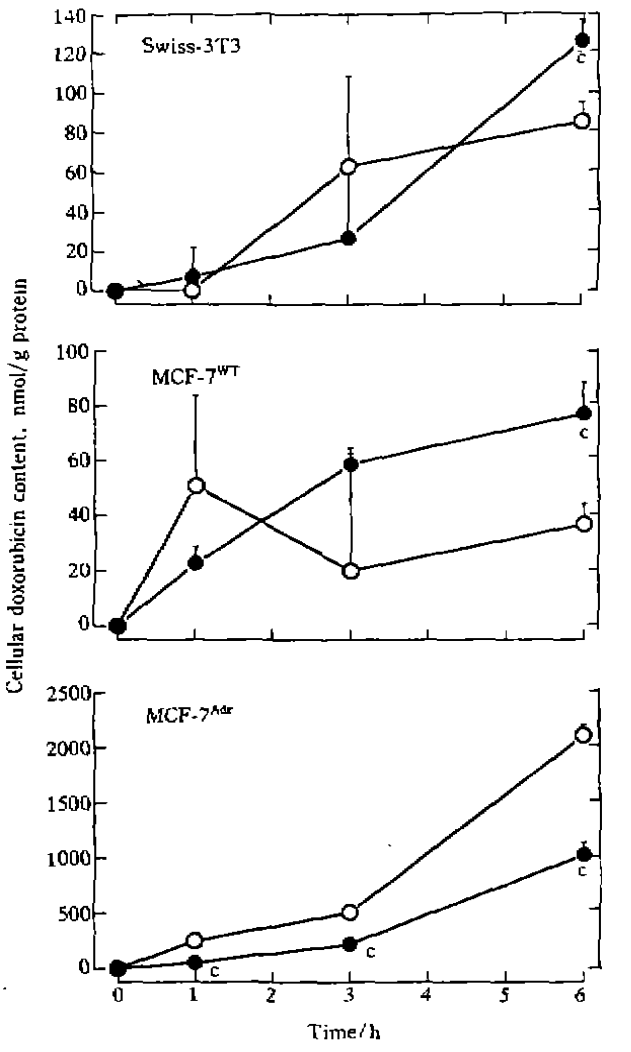

Fig 2. **Accumulation of Dox by cells in the absence (○) and presence (●) of Ver (3 μmol · L⁻¹ for Swiss-3T3, 6 μmol · L⁻¹ for the other two). The cells were loaded with IC_{50} of Dox. $n = 4$. $\bar{x} \pm s$. $^cP < 0.01$ vs absence of Ver.**

(6 μmol · L⁻¹) reduced by 20 %, 40 %, and 50 %, compared to that without Ver, at 1 h, 3 h, and 6 h incubation of Dox of IC_{50}.

Drug retention Continuing treatment with Ver after withdrawal of Dox increased the retention in MCF-7^{Adr}. The retention of Dox in Swiss-3T3 cells increased at 10 min and then decreased in the continuing presence of Ver compared to absence of Ver, which showed a dramatically similar pattern as that in MCF-7^{WT}(Fig 3).

RNA slot blot Slot blot analysis revealed no evidence of *mdr*-1 message in the Swiss-3T3 cells (Fig 4). *mdr*-1 Gene transfected Swiss-3T3 cells were included as positive control which showed *mdr*-1 mRNA expression.

DISCUSSION

Changes in cellular drug levels have been accounted for the expression of drug resistance and its reversal with calcium antagonists in Pgp expressed MDR cells[1]. In a typical MDR cell line MCF-7^{Adr}, diminished Dox accumulation and retention could be seen when these values were expressed as ratio of intracellular to extracellular

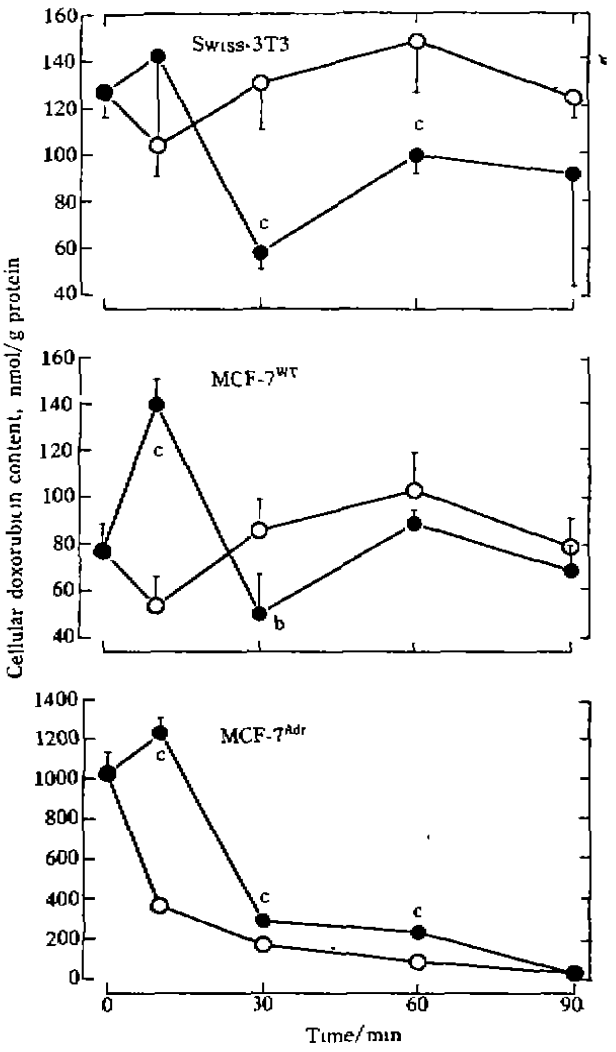

Fig 3. Dox retention in cells preloaded with IC_{50} of Dox in the absence (○) or presence (●) of Ver. $n = 4$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* (○).

drug concentrations (data not shown) compared with those in its parent cell line MCF-7^{WT}, which was partially restored by Ver. In our study IC_{50} concentrations of Dox were used in order to compare the pharmacokinetic processes at similar levels of biological action of the drug on these cells. The chemosensitizing effect of Ver on Dox cytotoxicity was observed in Swiss-3T3 cells in which *mdr*-1 gene expression was not detected. The cellular Dox level was altered in the presence of Ver during the accumulation and/or retention phase in this cell, the pattern and magnitude of alterations were strikingly similar to that in drug sensitive MCF-7^{WT} on which no sensitizing effect of Ver was observed. While

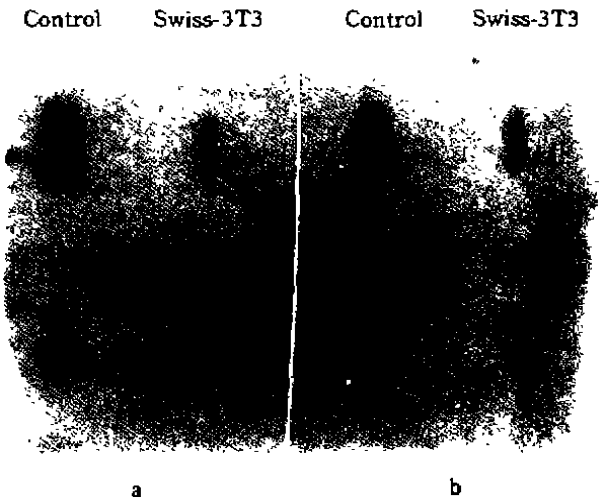

Fig 4. Slot blot analysis of Swiss-3T3 RNA. Total cellular RNA was applied to the nylon membrane by 1/7 dilution (20 μg at upper and 3 μg at lower). *mdr*-1 Gene transfected Swiss-3T3 cells were used as positive control. Hybridization with *mdr*-1 cDNA probe (a) or β-actin cDNA probe (b).

the alteration in cellular Dox pharmacokinetics in MCF-7^{Adr} was quite different from Swiss-3T3 and MCF-7^{WT} cells. Ver enhanced drug accumulation and retention was observed in other sensitive cells and was attributed to the nonspecific action of Ver on plasma membrane[15]. Our results suggested that sensitizing effect of Ver on Swiss-3T3 cells was not attributed, at least mainly, to cellular pharmacokinetic changes. Intracellular Ver enhanced sensitizing mechanisms could be involved in such effect as it has been reported that Ver potentiated cytotoxicity of Dox was associated with an increase in DNA-breakage in two human ovarian tumor cell lines[7]. There could be great difference between cell lines in mechanism of response to Ver, as shown in Swiss-3T3 cells and MCF-7 cells.

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402-406

阿霉素细胞水平药物动力学在维拉帕米对 Swiss-3T3 细胞的化学增敏效应中不起作用

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R 969.1

王 楠, 汤仲明, 章扬培, 丁清明
(北京放射医学研究所, 北京 100850, 中国)

关键词 阿霉素; 维拉帕米; 抗药性; 药物动力学; 成纤维细胞; 培养的肿瘤细胞

A **目的**: 确定维拉帕米(Ver)对药物敏感细胞化学增敏效应中, 阿霉素(Dox)细胞药物动力学是否起作用. **方法**: 测定合并 Ver 与否时, Dox 对 Swiss-3T3 细胞的毒性, 蓄积和残留期细胞内 Dox 含量, 并与多药抗药细胞 MCF-7^{Adr} 及敏感细胞 MCF-7^{WT} 比较. 分析 Swiss-3T3 中 *mdr*-1 mRNA 的表达 **结果**: Ver 增强 Dox 对 Swiss-3T3 和 MCF-7^{Adr} 的毒性, 而不增强 MCF-7^{WT} 的毒性. 存在 Ver 与反之相比, Swiss-3T3 和 MCF-7^{WT} 对等效浓度 Dox 的蓄积于孵育 6 h 增高, 残留量在 10 min 内升高, 而后下降; 而 MCF-7^{Adr} 对 Dox 的蓄积迅速降低, 残留则被 Ver 提高. 狭缝杂交未见 Swiss-3T3 有 *mdr*-1 基因表达. **结论**: Dox 在细胞内蓄积与残留的变化, 不能说明 Ver 对 Swiss-3T3 的化学增敏作用.