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小檗胺对猪基底动脉收缩及钙内流的影响

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提要 本文观察了小檗胺(Ber)对 KCl 和 5–HT 所致猪 BSA 收缩的影响。结果表明：Ber 可明显松弛高 KCl 引起的 BSA 收缩，并可被细胞外 Ca^{2+} 所拮抗；Ber 抑制 5–HT 收缩 BSA，对 STC 作用显著，FPC 相对不受影响。5–HT 收缩 BSA 依赖于细胞外 Ca^{2+}，STC 尤为明显；而 FPC 为内 Ca^{2+} 释放所致。Ber 明显抑制 5–HT 引起的 Ca^{2+} 内流。提示 Ber 对 PSCs 和 ROCs 均有阻断作用。

关键词 小檗胺；氯化钾；血清素；尼莫地平；基底动脉

BIBLID: ISSN 0253–9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Sep; 13 (5) : 416–419

Reversal of doxorubicin resistance by tetrandrine in Chinese hamster ovary cell line[1]

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ABSTRACT Tetrandrine (Tet) 0.5 $\mu g \cdot ml^{-1}$ and 1 $\mu g \cdot ml^{-1}$ potentiated 2.88– and 4.3–fold growth–inhibitory effects of doxorubicin (Dox) in Chinese hamster ovary cell line (CHO), respectively, while Tet 1 $\mu g \cdot ml^{-1}$ and 2.5 $\mu g \cdot ml^{-1}$ potentiated 7.3– and 8.4–fold in its resistant cell line (CHO / Dox), respectively. The colony–forming efficiencies were reduced in CHO and CHO / Dox when the cells were treated with noncytotoxic doses of Tet 2.5 $\mu g \cdot ml^{-1}$ and 5 $\mu g \cdot ml^{-1}$ in combination with different concentration of Dox. Increase in accumulation of Dox in CHO / Dox cells was shown by fluorometry. The result indicated that Tet reversed the resistance to Dox in CHO / Dox cells.

KEY WORDS tetrandrine; doxorubicin; drug resistance; ovary; transformed cell line; cricetulus

Drug resistant tumor cells are refractory to chemotherapy. Since the discovery of reversal of drug resistance in tumor cells by calcium antagonists and calmodulin inhibitors[1,2], a promising avenue to overcome drug resistance has been unfolded. Tetrandrine (Tet), a bisbenzylisoquinoline alkaloid

Received 1991–07–31 Accepted 1992–06–19
[1] Project supported by the National Natural Science Foundation of China, № 388002.
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from Stephania tetrandra, antagonizes voltage-dependent calcium slow channel[3] and inhibits calmodulin[4]. Thus, it is likely to potentiate the cytotoxicity of antitumor drugs in resistant cell lines by Tet. In this report we want to confirm whether Tet effectively reverses Dox resistance in Chinese hamster ovary cell subline (CHO / Dox) and increases Dox accumulation in CHO / Dox cells.

MATERIALS AND METHODS

Dox was purchased from Farmitalia Carlo Erba Ltd (Italy), dissolved in phorsphate buffer solutions (PBS). Tet, a gift of Prof CHEN Ning-Meng (Institute of Industrial Hygiene and Occupational Diseases, Chinese Academy of Preventive Medicine), was dissolved in 100 μl HCl 1 mol · L^{-1} with pH adjusted to 6.7. The drugs were stored at 4 ℃.

Cell lines CHO / Dox resistant cells were obtained from CHO by successive exposures to ascending gradient amounts of Dox. They were routinely cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10–12 % fetal calf serum (Xianqiao Farm Factory, Beijing) at 37℃ in an atmosphere of air+ 5% CO_2.

Inhibition of cell growth by the drugs Cells at exponential growth stage were seeded into 24-well plates. The final volume of the mixture per well was 1.1 ml with 2×10^4 cells. The cells were treated with Dox and Tet in quadruplicate for 72 h and then digested with 0.2% EDTA. Cell viability was detected by trypan blue exclusion. The number of the cells were counted by Coulter counter (England).

Cytotoxicity evaluation Cells at exponential growth stage were seeded about 100 cells / well in a 24-well plate. The clumps of 4–8 cells, 3 d later, were treated with the drugs for 1 h and washed 3 times with Phosphate Balanced Solution (PBS, NaCl 137, KCl 2.7, Na_2HPO_4 8.1, KH_2PO_4 1.3 mmol · L^{-1}, pH 7.4). The cells were then continuously cultured in an incubator till most of the colonies consisted of more than 50 cells. The colonies were counted under a stereomicroscope. The results were indicated as the number of colonies treated / number of control colonies × 100%.

Cellular accumulation of Dox Effect on the accumulation of Dox in the cell by Tet was quantified by measuring Dox fluorescence[5]. 5×10^6 Cells were incubated in Dox 10 μg · ml^{-1} for 1 h. The cell pellet was suspended in 3 ml mixture of 60 % EtOH and HCl 0.3 mol · L^{-1} (1:1, vol / vol), sonicated for 1.5 min in a Sonifier B30 (Branso Sonic Power Co), and then centrifuged at 12 000 × g for 30 min at 4℃. The Dox fluorescence was measured in a Hitachi 850 fluorospectrophotometer (Japan) at $\lambda_{ex} = 475$ nm and $\lambda_m = 575$ nm.

The crude membrane fractions from cells were prepared [6]. The content of membrane protein was determind by colorimetric method[7]. The experimental data were analyzed by t test.

RESULTS

Inhibition of cell growth CHO / Dox and CHO cells were treated with Dox 0.5 μg · ml^{-1} for 72 h. The inhibition rate in CHO cells was 72%, but only 10 % in CHO / Dox cells. Thus, CHO / Dox cells were resistant to Dox. Non-growth-inhibitory doses of Tet effectively reversed the Dox-resistance in CHO / Dox cells (Fig 1). Tet 1 μg · ml^{-1} shifted IC_{50} value of Dox from 2.78 to 0.38 μg · ml^{-1}. When the dose of Tet was increased to 2.5 μg · ml^{-1}, the IC_{50} value was further reduced to 0.33 μg · ml^{-1}. The indices

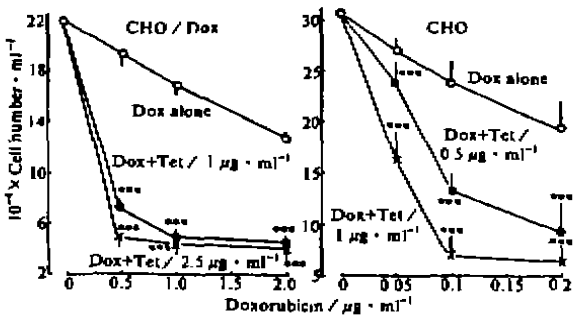

Fig 1. Effect of Tet on growth-inhibitory action of Dox in CHO / Dox cells and CHO cells. Cell numbers were counted after 72 h in culture. $n = 12$, $\bar{x} \pm s$. $^{***}P < 0.01$ *vs* **Dox group.**

of potentiation were 7.3– and 8.4–folds, respectively. In comparison with the IC_{50} value (0.26 $\mu g \cdot ml^{-1}$) of CHO cells, CHO / Dox cells remained partially resistant.

The potentiation phenomenon occurred in sensitive CHO cells when non–growth–inhibitory concentrations of Tet 0.5 or 1 $\mu g \cdot ml^{-1}$ were added with Dox into the culture (Fig 1). Tet 0.5 and 1 $\mu g \cdot ml^{-1}$ reduced the IC_{50} value of Dox from 0.26 $\mu g \cdot ml^{-1}$ to 0.09 and 0.06 $\mu g \cdot ml^{-1}$. The indices of potentiation were 2.88– and 4.3–folds, respectively.

Enhanced cytotoxicity of Dox In CHO / Dox and CHO cells The enhancing effect was shown by colony formation method. The colonies of CHO / Dox cells remained unchanged in Dox 0.5 $\mu g \cdot ml^{-1}$ for 1 h *vs* the control. When the cells were treated with noncytotoxic dose of Tet 5 $\mu g \cdot ml^{-1}$ in addition to Dox 0.5 $\mu g \cdot ml^{-1}$ for 1 h, the colony efficiency was reduced to 10 % in comparison with that of the control. Enhancing effect of Tet on the cells was more obvious with increasing concentrations of Dox. The colony forming efficiency of CHO / Dox cells treated with Dox 2.5 $\mu g \cdot ml^{-1}$ was 53 % in the absence of Tet, but was only 0.3 % in the presence of Tet 5 $\mu g \cdot ml^{-1}$ (Fig 2).

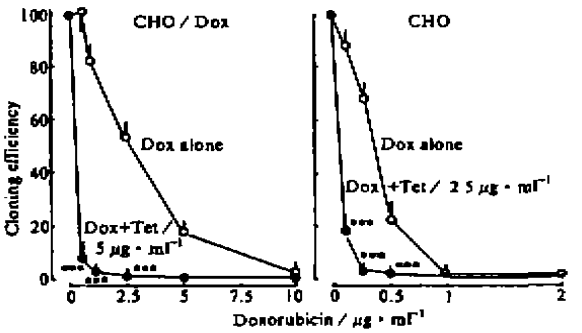

Fig 2. Effect of Tet on Dox cytotoxicity of CHO / Dox cells and CHO cells. Colonies (over 50 cells as a clone) were counted under a stereomicroscope. $n=8$, $\bar{x} \pm s$. $^{***}P<0.01$ *vs* **Dox group.**

Effect of Tet on Dox cytotoxicity to CHO cells was in agreement with its growth inhibitory effect. The colony forming efficiency of CHO cells treated with Dox 0.25 $\mu g \cdot ml^{-1}$ was 61 % in the absence of Tet, but only 0.8 % in the presence of Tet 2.5 $\mu g \cdot ml^{-1}$ *vs* Dox (Fig 2).

Effect on accumulation of Dox in cells Reduced intake of Dox into the resistant cells is one of the mechanisms for resistance to Dox[8]. The groups of CHO cells Dox alone, Dox + Tet (2.5 $\mu g \cdot ml^{-1}$), and CHO / Dox cells Dox alone, Dox + Tet (5 $\mu g \cdot ml^{-1}$) were tested with Dox 10 $\mu g \cdot ml^{-1}$. The Dox content of $0.70 \pm s$ 0.06 μg / mg membrane protein (MP) in CHO / Dox cells was lower than the Dox $0.87 \pm s$ 0.07 μg / mg MP in CHO cells ($P < 0.01$). Tet increasd the accumulation of Dox in CHO / Dox cells from $0.70 \pm s$ 0.06 to $0.94 \pm s$ 0.02 μg / mg MP ($P < 0.01$). The result indicated that reversal of resistance by Tet was due to increased accumulation of Dox in the CHO / Dox cells. Tet caused slightly increased accumulation of Dox in CHO cells ($P > 0.05$). The Dox amount of $0.94 \pm s$ 0.02 μg / mg MP in the CHO / Dox cells was close to that of $0.95 \pm s$ 0.08 μg / mg MP in the CHO cells treated by Tet and Dox.

DISCUSSION

The results indicated that Tet effectively reversed the resistance to Dox in CHO / Dox cells and caused the accumulation of Dox in the cells, which led to increased Dox cytotoxicity. This is in agreement with the increasing accumulation of [^{3}H]Dox in P388 / Dox[2], CHO / Dox cells [9] by calmodulin inhibitor trifluoperazine and calcium antagonists verapamil. Tet increased [^{3}H]vinblastin in membrane vesicles of KB / V1 resistant cells[10], 2–fold higher than verapamil did, the latter is normally used for overcoming drug resistance. Some bisbenzyl–

isoquinoline alkaloids, extracted from *Stephania tetrandra* S Moor may be selected to overcome drug resistance in tumor cells congenially.

The target affected by Tet in resistant cells is likely to be a P-glycoprotein (PGP). The overexpression of PGP in daunorubicin (a Dox analogue)-resistant CHO cells was reported[11]. It remains uncertain whether PGP is overexpressed in our resistant cells. There may be other mechanisms for the reversal of drug resistance by Tet as suggested by potentiation of Dox cytotoxicity to sensitive CHO cells by Tet. The characteristics of multidrug resistance in tumor cells is mediated by PGP which pumps out antitumor drugs in resistant cells. The relationship between Tet and PGP will be investigated.

AKNOWLEDGMENT Laboratory engineer LIU Chen-Gui of Department of Chemistry in Beijing Normal University offered a great help in the measurement of Dox fluorescence.

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(6) 416-419

粉防己碱逆转中国仓鼠卵巢上皮细胞系对阿霉素的抗性[1]

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提要 粉防己碱(Tet) 0.5 $\mu g \cdot ml^{-1}$ 和 1 $\mu g \cdot ml^{-1}$ 使阿霉素(Dox)对中国仓鼠卵巢上皮细胞(CHO)抑制作用增强 2.88 和 4.3 倍，而 Tet 1 $\mu g \cdot ml^{-1}$ 和 2.5 $\mu g \cdot ml^{-1}$ 使其抗性细胞(CHO / Dox)分别增强 7.3 和 8.4 倍。Tet 2.5 $\mu g \cdot ml^{-1}$ 和 5 $\mu g \cdot ml^{-1}$ 与 Dox 合用，明显降低 CHO 和 CHO / Dox 的克隆形成率，荧光法测定表明，Tet 增加 Dox 在 CHO / Dox 细胞内的积聚，说明，Tet 有效地逆转了 CHO / Dox 细胞对阿霉素的抗性。

关键词 粉防己碱；阿霉素；抗药性；卵巢；转化细胞系；仓鼠属