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基因重组人白细胞介素-2在小鼠的药物动力学和分布

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摘要 Iodogen 法制备^{125}I-rIL-2，放射纯度95 %。iv 后快速、慢速分布和消除 $T_{1/2}$分别为 < 2，30 — 120 和 6 — 15 h，AUC 与剂量呈正比。血尿原药占 81 ± 13 %。im 生物利用度0.57。iv 后15 min 浓度顺序为肝>胆汁>肾>血>肾上腺>血浆>肺>甲状腺>脾>小肠>肠系膜淋巴结>肠内容>卵巢>心>膀胱>胸腺>粪>肌肉>睾丸>脑>脂肪。24 h 排出 80 % 第 2 天 5 %。

关键词 白细胞介素-2；药物动力学；组织分布；小鼠

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Synergistic effect of probimane on anticancer cytotoxicity of doxorubicin *in vitro*

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ABSTRACT Using 3-(4, 5-dimethythiazole)-2, 5-diphenyltetrazolium bromide (MTT) method, the effect of probimane (Pro) on doxorubicin (Dox) cytotoxicity was studied. Pro 0.313, 0.625, and 1.25 $\mu g \cdot ml^{-1}$ potentiated cytotoxicity of Dox in Ehrlich ascites carcinoma (EAC) cells. Incubation of EAC cells with Dox 10 $\mu g \cdot ml^{-1}$ and Pro 116.5, 233, and 466 $\mu g \cdot ml^{-1}$ resulted in an increase in intracellular drug accumulation from 0.69 ± 0.06 to 1.08 ± 0.10 $\mu g/10^7$ cells. In S_{37}-bearing mice, Pro 23.3, 46.6, and 116.5 $\mu g \cdot ml^{-1}$ enhanced the malondialdehyde (MDA) formation in tumor and liver mitochondria and decreased MDA formation in liver mitochondria. These results suggested that the increases of Dox accumulation and MDA formation in tumor cells by Pro might be the reasons for synergistic effect of Pro on Dox cytotoxicity.

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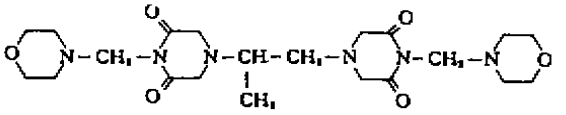
KEY WORDS doxorubicin; probimane; malondiadehyde; cultured tumor cells; drug synergism

Formation of free radicals of doxorubicin (Dox) *in vitro* may play a significant role in killing tumor cell[1,2]. Dexrazoxane (bisdioxopiperazine compound) was cytotoxic to certain tumor cells and potentiated antitumor activity of Dox in murine L_{1210}, S180, and HL-60 cells[3,4]. Probimane (Pro), [*dl*-bis (4-morpholinmethyl 3, 5-dioxopiperazin-1-yl) propane] first synthesized in China, is also a dioxopiperazine compound with antitumor activity[5], reduced the cardiotoxicity of Dox by scavenging the Dox-semiquinone free radical and increasing the contents of SOD and GSH-Px in rat heart[6,7]. We also found that Pro

potentiated the antitumor activity of Dox in sarcoma S_{37}, but the mechanism is not clear. The present study was carried out to investigate the potentiating effect of Pro on the antitumor action of Dox *in vitro*.



Probimane

MATERIALS AND METHODS

Chemicals and drug Dox was purchased from Farmitalia Carloerbra Ltd, Italy. Pro was synthesized by Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Thiobarbituric acid (TBA) was purchased from the Second Chemical Reagent Factory of Shanghai, NADH and 3-(4,5-dimethythiazole)-2,5-diphenyltetrazolium bromide (MTT) were purchased from the Sigma Chemical Co. Dox and Pro were dissolved in phosphate buffered saline (PBS, pH 7.4) just before use.

Microculture tetrazolium assay Ehrlich ascites carcinoma (EAC) cells were harvested from ascites of mice and incubated in RPMI-1640 medium with 10% fetal calf serum, penicillin 100 IU·ml^{-1} and streptomycin 100 IU·ml^{-1}. Cells were seeded in 96-well plates with the final volume 200 μl containing 1×10^5 cells per well. The cells were treated with Dox and Pro in quadruplicate tubes in 5% CO_2 at 37 ℃ for 24 h. After 100 μl of supernatant were removed, 10 μl MTT (0.05 mg dissolved in PBS) were added into each well. The plates were reincubated at 37 ℃ in 5% CO_2 for 4 h. The formazan crystals formed were dissolved in 100 μl Me_2SO and the absorbance at 570 nm (with reference wave at 630 nm) was measured by spectrophotometer. Wells containing no cell and no drug were used as a blank. The inhibitory rate (R) of drug was calculated.

$R=[1-(OD_{570-630}$ of tested sample/$OD_{570-630}$ of control)$]\times100\%$

the synergistic effect was showed as followed[8]:

$$q=(R_{D+P}/R_D+R_P-R_D\cdot R_P)$$

when $q>1$ representing synergistic effect. R_D, R_P were the inhibitory rates on tumor cells of Dox or Pro respectively.

Cellular accumulation of Dox Effect of Pro on the accumulation of Dox in EAC cells were quantified by measuring Dox fluorescence[9]. Cells 5×10^6 were incubated with Pro 116.5, 233, and 466 μg·ml^{-1} for 30 min and then Dox 10 μg·ml^{-1} was added. The test tubes were incubated for another 1 h at 37 ℃. After centrifugation at $300\times g$ for 10 min, the supernatant was discarded, and the cell pellets were re-suspended in 3 ml of 60% ethanol : HCl 0.3 mol·L^{-1} mixture, vortexed vigorously, sonicated for 90 s in a sonifier CSF-1A and centrifuged at $12\,000\times g$ for 30 min at 4 ℃. The Dox fluorescence was measured in Shimadzu RF-540 spectrofluorophotometer (Japan) at $\lambda_{ex}=475$ nm and $\lambda_{em}=575$ nm with slit 10 nm.

Assay for lipid peroxidation Kunming species mice, ♀, weighing 20±s 2 g were supplied by Centre of Laborotory Animals, He-nan Medical University, Mice were implanted with S_{37} sarcoma cell (5×10^5) and killed by cervical dislocation on d 10. Liver and tumor mitochondria were prepared[10]. Mitochondrial protein was determined colormetrically[11] using bovine serum albumin as the standard.

Pro (23.3, 46.6, and 116.5 μg·ml^{-1}) was preincubated with liver or tumor mitochondria protein 0.5 mg·ml^{-1} at 37 ℃ for 30 min, followed by the addition of Dox 27.2 μg·ml^{-1} and NADH 2.5 mmol·L^{-1}. After an incubation for an additional 1 h, the peroxidation reaction was terminated by adding 0.5 ml ice-cold 20% trichloroacetic acid, the precipitated proteins were removed by centrifugation, the supernatant was used for the determination of malondialdehyde (MDA) by TBA method[12].

RESULTS

Cytotoxicity The IC_{50} values following a 24-h exposure was 44.93 and its 95% confidence limits was 32.65 — 61.87 ng·ml^{-1} for Dox; the IC_{50} values of Pro was 33.86 μg·ml^{-1} and its 95% confidence limits was 29.94 — 38.30 μg·ml^{-1}. When the cells were treated with Dox plus Pro 0.313, 0.625, and 1.25 μg·ml^{-1}, the IC_{50} of Dox were 13.52 (95% confidence limits 9.25—19.74), 8.84 (95% confidence limits 4.33—18.04) and 6.27 ng·ml^{-1} (95% confidence limits 3.59—10.59), respec-

tively. The combination of Dox and Pro showed a synergistical cytotoxic effect (Tab 1).

Tab 1. Effects of Dox, Pro, or their combination on cytotoxicity of EAC cells. $n=4$, $\bar{x}\pm s$.

Dosage $\mu g\cdot ml^{-1}$ Doxorubicin	Probimane	Inhibitory rates %	q value
—	—		
0.006	—	10.42	
0.013	—	33.33	
0.05	—	45.83	
—	0.313	8.33	
—	0.625	14.58	
—	1.250	16.67	
0.006	0.313	29.17	1.67
0.013	0.313	64.58	1.66
0.05	0.313	52.08	1.03
0.006	0.625	37.50	1.60
0.013	0.625	66.67	1.55
0.05	0.625	66.67	1.24
0.006	1.250	45.83	1.81
0.013	1.250	68.75	1.55
0.05	1.250	66.67	1.22

Effect on the accumulation of Dox in cells

The EAC cells with Dox 10 $\mu g\cdot ml^{-1}$ alone, Dox 10 $\mu g\cdot ml^{-1}$ + Pro 116.5, 233, and 466 $\mu g\cdot ml^{-1}$ treatments were determined for Dox contents. The Dox content in the Dox alone group was 0.69 ± 0.06 $\mu g/10^7$ cells, Pro increased the Dox content in EAC cells concentration-dependently up to 1.08 ± 0.10 $\mu g/10^7$ cells (Tab 2).

Tab 2. Effect of probimane on Dox (10 $\mu g\cdot ml^{-1}$) accumulation in EAC cells. $n=6$, $\bar{x}\pm s$. $^cP<0.01$ *vs* **control.**

Pro/$\mu g\cdot ml^{-1}$	Intracellular Dox ($\mu g/10^7$ cells)	
—	0.69 ± 0.06	(100%)
116.5	0.79 ± 0.02^c	(115.5%)
233.0	0.93 ± 0.04^c	(136.2%)
466.0	1.08 ± 0.10^c	(158.1%)

Effect on MDA in liver and tumor mitochondria Dox 27.2 $\mu g\cdot ml^{-1}$ produced an increase of MDA contents in liver and tumor mitochondria systems ($P<0.05$). The addition of Pro to the liver mitochondria in the presence of NADH 2.5 $mmol\cdot L^{-1}$ lessened the enhancement of MDA induced by Dox in a concentration-dependent manner. But in tumor mitochondria system, only 116.5 $\mu g\cdot ml^{-1}$ of Pro increased the Dox-enchancement of MDA (Tab 3).

Tab 3. Effects of probimane on MDA formation in liver and tumor mitochondria of mice. $n=4$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **Dox.**

Dosage/$\mu g\cdot ml^{-1}$ Dox	Probimane	MDA ($nmol\cdot min^{-1}$/mg protein) Liver	Tumor
—	—	1.60 ± 0.26	1.99 ± 0.57
27.2	—	4.23 ± 0.69	3.25 ± 0.30
27.2	23.3	3.22 ± 0.41^b	3.31 ± 0.44^a
27.2	46.6	3.06 ± 0.37^b	3.38 ± 0.39^a
27.2	116.5	2.45 ± 0.50^c	4.84 ± 0.82^b

DISCUSSION

In the present studies, the combination of probimane with Dox showed markedly synergistic effect against the EAC cell *in vitro*. Walder[3] and Monti[4] indicated that dexrazoxane interacts synergistically with Dox in killing the murine sarcoma S180 and Human leukemia HL-60 cells.

Our study indicated that probimane enhanced MDA formation from Dox in tumor mitochondria. Monti[4] also found that dexrazoxane significantly enhance. OH formation by Dox as detected by DMPO spin trapping. They suggested that in certain tumor cells, the interaction of dexrazoxane and Dox-dependent cell kill may be independent of metal irons[4]. In addition, MnSOD and GSH-Px activities were lower in tumor tissues than that in the liver[13]. Perhaps this may be the reason of increase content of MDA in tumor mitochon-

dria by probimane.

By means of spectrofluorophotometry, we found that probimane can increase cellular Dox accumulation in EAC cells. It is different from that of dexrazoxane. Intracellular cation, such as calcium and iron, may modulate the antitumor effects of Dox by a variety of mechanisms such as alteration in intracellular disposition of Dox. Agents which alter intracellullar cation concentration may either augment or block the cytotoxic effects of the drug. Probimane, a derivative of EDTA and a potent chelating agent, could alter the intracellular cation millieu and disposition of Dox.

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吗丙嗪增强阿霉素的体外抗肿瘤细胞毒作用

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摘要 吗丙嗪(Pro) 0.313，0.625和1.25 $\mu g \cdot ml^{-1}$可增强阿霉素(Dox)体外对艾氏腹水癌(EAC)的细胞毒作用；Pro 116.5，233和466 $\mu g \cdot ml^{-1}$也可明显增加EAC细胞中的Dox含量，在S_{37}荷瘤小鼠中Pro可降低肝细胞线粒体而增加肿瘤细胞线粒体中Dox诱发的MDA含量．提示Pro的增效作用可能与其增加肿瘤细胞中Dox积聚有关，或许与MDA含量亦有关．

关键词 阿霉素；吗丙嗪；丙二醛；培养的肿瘤细胞；药物协同作用