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论著

# 浙贝母总生物碱对人肺腺癌A549/顺铂细胞耐药性的逆转作用

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摘要 目的 研究浙贝母总生物碱(TAF)对人肺腺癌A549/顺铂(DDP)细胞DDP耐药性的逆转作用。方法 1 离体实验: 采用MTT法观察TAF(12.5~200 mg·$L^{-1}$)对A549和A549/DDP细胞的毒性作用;采用MTT法检测TAF 9 mg·$L^{-1}$对A549/DDP细胞耐药的逆转作用,同时设环孢菌素A(Cys A)1 mg·$L^{-1}$和汉防己甲素(Tet)1 mg·$L^{-1}$为阳性对照;实时荧光定量PCR检测A549/DDP细胞多药耐药基因1(MDR1) mRNA相对表达;Western蛋白免疫印迹法检测A549/DDP细胞P-糖蛋白(P-gp)相对表达。2 在体实验: 制备BALB/c裸鼠A549/DDP移植瘤模型,随机分为模型对照、DDP 5 mg·$kg^{-1}$、TAF 2 mg·$kg^{-1}$、DDP 5 mg·$kg^{-1}$+TAF 0.5,1和2 mg·$kg^{-1}$联用组,每两天ip给予DDP,每天ig给予TAF,持续13 d,检测移植瘤体积和质量的变化。结果 TAF作用72 h抑制A549和A549/DDP细胞存活的$IC_{50}$值分别为141±5和(298±22)mg·$L^{-1}$;$IC_{10}$值分别为15.3±1.9和(9.0±1.2)mg·$L^{-1}$。DDP 0.01~100 mg·$L^{-1}$与TAF 9 mg·$L^{-1}$合用后,DDP抑制A549/DDP细胞存活的$IC_{50}$值由(14.06±3.72) mg·$L^{-1}$降至(0.79±0.14)mg·$L^{-1}$,抑制A549细胞存活的$IC_{50}$值无明显变化;TAF对A549/DDP细胞DDP耐药性的逆转倍数为17.80倍,高于Cys A(10.16倍)和Tet(14.05倍)。TAF可明显降低A549/DDP细胞MDR1 mRNA及P-gp相对表达($P<0.01$)。DDP 5 mg·$kg^{-1}$体内抑瘤率为49.9%,与TAF 2 mg·$kg^{-1}$合用后抑瘤率增至67.4%($P<0.01$)。结论 TAF在体内外均可逆转A549/DDP细胞对DDP的耐药性,可降低MDR1 mRNA和P-gp蛋白表达。

关键词 总生物碱 浙贝母 多药耐药性 P-糖蛋白
分类号 R285

# Multidrug resistance reversal activity of total alkaloid from Fritillaria thunbergii on cisplatin-resistant human lung adenocarcinoma A549/DDP cells

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**Abstract**

**OBJECTIVE** To explore the effect of total alkaloid from *Fritillaria thunbergii* (TAF) on reversing multidrug resistance (MDR) of human lung adenocarcinoma A549/DDP cells. **METHODS** 1 *In vitro* Cytoxicity and proliferation inhibitory rate of TAF (12.5-200 mg·$L^{-1}$) was assessed by MTT method. TAF 9 mg·$L^{-1}$ was used in subsequent reversal experiments. Cyclosporine A (Cys A) 1 mg·$L^{-1}$ and tetrandrine (Tet) 1 mg·$L^{-1}$ acted as positive control group. The amount of MDR1 mRNA and P-glycoprotein (P-gp) of A549/DDP cells was measured by real time polymerase chain reaction and Western blotting, respectively. 2 *In vivo* BALB/c nude mice were used to establish an A549/DDP tumor model. The mice were randomly divided into vehicle, DDP 5 mg·$kg^{-1}$, TAF 2 mg·$kg^{-1}$, DDP 5 mg·$kg^{-1}$ plus TAF 0.5, 1 and 2 mg·$kg^{-1}$ groups. DDP was ip given every two days and TAF was ig given once a day. Tumor volume was measured every four days and tumor mass was detected after 13 d. **RESULTS** After incubation with TAF for 72 h, $IC_{50}$ of TAF to A549 and A549/DDP cells was 141±5 and (298±22) mg·$L^{-1}$, respectively, and $IC_{10}$ of TAF to A549 and A549/DDP cells was 15.3±1.9 and (9.0±1.2) mg·$L^{-1}$. $IC_{50}$ of DDP without or with TAF 9 mg·$L^{-1}$ on A549/DDP cells was 14.06±3.72 and (0.79±0.14)mg·$L^{-1}$, respectively, while there was no significant change in $IC_{50}$ of A549 cells. TAF reversed DDP resistance of A549/DDP cells with fold-reversal 17.80, which was higher than that of Cys A (10.16) and Tet (14.05).

Compared with A549/DDP cell vehicle group, MDR1 mRNA and P-gp expression in A549/DDP cells was decreased by TAF ($P$<0.01). The tumor inhibitory rate of DDP 5 mg • $kg^{-1}$ *in vivo* was 49.9%. The combination of DDP 5 mg • $kg^{-1}$ and TAF 2 mg • $kg^{-1}$ increased the tumor inhibition rate to 67.4%. **CONCLUSION** TAF enhances MDR reversal effect of DDP on A549/DDP cells *in vitro* and *in vivo*, and down-regulates MDR1 mRNA and P-gp expression in A549/DDP cells.

**Key words** total alkaloid *Fritillaria thunbergii* multidrug resistance P-glycoprotein

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