The Role of Reactive Oxygen Species in Cisplatin-induced Apoptosis of Esophageal Cancer Cell Line EC-109

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[ABSTRACT] BACKGROUND & OBJECTIVE: Reactive oxygen species (ROS), in vivo oxygen metabolites and important signaling molecules, play a vital role in cell apoptosis. This study was to investigate the role of ROS in cisplatin (DDP)-induced apoptosis of esophageal cancer cell line EC-109, and explore the mechanism. METHODS: EC-109 cells were treated with different concentrations (0, 1, 5, 10, and 15 μg/ml) of DDP. MTT assay was used to evaluate the influence of DDP on cell proliferation. Flow cytometry was used to test ROS levels, intracellular mitochondrial transmembrane potential (∆ψm), and hypodiploid apoptosis peak in EC-109 cells. Cell apoptosis after pretreatment with hydrogen peroxide-scavenging enzyme catalase (CAT) was also detected. RESULTS: DDP obviously suppressed proliferation of EC-109 cells. When treated with 0, 1, 5, 10, 15 μg/ml of DDP for 2 h, ROS levels were (3.3±1.0)%, (21.6±2.0)%, (32.6±3.2)%, (44.7±2.2)%, and (53.1±3.6)%, respectively; when treated for 12 h, ∆ψm were (97.2±1.9)%, (90.6±1.9)%, (85.5±1.4)%, (67.8±2.0)%, and (62.4±3.0)%, respectively; when treated for 24 h, cell apoptotic rates were (3.4±1.2)%, (16.2±2.3)%, (28.1±1.5)%, (33.2±3.9)%, and (45.5±3.8)%, respectively. Pretreatment with CAT significantly rescued cells from apoptosis (P<0.05). CONCLUSION: DDP generates ROS in esophageal cancer EC-109 cells, which causes mitochondrial membrane permeabilization and ∆ψm decrease, thereby, leads to apoptosis of EC-109 cells.

KEYWORDS: Reactive oxygen species; Mitochondrial transmembrane potential; Apoptosis; Esophageal neoplasia; EC-109 cell line; Cisplatin
供有益的探索

### 材料与方法

#### 1 加药组平均

食管癌是我国常见的恶性肿瘤之一，确诊时已多属于中晚期，患者失去了手术治疗和局部放疗的机会。加药组平均加药组平均加药组平均加药组平均

### 结果

#### 1.1 DDP

DCF staining (flow cytometry, FCM) DDP

#### 1.2 EC-109

EC-109 EC-109 EC-109 EC-109

#### 1.3 MTT

### 428
Figure 1  Inhibitory effects of cisplatin (DDP) on growth of EC-109 cells

Figure 2  Changes of intracellular reactive oxygen species (ROS) levels

A, control; B, DDP, 1 μg/ml; C, DDP, 5 μg/ml; D, DDP, 10 μg/ml; E, DDP, 15 μg/ml.
2.5 CAT DDP

CAT 10 μg/ml DDP
CAT+10 μg/ml DDP

(45.7 ± 2.5)% (18.4 ± 2.2)% (F = 368.93, P < 0.01),
ΔΨm (80.9 ± 2.4)% (91.8 ± 1.5)% (F = 68.63,
P < 0.01) (33.1 ± 4.2)% (12.4 ±
信号增加且在一定的浓度范围内呈浓度依赖。活性氧是真核细胞有氧呼吸过程中形成的活性氧主要成分。结果表明细胞内活性氧减少后细胞凋亡并未完全被抑制。由此我们推测，在药物作用早期到线粒体决定凋亡的深刻变化期，活性氧起到放大倍增的信号作用。

发现在药物作用早期线粒体通透性转换孔（permeability transition pore, PTP）的开启和由此引起细胞凋亡不可逆，最终导致凋亡信号的 свone。在细胞凋亡的调控中扮演着重要的角色，它可作用于线粒体，使细胞内活性氧产生，其中一氧化氮和过氧化氢具有较强的氧化性，是活性氧的主要成分之一。它的降低是细胞凋亡的早期重要变化，一旦活性氧的降低和抑制超过阈值，它还能与活性氧的形成凋亡体，使染色质浓缩，而染色质浓缩又可作用于线粒体。

根据大量的研究报道及自己的研究成果提出，活性氧是细胞凋亡中细胞凋亡途径诱导凋亡。

最新的研究发现，细胞凋亡受到显著抑制。根据我们的研究，尽管加入抗活性氧的药物，效果并不理想，但我们在处理细胞前加入抗活性氧的药物，其作用是值得进一步研究的。