



Regression of cervical intraepithelial neoplasia by zerumbone in female Balb/c mice prenatally exposed to diethylstilboestrol: Involvement of mitochondria-regulated apoptosis

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Abstract

Background: Cervical cancer is the second most common cause of cancer death in women. We have demonstrated previously that zerumbone (ZER) has an anti-cancer effect toward human cervical cancer cells (HeLa). **Methods:** Anti-cancer properties of ZER were investigated using female Balb/c mice exposed prenatally to diethylstilboestrol. Female offspring have been treated with ZER (4, 8 and 16 mg/kg), normal saline and cisplatin (10 mg/kg; positive control). The anti-cancer properties of ZER were evaluated using histopathology, TdT-mediated dUTP nick end labeling (TUNEL) Assay and immunohistochemical staining of Bcl-2-associated X protein (Bax), a key protein in mitochondrial pathway of apoptosis. In addition, laser capture microdissection microscopy isolated RNA was amplified using reverse transcriptase polymerase chain reaction (RT-PCR) based on the specific primer of B-cell lymphoma 2 (Bcl-2). **Results:** Treatment with ZER resulted ($P < 0.05$, w_2 statistics) in the regression of cervical intraepithelial neoplasia (CIN) resembling cisplatin effect (10 mg/kg). TUNEL micrographs showed the absence of apoptosis in cancerous tissues treated with normal saline compared to ZER and cisplatin where abundant apoptotic cells were noticed. A post hoc analysis showed a significant ($P < 0.01$) difference in mean percentage of apoptosis between normal saline treatment (0%), ZER (15.7%) and cisplatin (21.7%). Immunohistochemical staining of Bax protein revealed that ZER modulates the expression of this apoptosis marker. Administration of ZER has also modulated the expression of Bcl-2 gene. **Conclusion:** These findings showed that ZER induces apoptosis efficiently in cervical tissues from female Balb/c mice treated prenatally with diethylstilboestrol. This suggested that ZER, a plant-derived compound, could be introduced as a new chemo-preventive agent for CIN in future. © 2009 Published by Elsevier GmbH.

Keywords: Zerumbone; Cisplatin; Cervical intraepithelial neoplasia; Apoptosis; TUNEL assay; Bax protein