Clinical Research

The pathological pattern of seven malignant cancers following Demethylcantharidin

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Abstract

Objective: To study effect of Cantharidin which was contained in *Mylabris phalerata* or *M. cichoris* L. in China or *Cantharis vesicatoria* in European, one kind of toxic insect, on different types of cancer patients. **Methods:** In this study, using comparative analyses, 6 of 7 cancers patient having malignant "nest" cells appears to be pathologically degenerated and necrosis occurs in diverse degree after undergoing demethylcantharidin. Demethylcantharidin 12-16mg added in 5% glucose or GNS solution and given intravenous daily, 2-8 weeks/one course. Cantharidin was treated over three weeks in 4 patients (total dosage over 300mg), cantharidin up to 15 days in 1 case (total dosage 210mg), and cantharidin within 10 days (total dosage 100mg) in 2 cases. Histologically and laparotomy were performed in 7 patients after 1 to 3 days of cantharidin. The pathological changes were measured by score analysis. **Results and conclusion**: The object response was proportion to the dosage of cantharidin. Moreover, there was coexistence of degeneration and coagulative necrosis of malignant "nest" cells, the phenomenon of lymphocyte infiltration and fibrosis around "nest" cells. *In vitro* cantharidin was remarkably sensitive to the inhibition of DNA, RNA synthesis, stimulate lysozyme activity in PHC cell line and xenograft tumors. The findings implicate that cantharidin is clinical therapeutically benefits.

Keyword: Cancer, hepatocellular carcinoma, Cantharidin, pathological changes

Introduction

Traditional system of medicine used all over the world even traditional medicine also used in cancer and plant products for therapeutic purposes. As compared to each drug alone, for instance, the combination treatment of glioblastoma multiforme (GBM) cell lines with anti-malarial artesunate and oncogenic receptor EGFRvIII inhibitor OSI- 774 (Efferth et al., 2004; Al-Nedawi et al., 2008; Zhu et al., 2016 & 2017) can increase growth inhibition of GBM cells. Another topical application of cantharidin to treat pediatric molluscum contagiosum and warts caused by *Molluscum contagiosum* virus (MCV) has been found effective (Silverberg, 2003; Smolinski, 2005). Blister bettle and cantharidin has long tradition in Asian medicine such as china and vietnam to treat cancer (Rauh et al., 2007). On the basis of report, in a recent year developed a set of cantharidin derivatives of new anticancer drugs. Demethylcantharidin (Norcantharidin), norcantharidin proliposome (Miao et al., 2006) and sodium cantharidate that belongs to this categories. Novel TCM-platinum compounds [pt (C (18) H (8) O (5) (NH (2) R) (2)) 1-5] (To et al., 2004), derived from integrating demethylcantharidin, a modified component from a traditional medicine with a platinum moiety, possess anticancer effect.

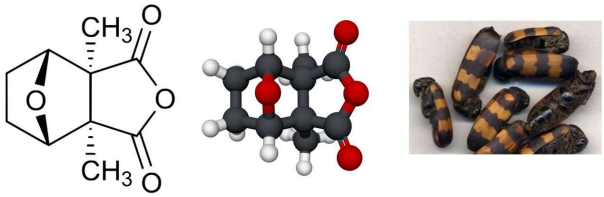

Figure 1. The chemical structure of cantharidin (2,6-Dimethyl-4,10-dioxatricyclo-[5.2.1.0]decane-3,5-dione)

Demethylcantharidin (EXO-cis-3.6 epoxyhe-Xahydro-Phthalic anhydride, (figure 1) was made in 1978. The true efficacy of demethylcantharidin was first to be reported by Professor BY Yang in 1983 (Zhu et al., 2017), who conducted CR (complete remission) in a patient with jaundice hepatocellular carcinoma, stage III. Total dosage of cantharidin was 1872mg. In the follow up, the patient with 6 years of disease-free survival remained well. Later, in May, 1998, Zhu started cantharidin in research program on unresectable advanced colon carcinoma (2 cases) in

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order to improve the symptoms, but no tumor regression (one colon cancer cantharidin 580mg). In April, 1999, a short CR (8+months) was obtained by using FAH (5-Fu, Ara-C, homoharringtonine) plus cantharidin (180mg) in an advanced gastric adenocarcinoma (8x10cm) complicated with intrahepatic metastasis. In May 2000, a CR (18+months, died of other disease) was also obtained through the combination of combined chemotherapy (VCR, CYT, 5-Fu, MMC, ADM) plus cantharidin (150mg) in an advanced lymphoma with intrahepatic lesion. In April, 2004, under gastroscope, another (ulcer) gastric cancer obtained a short CR via small dosage of 5-Fu, cantharidin and traditional medicine. Three months later, repeated B ultrasound scan presented no abnormality of gastric lesion. Moreover, Zhu (2002) conducted CR in an earlier viral hepatocellular carcinoma. After total dosage of cantharidin 1200mg, serum bilirubin was from 31.7umol/l declined to normal range (4-23.9umol/l) and serum AFP was from 8.7ng/ml declined to 2.05ng/ml. A 15-year follow up, he remained well. Cantharidin was also used in nasopharynx cancer (2 cases), lung cancer (490mg in 1 case), and metastatic breast cancer (1 case). These data indicated partly cantharidin in the role of cancer treatment especially in predispose to the indication of primary hepatocellular carcinoma (PHC) and gastric cancer. In this paper, we concluded the pathological changes in earlier, 7 malignant tumours following demethylcantharidin therapy.

Materials and methods

All 7 patients were in progressive at hospitalization.7 patients were PHC 1 case, esophagus cancer 1 case, gastric cancer 1 case, colon cancer 1 case, and carcinoma of gastric cardia 3 cases.

An approach to therapeutical protocol was that demethylcantharidin 12-16mg added in 5% glucose or GNS solution intravenous daily, 2-8 weeks/one course. Cantharidin was treated over three weeks in 4 patients (total dosage over 300mg), cantharidin up to 15 days in 1 case (total dosage 210mg), and cantharidin within 10 days (total dosage 100mg) in 2 cases. Histologically and laparotomy were performed in 7 patients after 1 to 3 days of cantharidin stop and biopsies from liver mass under gross inspection was quickly removed, regulatory dehydrated, fixed in 10% neutral buffered formalin, embeded in paraffin and stained with hematoxyline/eosin (HE), and evaluated using light microscope. The pathological changes were measured by score analysis. If an area of pathological changes was observed, tissues were serially sectioned.

Grade I. Disappearance of massive proliferation of malignant "nest" cells with degeneration and necrosis, replaced by fibrosis, rarely scattered malignant cells but without proliferation.

Grade II. Decreased massive proliferation of malignant "nest" cells with scattered degeneration and necrosis.

Grade III: local degeneration and necrosis of malignant "nest" cells, with distorted lobular architecture.

Grade IV. No any changes above. A massive proliferation of malignant "nest" cells without any degeneration and necrosis.

Interstitial reaction included strong, moderate, weak and no reaction according to the infiltration of lymphocytes, plasmacytes and fibrogenesis.

Another 60 cancers without cantharidin treatment was designed as control group. There were gastric cancer 26 cases, esophageal cancer 15 cases, carcinoma of gastric cardia 12 cases, colon cancer 6 cases, and hepatocellular carcinoma 1 case. Tissue specimens section was pathologically examined.

Results and discussion

In seven patients, complete remission (Grade I) was achieved in 2 cases according to score analysis. Grade II (partial remission, PR) in 3 cases, Grade III (minor remission, MR) in 1 case. No objective response (Grade IV) was noted in only 1 case. The detail results listed in table 1, figures 2 and 3.

Table 1. Patients characteristics

Cases	Diagnosis	Days for cantharidin	Total dosage of cantharidin	Pathological results
1	Primary hepatocellular carcinoma	23	352mg	Grade I
2	Colon carcinoma	29	404mg	Grade I
3	Carcinoma of gastric cardia	22	344mg	Grade II
4	Gastric cancer	27	328mg	Grade II
5	Carcinoma of gastric cardia	15	210mg	Grade II with moderate interstitial reaction
6	Esophagus cancer	8	96mg	Grade III
7	Carcinoma of gastric cardia	7	84mg	Grade IV

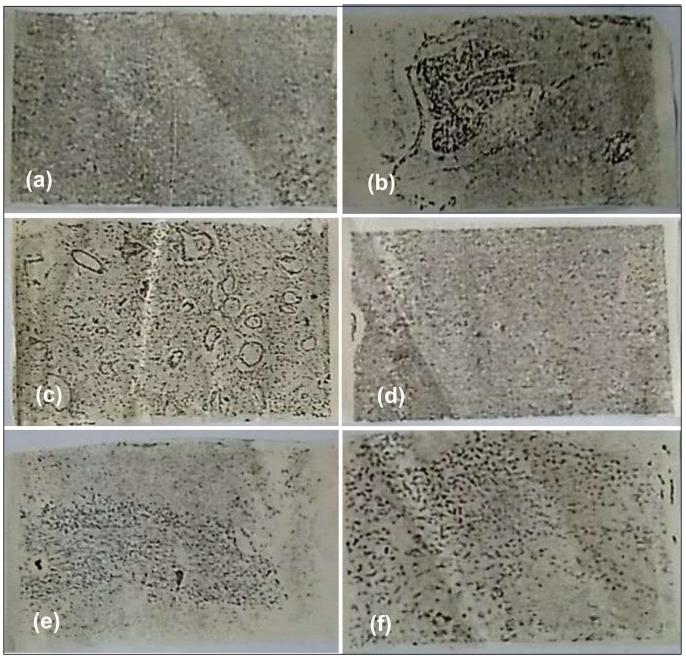

Figure 2. (a) A massive necrosis (histologically grade I) within malignant "nest" cells in a hepatocellular carcinomas following demethylcantharidin (352mg) (No.7492); **(b)** A massive necrosis (histologically grade I) within malignant "nest" cells in a colon cancer following demethylcantharidin (404mg) (No.7614); **(c)** A sheet of degeneration and necrosis (histologically grade II) was noted in a adenocarcinoma of gastric cardia following demethylcantharidin (344mg) (No.7044); **(d)** Area of clear vacuoles and local necrosis (histologically grade II) was noted in a gastric cancer following demethylcantharidin (328mg) (No.7342); **(e)** A sheet of coauglutive necrosis within malignant "nest" cells in carcinoma of gastric cardia following demethylcantharidin (210mg) (No.7358); **(f)** An area of lymphocyte infiltration around malignant "nest" cells was noted (immune defensive response) (No.7358).

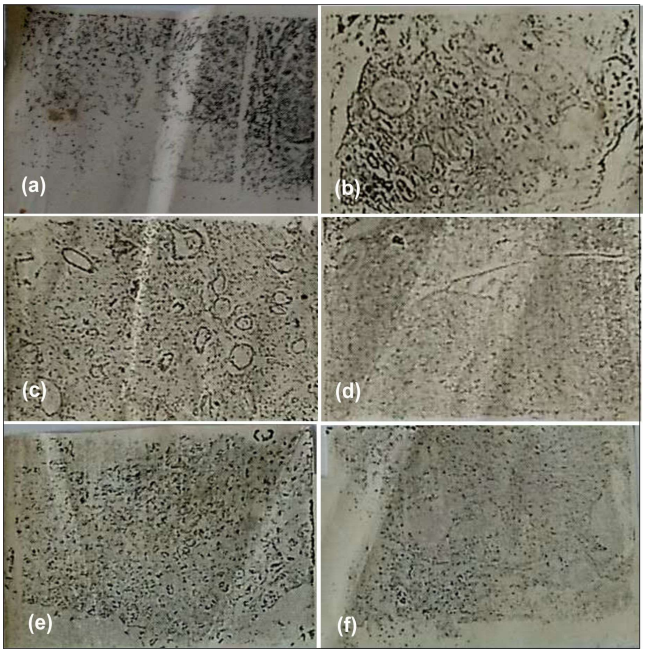

Figure 3. (a) An area of fibrosis around malignant "nest" cells was noted (immune defensive response) (No.7358); **(b)** A local degeneration and necrosis (histologically grade III) was noted in an esophageal carcinoma following demethylcantharidin (96mg) (No.9817); **(c)** No pathological changes in a carcinoma of gastric cardia following demethylcantharidin (84mg) (No.7121); **(d)** Control biopsies from a hepatocellular carcinoma. No pathological changes following 5-Fu (5000mg) and VCR (4mg) (No.7121); **(e)** Control biopsies, non-treated with demethylcantharidin from a esophagus cancer, no obvious changes was pathologically noted. (No.7652); **(f)** Control biopsies,non-treated with demethlycantharidin from a carcinoma of gastric cardia,no obvious changes was pathologically noted. (No.9307)

In this study, 6 of 7 cancers the malignant "nest" cells appears to be pathologically degenerated and necrosis in diverse degree after undergoing demethylcantharidin. The objective response was proportion to the dosage (300mg) of cantharidin, for the reason no any pathological changes in those patients using low dose.

In comparative control group, sixty cancers without cantharidin therapy tissues were serially inspected. The results were pathologically grade I, 2 cases (3.3%), grade II, 1 case (1.6%), grade III. 1 case (1.6%), lymphocytosis 3 cases (5%), and infectious necrosis (necrotic tissues with polynucleated cells) 6 cases (10%) respectively. There was statistically significance when the comparison of patients with cancers undergoing demethylcantharidin (6/7) with non-cantharidin controls (4/60).

Cantharidin and norcantharidin treatment causes DNA strand break in CCRF-CEM cells (Efferth et al., 2005) and in oral cancer KB cells (Kok et al., 2002). Cantharidin induces apoptosis by a p53-dependent mechanism on leukemic cells (Rauh et al., 2007). Cantharidin and norcantharidin are inhibitors of protein phosphatase (PP1) and protein phosphatase 2A (PP2A), PPP1R13B, the regulatory subunit 13B of PP1 (Li et al., 1992; Honkanen, 1993; Eldridge and Casida, 1995; McClusker et al., 2001; Laidley et al., 1997; McCl- usker et al., 2003; To et al., 2004; Rauh et al., 2007). PPP1R13B plays a central role in the regulation of apoptosis via its interaction with the tumor suppressor gene p53. Therefore, PP1 is one of the four major serine/threonine protein phosphatases, and the role of PP1 in the repair of UV-induced DNA lesions (Herman et al., 2002). PPP1R13B has a specific function in cantharidin induced DNA repair and apoptosis. Protein phosphatases are involved in the regulation of multiple cellular processes including signal transduction pathways, cell cycle progression, apoptosis, glucose metabolism and calcium transport (Wera and Hemmings, 1995; Rauh et al., 2007).

Moreover, in vitro cantharidin was remarkably sensitive to the inhibition of DNA, RNA synthesis, stimulate lysozyme activity in PHC cell line and xenograft tumors,

leading to cell shrinkage, nuclear condensation, degeneration and necrosis of cancer cells. We experienced that under moderate dosage of cantharidin, there was coexistence of degeneration and coagulative necrosis of malignant "nest" cells and the phenomenon of lymphocyte infiltration and fibrosis around "nest" cells in case 5, which indicate the inhibition of cancer cells without immune defense damage under this dose. Further, cantharidin stimulates host immune lymphocyte activity in vitro and *in vivo*, inducing the biological activity, was under investigation.

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