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Synthesis and synergetic anti-tumor activity evaluation of dihydroartemisinin-organogermanium(IV) compound

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ABSTRACT

Dihydroartemisinin (DHA), a semi-synthetic derivative of the herb artemisinin, has shown commendable bioactivity. In this paper, a novel dihydroartemisinin-organogermanium (DHA-Ge) compound was synthesized, characterized and its potential anti-tumor activity was evaluated by various methods. MTT results demonstrated that DHA-Ge could effectively inhibit the proliferation of HepG2 cells and showed their dose-dependent properties. The IC_{50} value of inhibition effect on HepG2 cells of DHA-Ge was 10.23 μg/ml which was lower than 39.44 μg/ml of DHA. Flow cytometric results suggested that DHA-Ge could induce apoptosis of HepG2 cells and the apoptosis rate was 20.26% after 24 h treatment with 56.8 μg/ml DHA-Ge concentration. Atomic force microscopy images showed that HepG2 cells were collapsed and the cell nucleus were fragmented after 24 h treatment. All these results together showed that the DHA-Ge possessed desirable synergetic enhanced anti-tumor effects and could be developed as a suitable tumor therapeutic agent.

Germanium (Ge) is an active ingredient of many Chinese medicines, such as ginseng, which plays a significant role in the pharmacological effects of the plants.[1,2] Germanium was used as dietary supplements for cancer and AIDS, but it is reported that many germanium compounds have potent toxicity.[3,4] Studies showed that organic germanium compounds not only had much lower toxicity, but also possessed immunomodulatory activity, anti-oxidation, antibacterial and anti-tumor activity.[5–9] Since Ge-132 (carboxyethylgermanium sesquioxide) was found.[10] Great efforts have been made to investigate organic germanium compounds for low-toxic and effective antitumor drugs,[11–17] but most of these achievements fell short of the desired results or prematurely terminated research on clinical uses.[18,19] Accordingly, drugs with better antitumor effects are required to fight against cancer cells. It was found that some of Ge-132 derivatives showed stronger anticancer activities than Ge-132,[20,21] therefore selecting an appropriate modify molecule offers a thrill route to devise anti-tumor reagent with enhanced activity.

As traditional Chinese herbal, artemisinin has been applied to treat fever for many centuries[22] and used to synthesize some modern anti-malarial drugs.[23] But its application was limited due to poor solubility. Dihydroartemisinin (DHA) is the main active derivative of artemisinin which possess a better bioactivity than artemisinin.[24] Recently, it was proved that DHA inhibited cell proliferation and induced apoptosis in various tumor cells.[25–29] A variety of classes of DHA derivatives had been made and their effect towards diverse tumor cell lines was compared with that of artemisinin and anti-tumor drugs.[25,29]

In order to develop an anti-tumor reagent with enhanced bioactivity and take advantage of the synergetic effects of Ge-132 and DHA, a dihydroartemisinin-organogermanium (DHA-Ge) compound was prepared. The scheme of synthesis route is showed in Figure 1. ESI-MS, ^{1}H NMR, Cyclic voltammetry, FT-IR and elemental analysis were performed to characterize the synthesized compound. The ESI-MS information in Supplementary data (Fig. S1) showed the fragment ions in agreement with the expected structure of DHA-Ge compound. The disappearance of the signal at 3.70 ppm originated from HO- of DHA in the ^{1}H NMR (Fig. S2) demonstrated that the DHA and the Ge-132 were connected. In the FT-IR spectra of DHA-Ge compound (Fig. S3), there is a peak at 1739 cm^{-1} attributed to the stretching vibration of C=O, which further indicated the binding of DHA with Ge-132. While the peak at 460 cm^{-1} corresponded to the stretching vibration of Ge-C[30] indicated the existence of Ge-132. Furthermore, the cyclic voltammogram (Fig. S4) showed the endoperoxide-bridge of DHA in DHA-Ge molecule retained intact during the synthesis. The elementary analysis data (Table S1) showed that the experimental results of the DHA-Ge compound were in accord with the theoretical ones. All of these results demonstrated the successful preparation of DHA-Ge compound.

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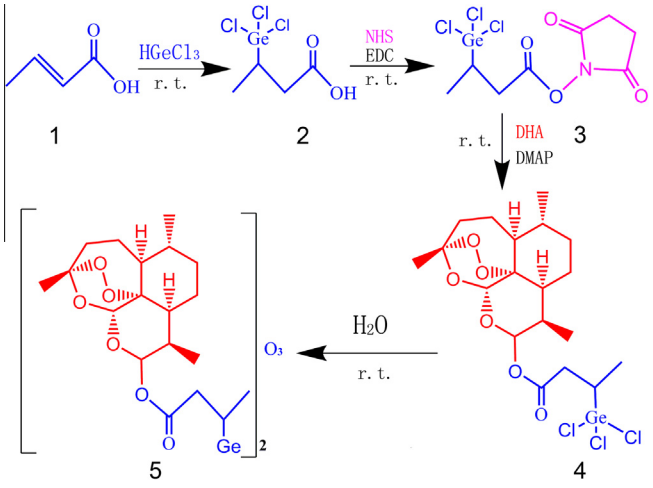

Figure 1. Schematic representation of the synthesis of DHA-Ge. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP), dihydroartemisinin (DHA).

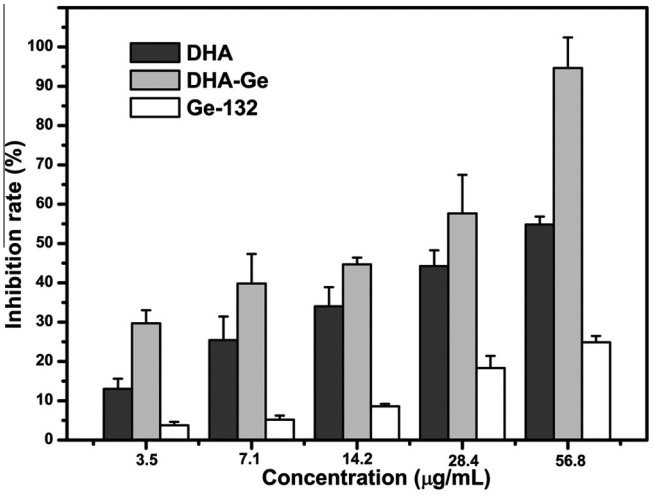

Figure 2. Compared inhibitory effects of DHA, DHA-Ge and Ge-132 on HepG2 cells by MTT assay.

The inhibitory effects on proliferation of HepG2 cells after exposure of DHA-Ge were evaluated by the MTT assay. Ge-132 and DHA were evaluated as compare subjects. The data (Fig. 2) showed that the DHA-Ge could effectively inhibit the growth and proliferation of HepG2 cells. The survival rate of HepG2 cells was decreased with the increased concentration of DHA-Ge compound, which suggested their concentration-dependent properties. The value of IC_{50} of DHA-Ge compound for HepG2 cells was 10.23 μg/ml which was lower than 39.44 μg/ml of DHA, which indicated that the inhibition of DHA-Ge compound was more obvious than that of DHA. These results suggested that the DHA-Ge compound possessed much higher antitumor activity compared with DHA and Ge-132. The better antitumor activity derived from DHA-Ge compound retained the basic structure of DHA and Ge-132 and they were well functioned, DHA and Ge-132 played a synergetic enhanced effect in DHA-Ge compound.

Inverted microscope was used to obtain morphology of HepG2 cells which were treated with different concentration of DHA-Ge compound for 24 h. The cells in the control group showed normal morphological characteristics and were full in shape and were evenly distributed (Fig. 3). But cell morphology changed to round from fusiform and the number of cells in round shape increased

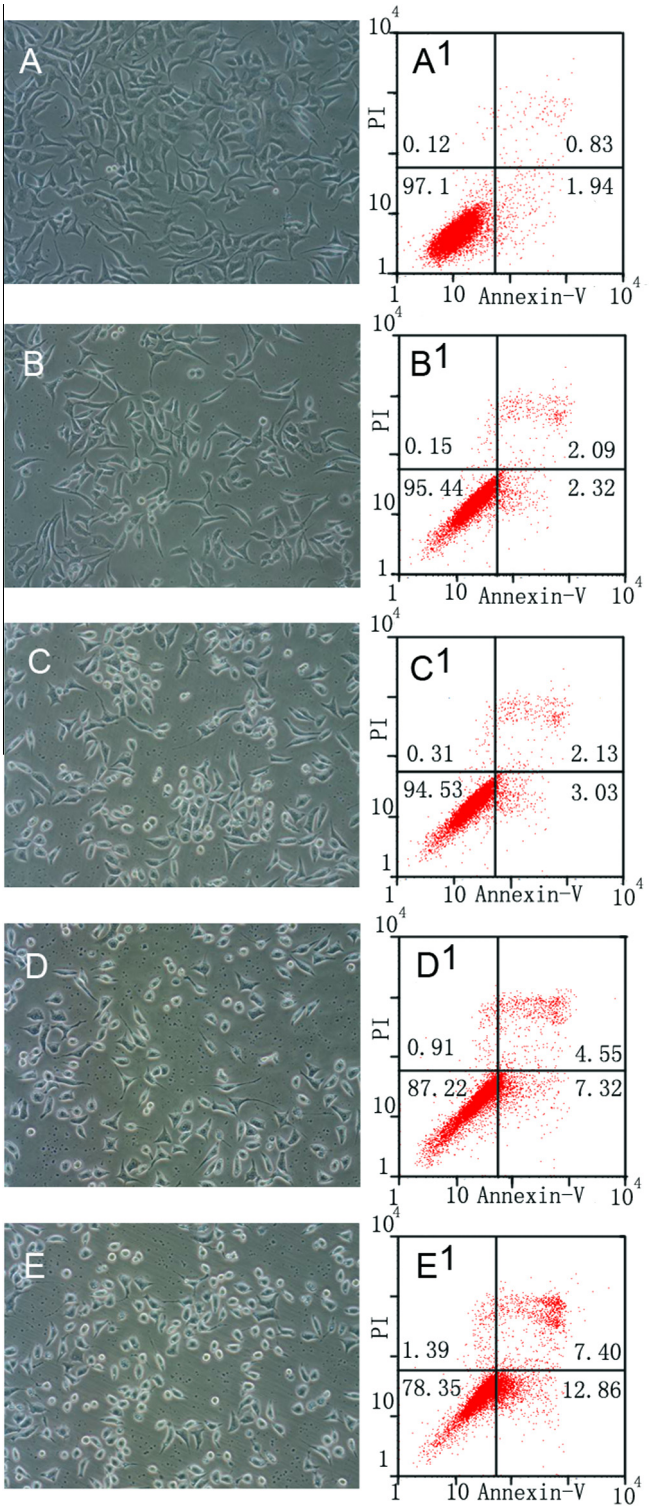

Figure 3. DHA-Ge stimulates morphological changes of HepG2 cells (A–E) by inverted microscope and effects of DHA-Ge on cell apoptosis by flow cytometry (A1–E1) after 24 h treatment. Concentration of DHA-Ge (A/A1) 0 μg/mL; (B/B1) 7.1 μg/mL; (C/C1) 14.2 μg/mL; (D/D1) 28.4 μg/mL; (E/E1) 56.8 μg/mL.

with the increased concentration of DHA-Ge compound after 24 h treatment. HepG2 cells were in a state that these cells were apoptosis at early phase. The apoptosis of HepG2 cells induced by DHA-Ge compound after 24 h of exposure was determined by flow cytometry. The result suggested that apoptosis of HepG2 cells depended on the concentration of DHA-Ge compound and the rate