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2–[(3–CARBOXY–1–OXOPROGY1) AMINO]–2–DEOXY–D–GLUCOSE INDUCES APOPTOSIS IN Hep G2 CELLS

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***Aim*: To determine the effect of 2-[(3-carboxy-1-oxoprogy1) amino]-2-deoxy-D-glucose (COPADG) on proliferation and apoptosis of human hepatocellular carcinoma cells (Hep G2). *Methods*: Hep G2 cells were cultured in RPMI-1640 medium in the presence of various concentrations of COPADG. Cell proliferation was determined by MTT assay. Apoptosis was determined by fluorescence microscopy, transmission electron microscopy, agarose gel electrophoresis of DNA fragmentation, and flow cytometry. *Results*: At the concentration ranging between 1–30 μM, COPADG potently inhibits growth and induce apoptosis of Hep G2 cells. *Conclusions*: COPADG could effectively induce apoptosis in Hep G2 cells. It may be potentially useful as a new agent for treatment of human hepatocellular carcinoma.**

***Key Words*: apoptosis, hepatoma, Hep G2, 2-[(3-carboxy-1-oxoprogy1)amino]-2-deoxy-D-glucose, COPADG.**

Apoptosis plays an important role in the elimination of unwanted or damaged cells. A delicate balance between apoptosis and cellular proliferation is critical in the maintenance of physiological homeostasis. On the other hand, dysregulation of apoptosis in response to various physiological and pathological stimuli has been associated with certain diseases such as neural degenerative disorders and cancers. In recent years, apoptosis has been increasingly recognized as an important type of cell death in cancer therapy, and thus agents or treatment modalities that result in apoptosis have become a new focus in cancer therapy [1–5]. Resistance to undergo apoptosis is one of the important mechanisms that lead to treatment failure in cancer [6, 7].

2–[(3–carboxy–1–oxoprogy1) amino]–2–deoxy–D–glucose (COPADG) is a derivative of D–glucose, a product of Chitosan's degradation process. Previous studies have discovered that some D–amine–glucose derivatives were able to induce leukemia cells K562 to differentiate into macrophages [8]. However, questions such as whether derivatives of D–amine–glucose can induce apoptosis in tumor cells, and if so, what are the molecular mechanisms involved, remain to be answered. In this study, we tested whether COPADG have any killing effects on human hepatocellular carcinoma cells *in vitro*.

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Abbreviations used: AO — acridine orange; DMSO — dimethylsulfoxide; FCM — flow cytometry; FCS — fetal calf serum; MOPS — [3–N–(morpholino)propane sulfonic acid]; HCC — hepatocellular carcinoma; MTT — 3–(4,5–dimethylthiazol–2–yl)–2,5–diphenyltetrazolium bromide; PBS — phosphate buffered saline; PI — propidium iodide.

MATERIALS AND METHODS

COPADG was synthesized by the Lanzhou Institute of Chemical Physics, China Academy of Sciences, China. Human hepatocellular carcinoma cells (Hep G2) were cultured in RPMI–1640 medium supplemented with 10% heat inactivated FCS, 100 μg/ml streptomycin and 100 μg/ml of penicillin (InVitrogen) and maintained in a humidified atmosphere of 5% CO_2 95% air at 37 °C.

MTT colorimetric assay. Cell viability and growth inhibition were determined by MTT assay, as previously described [9]. Briefly, after treatment with indicated concentrations of COPADG, cells were incubated with 5 mg/ml of MTT for 4 h. MTT was solubilized with dimethylsulfoxide (DMSO), and the optical densities (Å) were determined using a microplate reader at 490 nm. Cell growth inhibition was calculated as follows:

% growth inhibition = 1–Å (treated cells)/Å (control cells) × 100%.

Detection of apoptosis. Effect of COPADG on cell death was determined by staining with acridine orange (AO). After treatment with various concentrations of COPADG, cells were fixed and stained with 0.01% AO for 30 min at room temperature. Apoptotic cells were counted under the fluorescence microscope, and expressed as a percentage of the total number of cells counted.

The procedures for detection of DNA fragmentation by agarose gel electrophoresis was performed a previously reported [10]. Briefly, cells were lyzed and genomic DNA extracted. Five μg of the extracted DNA

which COPADG inhibits proliferation and induces apoptosis in cancer cells.

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ИНДУКЦИЯ АПОПТОЗА В КЛЕТКАХ ЛИНИИ Hep G2 ПРИ ВОЗДЕЙСТВИИ 2–[(3–КАРБОКСИ–1–ОКСОПРОГИ1) АМИНО]–2–ДЕЗОКСИ–D–ГЛЮКОЗЫ

***Цель*: определить влияние 2-[(3-карбокси-1-оксопрги1) амино]-2-дезокси-D-глюкозы (COPADG) на пролиферацию и апоптоз клеток гепатоцеллюлярной карциномы человека линии Hep G2. *Методы*: клетки Hep G2 культивировали в среде RPMI-1640 в присутствии различных концентраций COPADG. Пролиферацию клеток оценивали в МТТ-тесте, апоптоз — с использованием флуоресцентной и трансмиссионной электронной микроскопии, в агарозном гель-электрофорезе для выявления фрагментации ДНК и проточной цитометрии. *Результаты*: в концентрации 1–30 μM COPADG ингибировал рост и индуцировал апоптоз в клетках линии Hep G2. *Выводы*: COPADG может индуцировать апоптоз в клетках линии Hep G2, что свидетельствует о возможности его потенциального использования в терапии гепатоцеллюлярной карциномы человека.**

***Ключевые слова*: апоптоз, гепатома, Hep G2, 2-[(3-карбокси-1-оксопрги1) амино]-2-дезокси-D-глюкоза.**