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Research Article

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Effect of xanthotoxin on SGC-7901 cells proliferation and Autophagy

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ABSTRACT

Discussion of xanthotoxin (8-MOP) inhibiting effect on human gastric carcinoma SGC - 7901 cells, its effection on autophagy and its mechanism in vitro. Determined by MTT assay to detect different concentrations (10,20,40,80,160 μg / mL) of 8-MOP effected SGC-7901 cells after 48 h. Autophagosome was observed by transmission electron microscope.The contents of calcium ions was assayed by confocal laser.8-MOP obviously inhibited the SGC-7901 cell proliferation, its inhibition was in relationship with concentration-response. Transmission electron microscope observation showed that it generated a lot of autophagosome. 8 - MOP made intracellular calcium ion concentration elevated and it was dose-dependent manner. Its mechanism might be associated with the increase of intracellular Ca^{2+} concentration.

Keywords: Xanthotoxin ; gastric cancer ; proliferation; autophagy ; calcium ions

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INTRODUCTION

8-MOP is first isolated from the fruit of big Ami,it is Umbelliferae and Rutaceae plants such major linear furanocoumarins. It is more common in Chinese prickly ash, star anise and other seasoning, has very strong photosensitivity, mainly used in clinical treatment of angina pectoris and PUVA therapy in the treatment of vitiligo, psoriasis and other skin diseases[1-3]. Studies have shown that xanthotoxin can have inhibitory effect on tumor cells, but the mechanism remains unclear[4]. This experiment in vitro antitumor experimental observation xanthotoxin in human gastric adenocarcinoma SGC - 7901 cell proliferation inhibition, and study the possible mechanisms of inhibition of growth of SGC - 7901 cells, providing theoretical basis for the widely application of xanthotoxin.

EXPERIMENTAL SECTION

1. Experimental materials

1.1 Tumor cell lines

Human gastric cancer SGC-7901 cells by the Harbin University of Commerce Institute of Materia Medica Postdoctoral passaged conservation .

1.2 The main drugs and reagents

8-MOP (Mass fraction of 98% , Nanjing Zelang Medical Technology Co., Ltd.); 5 – fluorouracil(5-FU)(Tianjin Jinyao Amino Acid Co., Ltd.) ;RPMI1640(Beijing solarbio company); Thiazolyl Blue(MTT)(America Sigma company);Fetal bovine serum(America Hyclone company);trypsin(America Sigma company);Dimethyl sulfoxide(DMSO)(America Sigma company);Fluo-3/AM(Beijing Beyotime company).

1.3 The main experimental apparatus

MC0175 CO_2 incubator（Sanyo company）; SW-CJ-2FD clean bench（Sujing group）; SUNRISE microplate reader （TECAN company）; H-7650 transmission electron microscope（HITACHI company）; SP-2 laser scanning

confocal microscope（Leica company）; IX70 inverted microscope（Olympus company）; Adventurer millionth electronic balance（Ohaus company）.

2 Experimental Methods

2.1 MTT method to detect xanthotoxin effect on SGC - 7901 cell proliferation

5×10^3 logarithmic growth phase of human gastric adenocarcinoma SGC-7901 cells were seeded in 96-well plates at 37 ℃, the volume fraction of 5% CO_2 incubator conditions cultured for 24 h, were added to each well 100μl drugs (blank Add 100μl of control BASIC) , the final concentration of the drug 10,20,40,80,160 μg / mL (each has six parallel holes) , the positive control group in a final concentration of 30μg/mL 5-FU, to after drug cells cultured in an incubator 48h, were added to 100μl concentration of 0.5 mg / mL of MTT solution was extracted Prudential and liquid, cultured 4 h, and then the supernatant was aspirated , each hole by adding dimethyl sulfoxide 100μL, gently shaking 10 min to allow bromide MTT completely dissolved , measured at 492nm wavelength microplate absorbance (OD) values , based on the measured the absorbance value of the cell viability , and then calculate the inhibition rate [5] .

2.2 Transmission electron microscopy to detect autophagy

3×10^5 logarithmic growth phase cells were seeded in 6-well plates at 37℃, the volume fraction of 5% CO_2 incubator conditions cultured for 24 h, added to a final concentration of 25μg/mL pepper toxins, the positive control group join 10μg/mL5-FU, negative control group was the same volume of BASIC . The cells were collected 48h after administration of the centrifugal tube , PBS washed twice with 2.5 % glutaraldehyde and 1% osmium tetroxide dual fixed , ethanol, acetone , dehydrated , embedded in epoxy resin , sliced, dioxygen acetate after the uranium and lead citrate double staining TEM photographs [6] .

2.3 Confocal detection SGC-7901 cells calcium concentration

3×10^5 logarithmic growth phase cells were seeded in 6-well plates at 37℃, the volume fraction of 5% CO_2 incubator conditions cultured for 24 h, add pepper to a final concentration of toxins were 12.5, 25 , 50. positive control group was added 10μg/mL 5-FU, negative control group was the same volume of BASIC . Cells were collected 48h after administration in the centrifuge tube , PBS wash again , add 200μl Fluo-3/AM fluorescent probe (4 μM), 37℃ dark incubated 60 min; laser scanning confocal microscope observation . Excitation wavelength of 488 nm, emission wavelength of 540 ~ 570 nm [7].

3 The experimental results

3.1 Xanthotoxin effect on SGC - 7901 cell proliferation

Xanthotoxin effect on SGC - 7901 cells after 48 h experimental results such as table 1, the results showed xanthotoxin on SGC - 7901 cells was significantly inhibited with an IC50 of 55.53μg/mL, and in a dose -dependent manner.

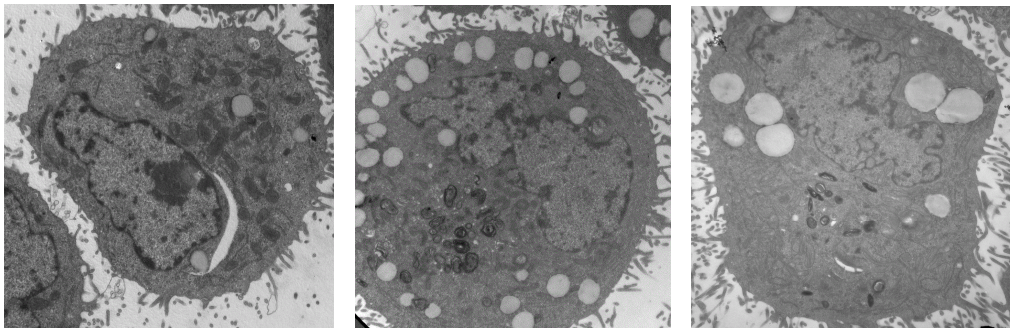
Table 1 Inhibitory rate of of 8-MOP on SGC-7901cells by MTT assay($\bar{X}$ ±s, n=6)

group	dosage(μg/mL)	OD value	IR(%)
Control	--	0.343±0.688	
8-MOP	10.0	0.331±0.027	3.50
8-MOP	20.0	0.271±0.032**	21.00
8-MOP	40.0	0.202±0.008**	41.11
8-MOP	80.0	0.126±0.032**	63.27
8-MOP	160.0	0.067±0.004**	80.47
5-FU	30.0	0.118±0.023**	65.60

***Compared with control P<0.01* **Compared with control P<0.05*

3.2 TEM observation of changes in the structure of SGC-7901 cells

Negative control group SGC-7901 cell membrane integrity, cell nucleus and organelles were normal clear ; with 5 - fluorouracil intervention SGC-7901 cells , nuclear condensation , chromatin condensation , and the emergence of autophagic vacuoles ; Xanthotoxin administration after 48h, SGC- 7901 a large number of double and multi-layer film structure , and gradually extended wrapped cytoplasmic components forming part of autophagy , cell vacuoles increased significantly.

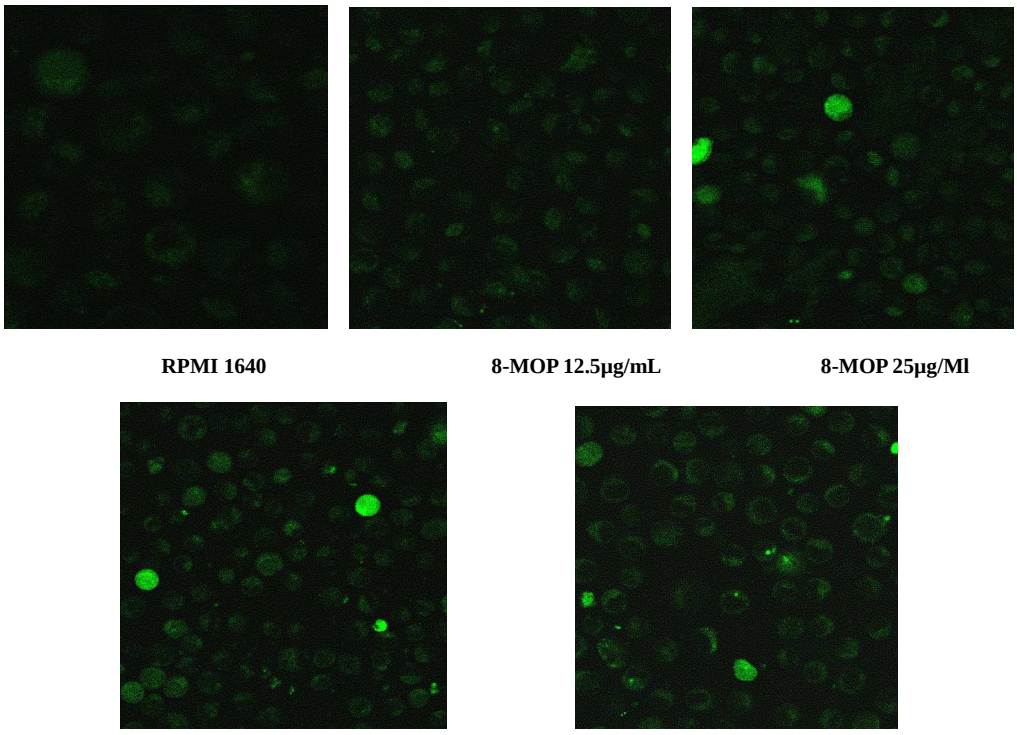


RPMI 1640 **8-MOP 25μg/mL** **5-FU 10μg/mL**

Fig.1 The ultrastructural changes of 8-MOP in-treated SGC-7901 cells were observed under transmission electronic microscope(×12000）

3.3 Xanthotoxin in the effect of calcium ion concentration in the SGC - 7901 cells

Different concentrations of Xanthotoxin on SGC-7901 cells after 48h, the calcium ion concentration increases with the dose of 8-MOP within the SGC-7901 cells, and compared with the control group was statistically significant (P <0.05).



RPMI 1640 **8-MOP 12.5μg/mL** **8-MOP 25μg/Ml**

8-MOP 50μg/mL **5-FU 10μg/mL**

Fig.2 Effect of 8-MOP on variation of $[Ca^{2+}]_i$in SGC-7901 cells observed

Tab.2 Effect of 8-MOP on Variation of $[Ca^{2+}]_i$ in SGC-7901 cells

group	dosage(μg/mL)	cell numbers	Variation of $[Ca^{2+}]$ (FI)
Control	--	30	12.091±0.715
8-MOP	12.5	30	20.900±1.335**
8-MOP	25.0	30	28.468±1.201**
8-MOP	50.0	30	34.292±0.927**
5-FU	10.0	30	32.740±1.235**

***Compared with control P<0.01* **Compared with control P<0.05*

DISCUSSION

Autophagy is mainly responsible for the removal of aging , damaged organelles, proteins and other cytoplasmic and longevity , excessive autophagy can also cause cell death , known as type Ⅱ programmed cell death [8]. Many

studies have shown that autophagy in tumorigenesis , development and treatment have played an important role in regulating autophagy through to influence tumor growth has wide application prospects mad[9-11] . The Xanthotoxin on a variety of tumor cell inhibition , and its mechanism is unclear.

This study first measured by MTT assay Xanthotoxin on SGC-7901 cells significantly inhibited the proliferation , can inhibit the proliferation of SGC-7901 cells via autophagy that Xanthotoxin simultaneously observed by transmission electron microscopy . Meanwhile confocal detection SGC-7901 cells, the calcium ion concentration display , pepper toxin SGC-7901 after 48h, intracellular Ca^{2+} concentrations generally increased cells, and an increase in the concentration of toxins and pepper into a significant dose-related .

Studies have shown that Ca^{2+} -induced cell cavity endoplasmic reticulum stress can regulate autophagy[12] . Intracellular Ca^{2+} increase in the cavity can inhibit the mTOR -dependent modulation of calcium through calcium-activated protein kinase kinase -β (CaMKK-β) -mediated reaction AMP protein kinase (5'-AMP-activated-protein kinase, AMPK) activation to induce autophagy occur. And studies have shown that cellular Ca^{2+} increases can not mediated by Ca^{2+} AMPK activation induced and directly induce the occurrence of autophagy and inhibit the degradation of autophagosome [13-16].

To sum up, Xanthotoxin to human gastric adenocarcinoma SGC - 7901 cells has a stronger inhibitory effect, and can induce SGC - 7901 cells to produce autophagy, its mechanism may be related to the increase of intracellular Ca^{2+} concentration.

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