RESEARCH ARTICLE

Inhibitory Effects of 3-Bromopyruvate on Human Gastric Cancer Implant Tumors in Nude Mice

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Abstract

Background: Gastric cancer is a common malignant tumor. Our previous study demonstrated inhibitory effects of 3-bromopyruvate (3-BrPA) on pleural mesothelioma. Moreover, we found that 3-BrPA could inhibit human gastric cancer cell line SGC-7901 proliferation *in vitro*, but whether similar effects might be exerted *in vivo* have remained unclear. Aim: To investigate the effect of 3-BrPA to human gastric cancer implant tumors in nude mice. Materials and Methods: Animals were randomly divided into 6 groups: 3-BrPA low, medium and high dose groups, PBS negative control group 1 (PH7.4), control group 2 (PH 6.8-7.8) and positive control group receiving 5-FU. The TUNEL method was used to detect apoptosis, and cell morphology and structural changes of tumor tissue were observed under transmission electron microscopy (TEM). Results: 3-BrPA low, medium, high dose group, and 5-FU group, the tumor volume inhibition rates were 34.5%, 40.2%, 45.1%, 47.3%, tumor volume of experimental group compared with 2 PBS groups ($p<0.05$), with no significant difference between the high dose and 5-FU groups ($p>0.05$). TEM showed typical characteristics of apoptosis. TUNEL demonstrated apoptosis indices of 28.7%, 39.7%, 48.7% for the 3-BrPA low, medium, high dose groups, 42.2% for the 5-FU group and 5% and 4.3% for the PBS1 (PH7.4) and PBS2 (PH6.8-7.8) groups. Compared each experimental group with 2 negative control groups, there was significant difference ($p<0.05$); there was no significant difference between 5-FU group and medium dose group ($p>0.05$), but there was between the 5-FU and high dose groups ($p<0.05$). Conclusions: This study indicated that 3-BrPA in vivo has strong inhibitory effects on human gastric cancer implant tumors in nude mice .

Keywords: 3-bromopyruvate -gastric cancer- nude mice- inhibitory effect

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Introduction

Gastric cancer is one of the most serious malignant tumor (Zare et al., 2013). In recent years, along with the application of comprehensive treatment, especially surgical operation, the prognosis of gastric cancer has improved, but the overall 5 year survival rate remains low, generally less than 40% except individual hospitals reported higher (Kim et al., 2014). In order to further improve the therapeutic effect of gastric cancer, it is necessary to find new effective anti-tumor drugs. Induction of tumor cell apoptosis is an important way for the study of anti tumor. Otto Warburg found that: compared with normal cells, tumor cells even in sufficient oxygen supply conditions were still in glycolysis as the main way to get the ATP (Warburg et al., 1956). Research suggested that, when inhibited glycolysis, ATP depletion can produce persistent DNA degradation, which induced tumor cells into apoptosis state (Geschwind et al., 2002; Danial NN et al., 2003). 3-BrPA is a strong alkylating agent, research has indicated that it can inhibit the enzyme activity by combined with the kinase active site of hexose, thereby inhibited glycolysis (Nelson et al., 2002).

Our previous study proved that the inhibitive effect of 3-BrPA to pleural mesothelioma (Zhang et al., 2009), moreover, found that 3-BrPA can inhibit human gastric cancer cell line SGC-7901 proliferation *in vitro* (Xian et al., 2013), but 3-BrPA *in vivo* whether can inhibit growth of human gastric cancer implant tumor in nude mice unclear.

Materials and Methods

Cell culture and animals

Human gastric carcinoma cell line SGC-7901 was purchased from cell bank of Xiangya School of Medicine, Central South University. Experimental animals were purchased from animal experimental center of Guangxi Medical University, female nude mice (BALB/c, nude), week old 4-5wk, weight 14-16g, specific pathogen free (SPF) conditions of feeding. The animal experimental process in the supervision of the ethics committee of Guangxi Medical University, experimental operation with animal ethical requirements.

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Proliferation assay and xenograft nude mouse models

After resuscitation, human gastric cancer cells SGC-7901 cultured in RPMI-1640 containing 10% fetal bovine serum, cultured in 37°C, 5% CO_2 constant temperature incubator, routine passage by centrifugation. Took SGC-7901 cells of logarithmic growth, diluted with saline to 2×10^6/ml cell suspension, in each nude mice subcutaneous the right inguinal region injection of 0.2ml cell suspension. Tenth days after planting, xenografts were produced (Jiang et al., 2013), tumor reached into standard (the diameter of tumor 0.5cm), animal models have been successfully established. Nude mices were randomly divided into 6 groups: high dose of 3-BrPA group (2.67 mg/kg), medium dose group (2.23 mg/kg), low dose group (1.85 mg/kg), positive control group 5-FU (15 mg/kg), PBS negative control group 1 (pH: 7.4), PBS negative control group 2 (pH value: 6.8-7.8), with 10 rats in each group. The 3-BrPA, 5-FU with sterile PBS diluted to concentrations in nude mice tumor peripheral injection, each nude mice injected 0.2ml, once per day, administered before NaOH and acetic acid 1mol/L regulation diluted solution of pH 7.4, The negative control group injected 0.2ml corresponding pH PBS, continuous injection for 4 weeks. Nude mice eating, defecating and mental state have no change were observed.

TEM and TUNEL

Administered before and 24h after the last administration, measured tumor volume, use vernier caliper to measure the diameter of tumor precision long a and short diameter b, tumor volume $=ab^2/2$. The inhibition rate of tumor volume (1- the average tumor volume / control group mean tumor volume)×100 %. 24 hours, after the last administration nude mice were killed by cervical dislocation, 1 minute took 1×2mm the size of the tumor tissue, by 3% glutaraldehyde, 1% osmic acid fixation, epoxy resin embedding, slicing, urany acetate and lead citrate staining, observed by H7650 transmission electron microscope. Removed the tumor after nude mice were killed, according to TUNEL kit instructions, DAB color, hematoxylin staining. Result: the nucleus or cytoplasm brown yellow granule as positive, combined with HE staining, HE staining confirmed the exclusion of dead cells; each tumor specimen from 5 sections, each section observed more than 10 high power field or counting more than 500 cells, apoptosis index (AI)=TUNEL staining cell number / positive tumor cells ×100%.

Statistical analysis

The statistical software SPSS16.0 was used to analyze the date. The measurement data using mean standard deviation ($\bar{x}\pm S$). Multi group compared was used to single factor analysis of variance, two-two compared was used to the SNK-q test; a p value of ≤0.05 was considered statistically significant.

Results

In this study, the eating, defecating and mental state of nude mice have no obvious abnormity. The experimental results showed: along with injection of 3-BrPA group and 5-FU group, the growth of gastric cancer tumor was slowed or even stop. To compare drug group with 2 PBS groups, the tumor volume was significant (p<0.05). To compare 5-FU group with 3-BrPA high dose group, the tumor inhibition rate has no significant difference (q =0.9705, p>0.05). (Table 1)

Table 1. The Comparison of Curative Effect by Using Drugs to Xenografts

Group	Nude mice (n)	Dose (mg/kg)	Tumor volume (mm^3) $\bar{x}\pm S$	Inhibition rate of tumor volume %
3-BrPA (high)	10	2.67	699.9±86	45.1*
3-BrPA (medium)	10	2.23	762.5±79	40.2*
3-BrPA (low)	10	1.85	835.1±98	34.5*
5-FU	10	15.00	671.9±52	47.3*
PBS 1	10	…	1293.8±93△	…
PBS 2	10	…	1257.2±89△	…

*: represents the comparison of drug group to PBS group respectively (p<0.05); △: represents the comparison of 2 PBS groups (q=1.3034, p<0.05)

The apoptosis of tumor cell observed by transmission electron microscope in experimental group is obvious, we can see the apoptotic cells were separated from other cells, chromatin was condensated (△), nuclear shape was narrow and volume was reduced (↑), and apoptotic bodies were found in the tumor cells (⇪). But in the control group, nuclear membrane of tumor tissue keep integrity, cells were connected closely, there was no obvious apoptosis expression. (Figure 1) The mean apoptosis index for each group: 3-BrPA low, medium, high dose group were

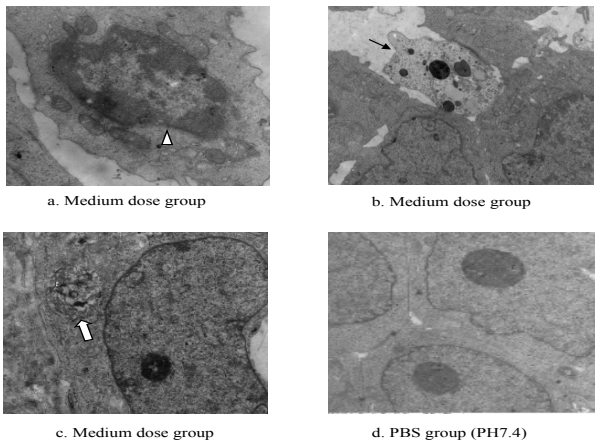

Figure 1. The Cell Structure Changes were Observated by Transmission Electron Microscope after Treatment of 3-BrPA and PBS

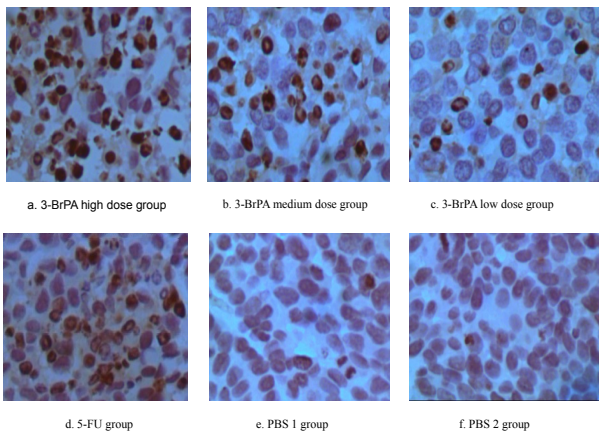

Figure 2. Apoptosis Index of Tumor Cell was Detected by TUNEL Method after Treatment. (400×)

28.7%, 39.7%, 48.7%, 5-FU group was 41.2%, PBS1 was 5.0%, PBS2 was 4.3%. Compared each injection of 3-BrPA with 2 negative control groups respectively, it has significant difference ($p<0.05$), there was significant difference between 3-BrPA high, medium, low dose group ($p<0.05$), there was no significant difference between 5-FU group and medium dose group (q=1.1632, $p>0.05$), and there was statistical significance between 5-FU group and high dose group (q=5.6608, $p<0.05$), there was no significant difference between the two negative control group (q=0.5469, $p>0.05$) (Figure 2).

Discussion

3-BrPA is an anti energy medicine, our previous study demonstrated that the inhibitive effect of 3-BrPA to pleural mesothelioma (Zhang et al., 2009). In recent years, studies have confirmed that it also had an inhibitive effect to liver cancer, breast cancer and other malignant tumors (Geschwind et al., 2002; Liu et al., 2009; Ganapathy et al., 2010; Ota et al., 2013; Xu et al., 2013), but the 3-BrPA *in vivo* whether can inhibit the growth of gastric cancer, inducing apoptosis of gastric cancer cells has not been reported; therefore, we establish animal models of human gastric cancer implant tumor in nude mice, to do the experimental research on 3-BrPA against human gastric cancer *in vivo*. Our experimental results show that: in the experimental drug dose range, inhibitive effect of 3-BrPA to tumor reinforced with increase of the drug dose, it has dose-effect relationship; 3-BrPA has similar anti-tumor effect to chemotherapeutic drug 5-FU; it proves that 3-BrPA has an obviouly inhibitive effect to human gastric cancer cell SGC-7901 xenografts in nude mice; in addition, there is no significant difference between 2 PBS groups as PH change, it means that the change of PH brings no obvious effect in apoptosis of gastric cancer cells, therefore it further illustrates the difference between experimental group and control group is the result of 3-BrPA.

The possible anti-tumor mechanism of 3-BrPA: tumor cells even in the enough oxygen condition still consider glycolysis as the main way to gain ATP, which is known as the "Warburg effect" (Chesney et al., 1999; Garber et al., 2004). Studies have shown that 3-BrPA can connect to XH (Pereira da Silva et al., 2009), XH is the hexokinase active part of the key enzymes of glycolysis (Sener et al., 1985), so that the enzyme activity is inhibited, the process of glycolysis in tumor cell is restrained, tumor cells become slow growth and stagnation. If they could not get enough ATP, the exhaustible ATP can produce persistent degradation of DNA, so that the cells into apoptosis state (Parks et al., 2013); moreover, mitochondrial permeability is increased by the inhibition of hexokinase, it can release cytochromeC and activate caspase, which induce apoptosis of cell (Ferraro et al., 2008; Zuo et al., 2011). It has been reported that 3-BrPA can inhibit VEGF (vascular endothelial growth factor), and inhibit the lymphatic vessels of tumor (Cao et al., 2008). However, recent proteomics studies showed that 3-BrPA is the inhibition of glyceraldehyde phosphate dehydrogenase (GAPDH), it through the GAPDH of alkylation to inhibit the glycolysis, pentose phosphate pathway, cell self ingest and transcription, so as to inhibit the growth of tumor cells, and induce the apoptosis of cells (Pereira da Silva et al., 2009; Ganapathy et al., 2010). In addition, whether 3-BrPA is also through other ways to inhibit the growth of tumor cells and induce the apoptosis of tumor cells remains to be further studied.

In summary, our studies show that 3-BrPA *in vivo* has the strong inhibitive effect to human gastric cancer implant tumor in nude mice. The strength of inhibitive effect has dose-effect relationship in the experimental dose range, there was no significant difference between 3-BrPA high dose and 5-FU in anti-tumor effcet. Base on the experiment of 3-BrPA *in vivo* offered evidence for the treatment of gastric cancer. There is the reason to believe that 3-BrPA is a promising novel anti-tumor drug.

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