EFFECT OF BAICALEIN ON PCNA EXPRESSION DURING THE DEVELOPMENT OF MURINE FORE-STOMACH CARCINOMA

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ABSTRACT

Introduction*: To investigate the effect of baicalein on Proliferating cellular nuclear antigen (PCNA) expression in dynamic process of murine fore-stomach carcinoma.*

Materials and methods*: Dynamic animal models of murine fore-stomach carcinoma were established with the equal volume mixing (abbreviated as NSEE) of Sacrosine-ehylester-hydrochlorid and Sodium nitrite as a mutagen. 90 NIH mice (SPF) was divided into blank control group, the negative control group, high (1000mg kg^{-1}), medium (500mg kg^{-1}) and low (200mg kg^{-1}) dose groups with baicalein (dissolved with 0.5% sodium carboxymethyl cellulose solution). In the experiment,all group were treated with NSEE, twice a week for six weeks by gavage,without the blank group (with equal volume of 0.9% saline). Meanwhile, all interventional groups with Baicalein were treated with baicalein solution by gavage once a day. The fore-stomach tissues were taken on 28th, 5 6th and 84th day for pathological observation. The PCNA expression in the fore-stomach tissues was observed by immunohistochemistry.*

Results*: Compared with the blank control group, for interventional group,fore-stomach tissues was gradually evolves from hyperplastic lesions to atypical hyperplasia,or even carcinoma in situ,and the PCNA expression in the negative control group increases significantly (P <0.01) by immunohistochemistry. Compared with the negative control group,the situation is the slightest in high dose group by pathological sections at 84d, the experimental results by immunohistochemistry showed that PCNA expression of high,medium and low dose baicalein intervention groups was significantly different from that of model group (P <0.01), but there was no significant difference among the groups.*

Conclusion*: High, medium and low doses of baicalein can significantly inhibit NSEE-induced fore-stomach cancer carcinogenesis in mice,which may be related to down-regulation of PCNA expression.*

Keywords*: Baicalein, Murine fore-stomach carcinoma, Proliferating cellular nuclear antigen (PCNA), Dynamic animal models.*

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Introduction

Human esophageal cancer is one of the common malignant tumors of the digestive tract. According to investigation, the incidence of esophageal adenocarcinoma in western countries has risen significantly over the past 30 years,and our country also have high incidences of esophageal cancer with the death rate accounting for over 50% of the global[1]. Most of today's clinical admission cases are advanced and middle-advanced, with high mortality[2]. Therefore, early diagnosis and intervention research of esophageal cancer is still of great significance.

Baicalein is a flavonoid compound found in the dried root of Scutellaria baicalensis Georgi, belong to Labiatae plant. It has been used to treat inflammation, cardiovascular disease and microbial infections[3-5]. There is increasing evidence that this flavonoid compound has antitumor activity against various human tumor cell lines[6-10]. Because human esophageal cancer is very similar to murine fore-

stomach carcinoma in phenotype[11], we established a dynamic animal model of murine fore-stomach carcinoma to simulate the carcinogenesis progression of human esophageal cancer, intervened it with different doses of baicalein to explore the effect of baicalein on PCNA in mouse fore-stomach tissue during fore-stomach cancer progression.

Materials and method

Experimental animals

90 NIH mice, (SPF male), provided by Beijing Vital River Laboratory Animal Technology Co., Ltd,were kept in an environmentally controlled room (22 ± 2°C, 55 ± 5% relative humidity, and 12 hours light-dark cycle) and received food and water ad libitum.All animal experiments were conducted in accordance with Principles of Laboratory Animal Care.

Major instruments and reagents

Baicalein was purchased from Xi'an Jianglin Biotechnology Co.,Ltd.The 0.5% sodium carboxymethylcellulose was used as the solvent to prepare solutions. Sacrosine-ehylester-hydrochlorid (SIGMA-ALDRICH Company, USA); Immunohistochemistry UltraSensitiveTM SP kit (Fuzhou Maxim Biotechnology Development Co., Ltd,China); PCNA monoclonal antibody (Beijing Biosythesis Biotechnology Co., Ltd ,China).

Experimental method

Animal model establishment

After one week's acclimatization, the mice were randomly divided into blank control group, the negative control group, low, medium and high dose baicalein group. All interventional groups were established with NSEE (The equal volume mixing was Sacrosine-ehylester-hydrochlorid (abbreviated as S, 2g · kg^{-1}) and sodium nitrite (abbreviated as N,0.3g · kg^{-1}) with 0.01mol · L^{-1} hydrochloric acid) as a mutagen by gavage twice a week for six weeks,but the blank control group was treated with equal volume of 0.9% saline.

Medicine intervention

The blank control group and the negative control group were treated with 0.5% sodium carboxymethylcellulose solution once a day by gavage. Meanwhile, the high (1000 mg · kg^{-1}), medium (500 mg · kg^{-1}) and low (200 mg · kg^{-1}) groups were treated with baicalein once a day by gavage.The mice were sacrificed in three batches at day 28, 56, 84, and forestomach tissue was collected.

Histopathology detection

The fore-stomac tissues of mice in each group were fixed with 4% paraformaldehyde, treated with paraffin-sectioning and H.E-staining.The pathological changes of the forestomach tissues were observed under light microscope.

Expression of PCNA in mouse fore-stomach tissue

The paraffin sections of fore-stomach tissue samples in each group were dewaxed and hydrated, and then the PCNA expression in mouse forestomach tissues was detected by immunohistochemical UltraSensitiveTM SP kit instruction.

Data Analysis

The positive part of the immunohistochemical section was analyzed with image analysis software Image-pro plus 6.0. Calculation method: total cell number and positive cells were calculated for each high power field and positive cell rate was calculated. The experimental data were expressed as mean ± standard deviation ($\bar{x}$± s), and the experimental data was statistically processed with SPSS18.0 software.

Results

H.E staining results

It can be found by observation of pathological sections (Figure 1) that, the mouse fore-stomach epithelial tissue of blank group shows normal histological morphology in each batch, the histological structure of each layer is obvious, the morphology is normal, and no obvious abnormal proliferation is observed. Compared with the blank control group,hyperkeratosis is found in fore-stomach epithelial tissue of all interventional groups, mainly simple hyperplasia on 28d, which is more than doubled thicker. On 56d, varying degrees of papillary hyperplasia and cauliflower-like hyperplasia are observed. On 84d, fore-stomach tissues was gradually evolves from hyperplastic lesions to atypical hyperplasia, or even carcinoma in situ,which is the most serious in the negative control group. Compared with the negative control group, on 84d, the sitution was slightest in high dose group still mainly with simple hyperplasia

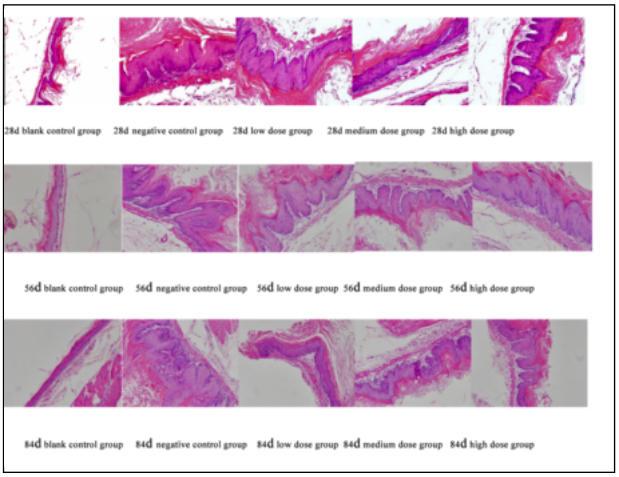

Fig. 1: 28d,56d,84d pathological sections (200×)

Immuno histochemistry results

Compared with the blank control group,the PCNA expression in each interventional group increases significantly at 28d,56d and 84d, especially in the negative control group (P <0.01, Table 1) by immunohistochemistry. Compared with the negative control group,the PCNA expression decreases in different dose groups of baicalein, with very significant difference (P <0.01, Table 1), but there was no significant difference among the groups.

	28d	56d	84d
blank control group	0.26562±0.04226	0.28298±0.06101	0.28496±0.04510
negative control group	0.64964±0.04241*	0.70044±0.05167*	0.74326±0.05474*
low dose group	0.33203±0.09037**	0.36043±0.02611**	0.40265±0.02851**
medium dose group	0.31084±0.05593**	0.36037±0.05595**	0.39580±0.02290**
high dose group	0.32524±0.02558**	0.35901±0.05103**	0.38353±0.04220**

Table 1: Positive Rate of PCNA ($\bar{x}$±s).

*Note: **Compared with the negative control group: P <0.01; *Compared with the blank control group: P <0.01*

Discussion

Epidemiological survey of the population shows that certain human cancers such as gastric cancer, esophageal cancer, liver cancer, colon cancer and bladder cancer may be related to nitrosamines[2]. Baicalein is a kind of flavonoid compound existing in the traditional Chinese medicine Scutellaria baicalensis, which has the functions of anti-oxidation and antitumor[12-13]. Therefore, in this experiment, NESS, a nitrosamine precursor,was used to establish dynamic animal models of fore-stomach cancer carcinogenesis in mice.

At the same time, low, medium and high doses of baicalein were given daily to observe its intervention effect in different development stages of mice fore-stomach cancer.

Many studies have shown that baicalein can inhibit the proliferation of a variety of tumor cells, and affect the cell cycle distribution (mainly by acting on the G2-M phase)[14-15]. Previous studies have shown that baicalein can inhibit the proliferation of liver cancer cells and induce cell apoptosis, and can inhibit gastric cancer proliferation by inhibition of gastric cancer cell vEGF and HGF expression[13]. Also, in vitro experiments have shown that baicalein can induce apoptosis of human breast cancer cells, prostate cancer cells, squamous cell lung carcinoma, human leukemia K562 cells, HL-60 cells, immature myeloma cells and human esophageal squamous cell carcinoma EC 109 cells[10].There is a dose-effect relationship in baicalein's induction of apoptosis of some tumor cells[14-15].

PCNA is an accessory protein of DNA polymerase δ, which participates in DNA replication during cell proliferation and reflects cell proliferation activity. It can be used as an indicator of cell proliferation status[17]. Studies have found that PCNA expression was significantly increased in esophageal cancer and many other proliferative lesions[16-17]. In this study, with the development of murine fore-stomach carcinoma the expression of PCNA was significantly increased, especially in the negative control group,which may be related to the enhancement of cell proliferation. Compared with the control group, the PCNA expression was significantly up-regulated in the model group both in early and mid-to-late stages with very significant deference (P <0.01), which was consistent with the results of the previous studies. It indicated that PCNA was involved in proliferation and carcinogenesis of mice forestomach tissue cell. However, in different baicalein dose groups, PCNA expression was down-regulated compared with the model group, and showed very significant difference (P <0.01) in the early stage of cancer induction. It suggests that baicalein may show anti-tumor activity by down-regulating PCNA expression (fig. 2).

This experiment shows that baicalein has a certain degree of inhibition effect on NSGF-induced mice fore-stomach tissue lesions, probably by affecting PCNA expression to inhibit abnormal proliferation of mouse fore-stomach tissue. There is a dose-effect relationship in the inhibition, but its

detailed mechanism of action still needs further study. Meanwhile, Whether this inhibitory effect is associated with influences of HSP70, P53, cyclin D1 and so on, also needs further investigation

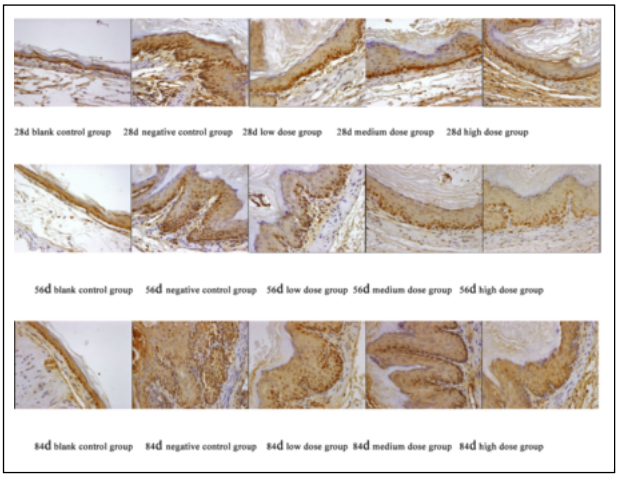

Fig. 2: 28d,56d,84d Expression of PCNA in mouse forestomach tissue (400×).

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