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Research Paper

The Chemopreventive Effect of Tamoxifen Combined with Celecoxib on DMBA chemically-Induced Breast Cancer

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Abstract

Objective: To investigate the chemopreventive effect of tamoxifen combined with a COX-2 selective inhibitor, celecoxib, on breast cancer in rats chemically induced by 7,12-dimethylben (a)anthracene (DMBA). **Methods**: DMBA was irrigated into the stomaches of SD female rats to build breast cancer model. A total of 120 rats were divided into four groups: control group, tamoxifen group, celecoxib group and combined group. The incidence rate, latent period, number and volume of breast cancer were detected and analyzed. **Results**: The tumor incidence rate of tamoxifen group (48.15%, 13/27) and celecoxib group (50.00%, 14/28) were lower than that of control group (85.71%, 24/28), but higher than that of combined group (21.43%, 6/28). The tumor's latent period of tamoxifen group (97.54±1.85 d) and celecoxib group (96.79±2.89 d) were longer than that of control group (89.50±5.99 d), but shorter than that of combined group (103.67±3.39 d). The average tumor number of tamoxifen group (1.77±0.73) and celecoxib group (1.71±0.61) were less than that of control group (3.50±1.62), but more than that of combined group (1.17±0.42). The average tumor volume of tamoxifen group (1.78±0.71 cm^3) and celecoxib group (2.05±1.04 cm^3) were smaller than that of control group (6.42±3.96 cm^3), but bigger than that of combined group (0.71±0.96 cm^3) ($P < 0.05$ respectively). **Conclusion**: Celecoxib and tamoxifen are effective drugs in preventing the occurrence of rat breast cancer chemically induced by DMBA. Furthermore, combination of them has better chemopreventive effect.

Keywords: breast neoplasm; chemoprevention; tamoxifen; cyclooxygenase-2

INTRODUCTION

Breast cancer is one of the most common malignant tumors of women. Generally accepted, treatment of precancerosis is the most convenient and effective method. Chemoprevention is to interrupt or reverse the processes of canceration so as to prevent carcinogenesis and reverse precancerosis[1]. This study investigated the chemopreventive effect of COX-2 selective inhibitor [2], celecoxib, combined with tamoxifen on breast cancers chemically induced by DMBA in rats, and underlying mechanisms.

MATERIALS AND METHODS

Animal modeling

Female SD rats uncopulated were provided by animal centre, medical college, Xi'an Jiaotong university (Animals' Certificate Number: No 08-005 of Shanxi medical animal test centre), age 45±5 days, weighting 110±10 g. 200 mg/kg 7,12-dimethylben (a)anthracene (DMBA) was irrigated into the stomaches of rats to build breast cancer models.

Animal groups

120 rats were randomly divided into 4 groups, control group(normal diet) was considered as negative control, tamoxifen group (4 mg/kg tamoxifen was put in drinking water), celecoxib group, 1000 mg/kg celecoxib disowed in oleum maydis in forage) and combined group (4 mg/kg tamoxifen in drinking water, 1000 mg/kg celecoxib was put in forage). The breasts of rats were examined twice a week, when palpable breast neoplasm appeared; the number of breast neoplasm and its changes in size were recorded.

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Specimen detection

After 120 days, the experiment was stopped. Vaginal smear examination was performed to confirm that the rats were in the dioestrus period, and rats were anesthetized by intraperitoneal injection of 5% urethane. Then, every mammary gland of the rats with surrounding skin and hypodermia were excized, the mammary glands were cut half open to observe the shape and count the number of tumors. The size of the tumors was measured tith 1mm precision sliding caliper. The tumor incidence rate of each group was calculated. The volume of tumors was calculated according to the equation as follows: length×weight×height×π/6[3]. If one rat had many tumors, the volume of tumors of this rat was the summation of all tumors.

Histopathology observation

The specimens were fixed in 10% neutral formalin, embedded in paraffin and stained with hematoxylin-eosin for histological examination. The diagnosis of rat breast cancer was performed according to the diagnostic code of experimental rat breast cancer[4].

Statistical Analysis

t test and χ^2 test was performed by SPSS10.0 for windows. $P < 0.05$ was considered significant.

RESULTS

Each group had 30 rats, in which the death before the end of experiment wasn't included. At last, 28 cases were collected from control group, compared with 27, 28, 28 cases from tamoxifen group, celecoxib group and combined group respectively.

Tumor incidence rate

The tumor incidence rate of each group respectively was: 85.71% (24/28) in control group, 48.15%(13/27) in tamoxifen group, 50.00%(14/28) in celecoxib group, 21.43% (6/28) in combined group. Statistical analysis showed that the tumor incidence rates of tamoxifen group, celecoxib group and combined group were lower than that of control group (χ^2=8.811, P=0.003; χ^2=8.187, P=0.004; χ^2=23.262, P=0.000 respectively). The tumor incidence rates of tamoxifen group and celecoxib group were obviously higher than that of combined group (χ^2=4.340, P=0.037; χ^2=4.978, P=0.026 respectively). The difference of the tumor incidence rate of tamoxifen group and celecoxib group didn't have statistical significance(χ^2=0.019, P=0.891).

Tumor latency period

The tumor latency period of each group respectively was: 89.50 ±5.99 days in control group, 97.54 ±1.85 days in tamoxifen group, 96.79 ±2.89 days in celecoxib group, and 103.67 ±3.39 days in combined group. Statistical analysis showed that the tumor latency periods of tamoxifen group, celecoxib group and combined group were longer than that of control group (t=4.689, P=0.000; t=4.253, P=0.000; t=5.526, P=0.000 respectively). The tumor latency periods of tamoxifen group and celecoxib group were shorter than that of combined group (t=5.157, P=0.000; t=4.648, P=0.000 respectively). The difference of tumor latency period of tamoxifen and celecoxib group hadn't statistical significance (t=0.799, P=0.432).

Average tumor number

The average tumor number of each group respectively was: 3.50±1.62(1~7)pieces in control group, 1.77±0.73 (1~3)pieces in tamoxifen group, 1.71±0.61 (1~2)pieces in celecoxib group, and 1.17±0.42 (1~2)pieces in combined group. Statistical analysis showed that the average tumor number of tamoxifen group, celecoxib group and combined group were less than that of control group (t=3.651, P=0.001; t=3.956, P=0.000; t=3.468, P=0.002 respectively). The average tumor number of tamoxifen group and celecoxib group were more than combined group (t=2.307, P=0.035; t=2.346, P=0.034 respectively). The difference of the average tumor number of tamoxifen group and celecoxib group showed no statistical significance(t=0.213,P=0.833).

Average tumor volume

The average tumor volume of each group respectively was: 6.42±3.96 cm^3 in control group, 1.78±0.71 cm^3 in tamoxifen group, 2.05±1.04 cm^3 in celecoxib group, 0.71±0.96 cm^3 in combined group. Statistical analysis showed that the average tumor volume of tamoxifen group, celecoxib group and combined group were smaller than that of control group (t=4.162,P=0.000;t=4.028,P=0.000;t=3.459,P=0.002 respectively). The average tumor volume of tamoxifen group and celecoxib group were bigger than that of combined group (t=2.428, P=0.043; t=2.772, P=0.019 respectively). The difference of the average volume of tamoxifen group and celecoxib group had no statistical significance(t=0.780,P=0.442).

Histopathology observation

The tissues of control group presented with Infiltrating ductal carcinoma, showing cancer nest, obvious nuclear atypia and nuclear division. There was

few gland-like structures and stroma(***Fig 1***). Medullary carcinoma was composed of cancer cells and had ductless glands. The specimens without tumorigenesis showed different degrees of lobuli mammae hyperplasia and glandular epithelium atypical hyperplasia. The light degree of hyperplasia showed intralobulus and interlobulus fibrous tissues expanded or increased, gland alveolus increased, but acinous cells were still in monolayer. The atypical hyperplasia showed acinous cells arranged in disorder and multilayer, increased karyoplasmic ratio and changed nuclear atypia(***Fig 2***).

In experimental Groups, the Infitrating ductal carcinoma had more gland-like structures and stroma, in which the cells were dispersed; there was few nuclear atypia and nuclear division(***Fig 3***). The specimens without canceration showed no or light degree of glandular epithelium hyperplasia, increased intralobulus and interlobulus fibrous tissues, also revealed less gland alveolus and only duct (***Fig 4***).

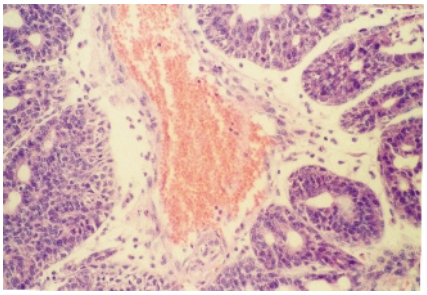

Fig 1 **Infiltrating ductal cancer in control group (HE, ×200)**

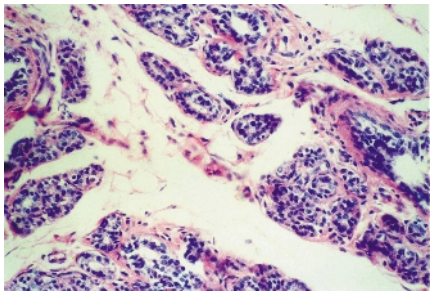

Fig 2 **Atypical hyperplasia in control group (HE, ×200)**

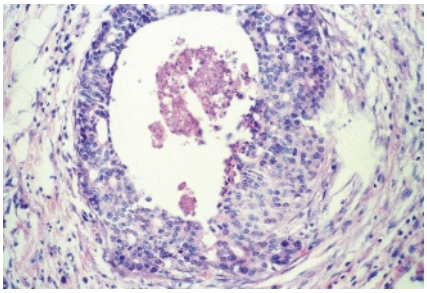

Fig 3 **Infiltrating ductal cancer in chemopreventive group (HE, ×200)**

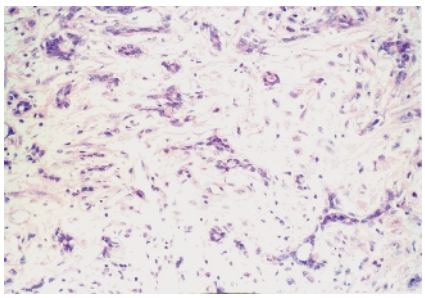

Fig 4 **Hyperplasia in chemopreventive group (HE, ×200)**

DISCUSSION

Cancer chemoprevention is to interrupt or reverse the processes of canceration by chemical. Combined chemoprevention use of two or more chemoprophylactic drugs with different antineoplastic mechanisms, to increase the curative effects and redcsce adverse reaction. This will be the best selectied scheme of chemopreventing tumorigenesis[5]. COX-2 expresses in various tumor cells and tissues, for example, colorectal, lung and breast cancer [6], meanwhile, it concerns with tumorigenesis and tumor development. Its mechanisms include external biological metabolism, neovascularity, apoptosis, immune function and tumor invasion[7,8]. Celecoxib combined with tamoxifen may increase the therapeutic efficacy of breast precancerosis and become an effective scheme of preventing breast cancer occurrence.

In this study, the time for DMBA to induce the earliest palpable breast cancer was 82 days. The tumor incidence rate was 85.71%, a little lower than that report abroad[4].

The phenomenon that tamoxifen can reduce the occurrence of breast cancer of rats coincides with the rule that tamoxifen prevents hursan breast atypical hyperplasia from developing breast cancer [5]. The major mechanism of tamoxifen prophylaxising breast cancer is estrogenic antagonism, simultaneously including inhibiting cancer cell secreting VEGF[9], decreasing oncogene expression, inducing tumor cell apoptosis through varions channels[10].

This study found that celecoxib could inhibit rat carcinogenesis and cancer development. Meanwhile. It might be useful in preventing breast cancer in the high risk. Other scholars had similar views. Nakatsug

et al [3] reported that 400ppm nimesulide could decrease the tumor incidence rate, the volume and the multisitus rate. Abou Issa *et al* [11] observed the preventive effect of celecoxib on rat breast cancer induced by DMBA among different dosage groups and found that low dose celecoxib can prevent breast cancer. Moreover, it was in a dose-dependent manner. Histopathology showed that the degree of rat breast atypical hyperplasia and duct epithelial hyperplasia in celecoxib group were obviously relieved, and the breasts of others which didn't cancerate were similar to normal breast gland. This phenomenon and COX-2 expression in precancerosis indicated that COX-2 overexpression was closely correlated with tumorigenesis. Liu *et al* [12] also introduced COX-2 overexpression stimulating tumor occurrence and growth, and antisense COX-2 significantly slowed tumor growth and improved efficacy of chemotherapeutic drugs[13]. The major mechanism of COX-2 stimulating tumorigenesis is its product, PGE2, that makes tumor cell proliferation, induces VEGF upregulation, inhibits tumor cell apoptosis and the immune function[14], Tari AM *et al*[15] reported that COX-2 used PGE2 to stimulate the activities of protein kinases A and C to induce selectively tamoxifen resistance in ER alpha-positive breast cancer cells, but COX-2 selective inhibitor, celecoxib, can inhibit tumorigenesis and tumor development through this way. And, celecoxib had been reported indepzndent of COX-2 with mechanisms[16].

The chemoprevention of breast cancer is one of the research hot spots. Celecoxib and tamoxifen both have good preventive effects on rat breast cancer induced by DMBA. These results demonstrated that the combined group had obviously lower tumor incidence rate, longer latency period, fewer number and smaller volume, besides, and tamoxifen combined with celecoxib could significantly reducle rat breast cancer occurrence and development induced by DMBA all the better. This research indicated that tamoxifen combined with celecoxib had better preve ntive effect on rat breast cancer.

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