Effect of Tea Polyphenols On Microvessel Density In Breast Cancer Tissue and Vital Organs Tissue

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Abstract **Objective** To study the effect of tea polyphynols on microvessel density in breast cancer tissue and normal tissues of vital organs (such as heart, brain, kidney), then judge the specificity of its inhibiting angiogenesis effect. **Methods** Mammary adenocarcinoma EMT6 was cultured and propagated, then implanted into female BALB/c mice. Tea polyphenols was peroral administrated and local injected, microvessel density in tumor tusuue and vital organ tissues (heart, brain,keney) was detected by immunohistochemical method. **Results** In local injection group and peroral administrated group the expression of microvessel density (MVD) of implanted mice breast cancer tissue decreased ($P<0.05$), while in heart, brain and kidney in treated group were no significant difference compared with model group ($P>0.05$). **Conclusion** Tea polypynols can selectively inhibit the information of microvessel in breast cancer tissue and have no significant effect on vital organs such as heart, brain and kidney.

Key words Tea polyphenols; breast cancer(EMT6); microvessel density(MVD); vital organs

There are some related reports about the effect of tea polyphenols inhibiting angiogenesis in recent years, which proved that green tea´s extractive or EGCG may inhibit secretion of VEGF in tumor cells and inhibit tumorous angiogenesis.[1~7] Based on observation on tea polyphenols´s inhibiting of new vessels in tumor tissue and tumor growth of implanted breast cancer and observation on tea polyphenols´s effect on microvessle density in tumor tissue. We also observe that its effect on microvessle density in vital organs such as heart, brain, and kidney to assess specificity of angiogenesis inhibited by tea polyphenols. Furthermore, We provid evidence for clinical application and direct new drug.

MATERIALS AND METHODS

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Materials

Animal and cell line 60 female BALB/c mouses, body weight(20±2)g, were purchased from in ShanHai KeLai Experimental Animal Limited Company[licence: SCXK 2003-0003], breed with barrier system -SPF circumstance of Experimental Animal Center of Nan-Jing Chinese Medical University. Buy breast cancer cells of EMT6 mouses in Experimental Animal Center of The Fourth Military Medical University.

Medicine Tea polyphenols was purchased from Zhe-Jiang east tea limited company, purity 98%(Batch No: 20050334); Comparision medicine: panaxsaponin Rg3 (ginseng one capsule,contain panaxsaponin Rg3 10 mg) was purchased from JilinYatai Limited Company(Batch No: 20040704).

Equipment Superclean bench is made in (Tlye sa-cj-zFD) SuZhou AnTai Air Technique Limited Company. Centrifugal machine is made in ShangHai AnTing Scientific Instrument Factory (Type KA-1000); Lai Ka inverted microscope is made in German LaiKa Company(Type DMIL); Forma constant temperature-incubator is made in American Forma Company(Type 3111).

Reagent Ⅷ-Rag polyclonal antibody is made in American DAKO company.(Batch No: A 0082), the immunity- histochemistry supersensitivity mouse tissue

kit(UltraSensitiveTMS-P)was purchased from Fuzhow MaiXing Biotechnology Development Limited Company.

Methods

Cell culture and inoculation EMT6 cells were cultivated in RMPI 1640 culture fluid (containing 10% calf serum´s), and digested with 0.25% trypsinization after the cells growing fully monolayer, after centrifugation, cells were collected and diluted to the concentration of 5×10^6 by PBS, then inoculate 0.2ml into mouse right back. Transplanted while the tumor grow to 1g after 12d.

Tumor transplantation Tumor-bearing mice were executed by breakdown-neck, and were placed on the superclean bench flat plate after soak by 75% alcohol for 3-5 min, cutting the tumor skin by autoclaving surgical instruments and separating tumor envelope, then wash by sterilized PBS after taking out of tumor and weigh, Dilute by isotonic Na chloride at the rate of 4ml each gram tumor. The tumor body was to be broken with scissors, and cell suspension was prepared by full homogenate, adjusted to the density of 2×10^6, Hypodermic inoculation 0.2ml every mouse in right back.

Groups and Medication Tumor-bearing mice (48 growth tumor)were divide at random 4 groups when tumor grew to 1 mm^3 after implanting 13th day: tea polyphenols intragastric administration group, tea polyphenols part- injection group, intragastric administration group of comparison medicine by panaxsaponin, model comparision group, 12 animals every group.

Determine all administration dosage with dosage conversion of human and animal: ① Tea polyphenols intragastric administration groups: Calculate according to 900 mg/d human therapeutic dose, convert to dosage of experimental medication(11.25 mg/kg), Calculate according to mouse body weight 20g. Concoct concentration of 5.625 mg/ml, intragastric administration 0.4 ml every day. ② Tea polyphenols local - injection group: Refer to tea polyphenols intraperitoneal injection LD_{50} 160 mg/kg. Take a quarter amount as experimental low dose group. Convert to dosage of 12 mg/kg experimental low dose and experimental low dose 24 mg/ml. Partly inject 0.1 ml every day. ③Panaxsaponin Rg3 group:Calculate according to human therapeutic dose (40 mg/d), convert to dosage of 0.1 mg/d per 20 g mouse, concoct concentration of 0.25 mg/ml, intragastric administration 0.4 ml every day. ④Model comparision group: isotonic Na chloride lavage, 0.4 ml every day.

Take successive administration for 10 days in each group, execute mouse in 11th day, and get tumor tissues and tissues of heart, brain, kidney to examine MVD

Detection of MVD

Put the tumor tissues and tissues of heart, brain, kidney into 10% Formaldehyde and fixed, dehydration in 2th day and paraffin imbedding, sliced serially with a thickness of 4μm, take one of them dyed with HE , Choose 3 histological sections in other each group to examine expression of MVD according to kit ´s explanation. Use PBS as anti-negative control. Positive staining is buffy, position is in endothelial cell endochylema, handle colored section with American TN-8502 image analytical system and determine IOD. Take average value of 3 histological sections and express by ($\bar{x}\pm s$)

Statistical Analysis

experimental data is dealed with SPSS 11.5 computer statistical soft ware. experimental data is dealed with t-test, $P<0.05$ indicate significant difference.

RESULTS

Determination of tumor tissues´MVD

Effect of MVD on breast cancer tissues were pre-

Table 1 MVD of mouse breast cancer tissues

Divided Group	IOD value ($\bar{x}\pm s$)
Model control group	151.69±15.58
Rg3 control group	141.76±14.38
Intragastric administration group	109.54±20.30*
Part-injection group	114.56±37.43△

Comparision to model control group: *$P<0.01$; △$P<0.05$

Table 2 MVD of vital origan(IOD value, $\bar{x}\pm s$)

Divided group	MVD of heart tissue	MVD of brain tissue	MVD of kindeytissue
Model control group	142.89±14.24	134.77±23.63	157.19±10.43
Tea polyphenols intragastric administration group	130.40±23.52	140.25±22.461	147.51±11.74

sented in Table 1 andFig.1 to Fig.4.

Result of graph above shows that there is statistically significant between tea polyphenols intragastric administration group or local-injection group and model control group ($P<0.01$, 0.05); there is not statistically significant between Rg3 group and control group ($P>0.05$). This show that its one of mechanism of action that inhibit breast cancer is possible to inhibiting angiopoiesis

Determination of vital organs´MVD

Effect of MVD on vital organ tissue (heart, brain, kindey)were presented in Table 2and Fig.5 to Fig.10.

From table 2, we can discern tea polypynols can selectively inhibit the information of new microvessel and have no significant effect on MVD of vital organs such as heart, brain and kidney. ($P>0.05$).

DISCUSSION

Examining MVD of tumor with immunohistochemical method can reflect new blood vessels hyperplasia. Hyperplasia of tumor blood capillary is close related to tumor invasion, transportation and prognosis of the patients. Tumor cell multiplication depend on new vessels which supply blood [8, 9]. The more MVD is, the faster tumor grow, therefore, MVD can directly reflect relationship of between tumor microvascular growth and tumorous depressive effect in implanted tumor animal experiment.

Factor Ⅷ related antigen express mainly in vascular endothelial cell, which has better specificity and can combine with endothelial cell, bone marrow - megakaryocyte, and tumor tissues originated in endothelial cell. But it do not respond to other interstitial substance or epithelial tissue tumor. Therefore it has well repeatability to examine MVD of tumor. Furthermore, background-intervention is fewer if use tumor or organ histological section-staining and show blood capillary very clear [10]. Bosari take follow-up investigation of 187 cases of breast cancer patients for 9 years, he find MVD is close correlated to prognosis of breast cancer. Furthermore, as a independent prognosis factor, Weinder discover MVD of breast cancer tissues is close related to axillary lymph nodes metastasis or distant metastasis [11 -12]. Our experimental result showed that tea polyphenols can inhibite the growth of implanted mouse breast cancer and its inhibiting mechanism is correlated to inhibiting tumor vasculogenesis .

Point of view in clinic, vasodepressor is divided into 2 category. One is specific or half-specific angiogenesis inhibitor which inhibit mainly EC hyperplasy or transferrence, but have no substantial effect on non-vascular endothelial cell. Another is non-specific angiogenesis inhibitor which has no high selectivity and its targeting is not certain, and it has generally effect on EC, tumor cells and normal tissue cells. Point of view of tumor therapy principle of killing all tumor cells, the non-specific angiogenesis inhibitor seem to be superiority, it can either inhibit tumor blood supply or have cytotoxic function on tumor cells. Because it can have effect on many kinds of cells, so it can bring about prediction adverse effect. For instance, while it inhibit angiogenesis of the tumor, and damage normal organs (vital organs) tissues at the same time which results in injuring organism, or functional patho-change bring new disease. Thus this limits clinical long-term application of non-specific angiogenesis inhibitor.

Based upon consideration above, in order to assess specificity of anti-new vessels of tea polyphenols, while examining MVD of implanted tumor tissues, we examine MVD of histological section of implanted mouse vital organ (heart, liver, kindey)simultanously. experimental result showed that the expression of MVD of

heart, liver, kindey were not statistically significant compared to model group ($P>0.05$), which indicated that tea polypynols can selectively inhibit the information of microvessel of breast cancer tissue and have no significant effect on normal organs. The mechanisms is possible to have effect on microvascular production by adjusting related angiogenesis factor. It limits in tumor tissues of hyperplasy in vasoformation stage. But it has no significant effect on blood capillary becaue the vasculogenesis mechanism is closed in normal tissue and organ, it has no influence on function of normal tissue and organ when it used clinically. However, we should set much store on the pathologic state and physiological state as follow: pregnancy, development, angiogenesis after wound, and cerbral infarction, blood capillary re-establish after heart infarction. Whether it has side effect when using of tea polyphenols under the physiology or pathologic state, there is no relative date to confirm and it need study and research further.

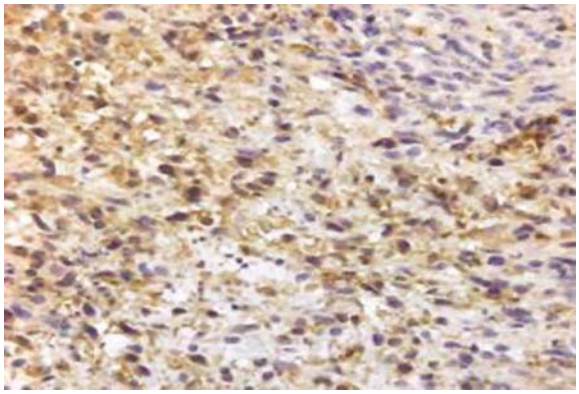

Fig. 1 MVD of tumor tissues of model control group (×200)

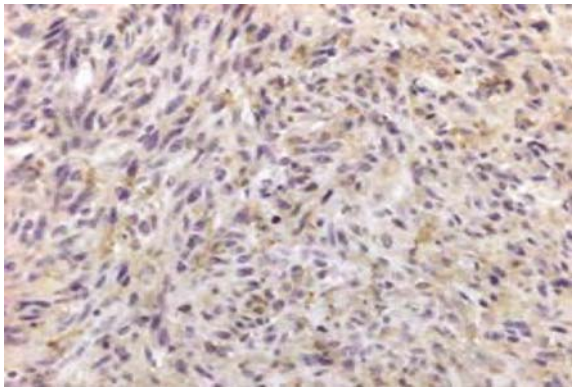

Fig. 2 MVD of tumor tissues of tea polyphenols intragastric administration group(×200)

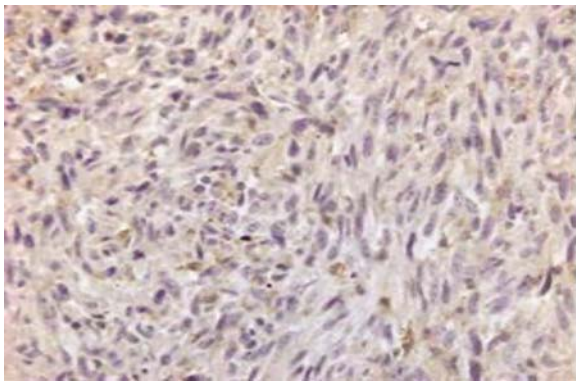

Fig. 3 MVD of tumor tissues of tea polyphenols Part-injection group(×200)

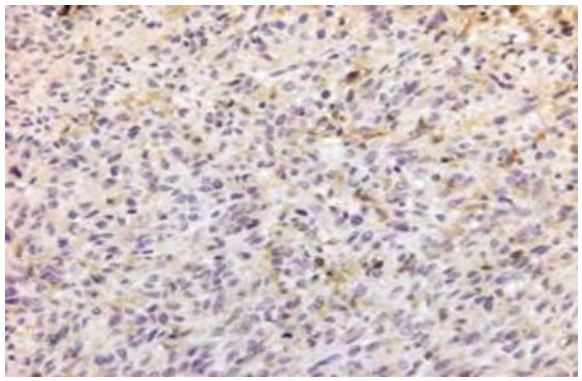

Fig. 4 MVD of Rg3 tumor tissues group(×200)

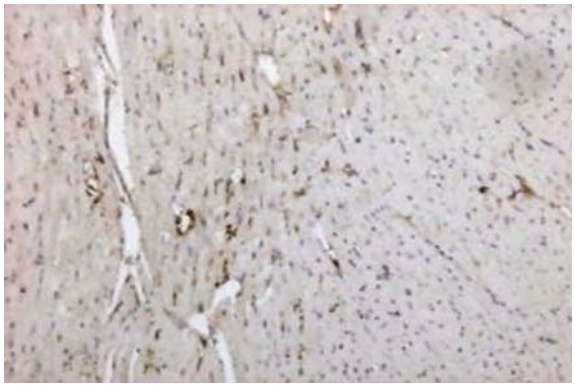

Fig. 5 MVD of heart tissue of model group(×200)

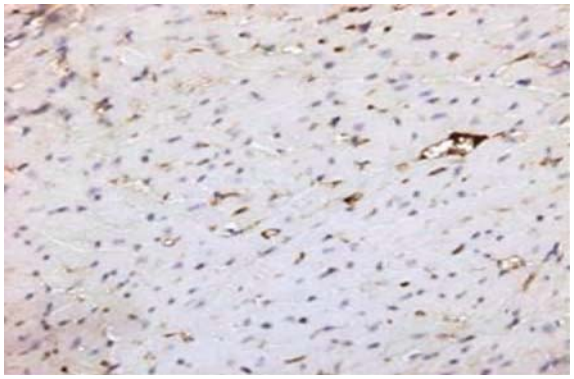

Fig. 6 MVD of heart tissue of tea polyphenols intragastric administration group(×200)

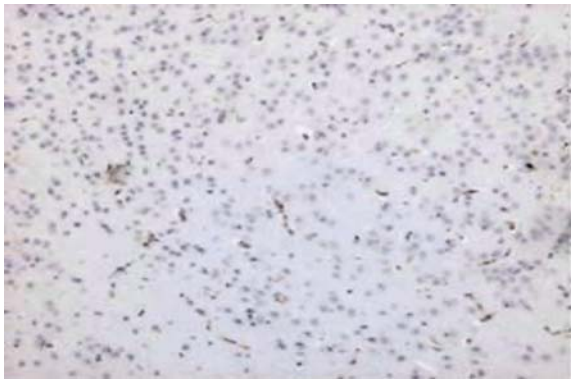

Fig. 7 MVD of brain tissue of model group(×200)

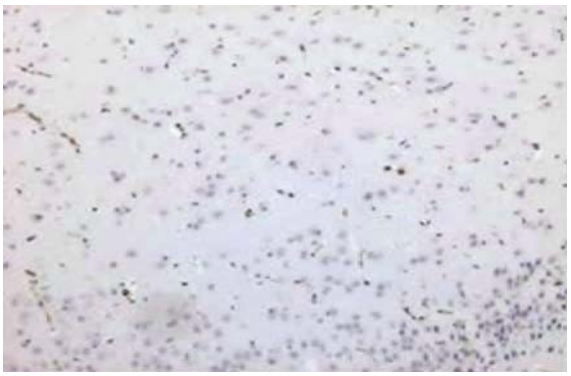

Fig. 8 MVD of brain tissue of tea polyphenols intragastric administration group(×200)

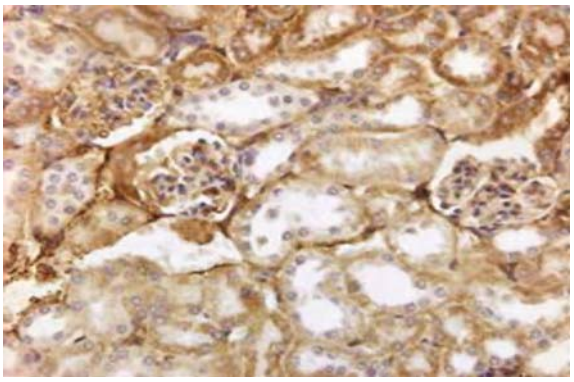

Fig. 9 MVD of kidney tissue of model group(×200)

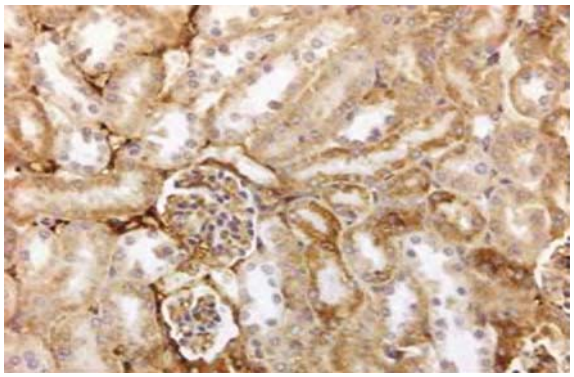

Fig. 10 MVD of kidney tissue of tea polyphenols intragastric administration group(×200)

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