RESEARCH ARTICLE

Solanum Nigrum Polysaccharide (SNL) Extract Effects in Transplanted Tumor-bearing Mice - Erythrocyte Membrane Fluidity and Blocking of Functions

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Abstract

Background: *Solanum nigrum* **L. has been used in traditional Chinese medicine because of its diuretic and antipyretic effects. The present research concerned effects of crude polysaccharides isolated from** *Solanum nigrum* **L. on erythrocyte membranes of tumor-bearing S_{180} and H_{22} in mice. Materials and Methods: Fluorescence-labeled red blood cell membranes were used with DPH fluorescence spectrophotometry to examine erythrocyte membrane fluidity, and colorimetry to determine degree of erythrocyte surface membrane blocking. Extent of reaction by tumor-bearing mice with the enzyme erythrocyte membrane bubble shadow detection of red cell membrane variation in the degree of closure before and after administration. Results:** *Solanum nigrum* **polysaccharide could significantly improve the S_{180} and H_{22} tumor-bearing mice erythrocyte membrane fluidity, compared with the control group, the difference was significant ($p<0.01$), SNL can significantly improve the red blood cell membrane and then S_{180} tumor-bearing mice sealing ability, compared with the negative control group, the difference was significant($p<0.05$, $p<0.01$). H_{22} tumor-bearing mice can increase red cell membrane and then sealing ability, the difference was significant ($p<0.05$). Solanum nigrum polysaccharide degree of fluidity and blocking two transplanted tumors in mice restored the ability to raise the red cell membrane has a significant effect. Conclusions: Solanum nigrum L.-type mice transplanted tumor can affect the red blood cell membrane fluidity and re-closed, through the red cell membrane of red blood cells to enhance the immune function of the possibility of erythrocyte immunity against tumor formation garland provide experimental basis.**

Keywords: Solanum nigrum polysaccharide - red cell membrane - membrane fluidity - membrane blocking degree

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Introduction

Red cell membrane is a barrier to life activities of cells play a protective role, change and maintain the normal function of the cell membrane is closely related to the characteristics of the drug to act on the cells through the cell membrane must first change will inevitably affect cell membrane function internal physiological functions the change, so from the perspective of the red cell membrane function were studied very meaningful (Hongsheng et al., 2014). Anti-tumor effects of red blood cells are mainly through the regulation of immune complexes include two aspects, one of the complement antigen antibody immune complex regulations, and the other is to complement antigen complex regulation. Many antigens such as tumor cells can be activated directly adhered complement and red blood cells by complement receptors are adhered to carry. In blood system of the body, red blood cells are much larger than the total number of white blood cells, the probability of contact with the tumor is relatively much greater chance of greater leukocyte adhesion, adhesion mainly through cell membranes and membrane effectors proteins or enzymes related to completed. The red cell membrane fluidity and reclosable enhanced ability to affect its adhesion and tumor cells, red blood cell membrane function and therefore considered to play a more important role in stopping cancer metastasis (Supabphol et al., 2009). Red cell immune function is made of red blood cells of the immune system as well as an important prerequisite, but the impact on red cell immune function of cell membrane still many issues that need further research, basic research both in experimental or clinical applications, have important significance (Narla et al., 2008).

Materials and Methods

Specimen

S_{180} and H_{22} mice were sacrificed by cervical dislocation after 7d~10d, placed at a concentration of 75%

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alcohol soaked 5min, the abdominal skin disinfection, peel the skin of the abdomen, with a sterile syringe to extract thick milky fused ascites (2~5ml), placed in a sterile centrifuge tube, the setting stored in the condensing agent. Another small amount of ascites was added with heparin tubes, cell morphology and cell counting, the number of tumor cells may use more than 97%, the ascites was diluted (1:2) with sterile saline, the tumor cells to appropriate concentration. Tumor cell suspension should be milky white translucent liquid, if bloody ascites unusable. Group received the same daily volume of normal saline, negative control group was given the same volume of saline, the positive control group was given astragalus polysaccharide 100mg/kg, SNL high dose group 125mg/kg, middle dose group 62.5mg/kg, low-dose group 31.25mg/kg. In S180 tumor-bearing mice inoculated intraperitoneally administered sterile next day, on H_{22} tumor bearing mice inoculated subcutaneously sterile next day, each mouse 0.4ml, continuous administration 7d, 24h withdrawal retrobulbar blood.

Cell erythrocyte membrane fluidity

Preparation of red cell membrane: Blood of tumor-bearing mice eyes after administration 7d, 3000rpm centrifuge 5mins, the supernatant was discarded, washed three times with pH 7.4PBS centrifugation, and then resuspended in PBS at a 1:1 ratio into erythrocytes, blood count plate count. Add 10mmol/L Tris-HCl (1:20), while protease inhibitors PMSF, 4°C hemolysis 1h, then centrifuged at 12000rpm 30min, the supernatant was discarded. 10mmol/L Tris-HCl and washed three times (same speed), draw a white precipitate, which was suspended in a 1:1 solution of pH 7.4 PBS (Marcela et al., 2008).

Fluorescently labeled red blood cell membrane: Take freshly prepared 2mmol/L DPH 1mL tetrahydrofuran solution were added to 500μL RBC membrane suspension (total membrane protein 100μg/ml, extraction and dilution according to different circumstances and different) and 2mL PBS buffer, rapid mixing, 37°C warm bath 30min, 3000rpm, centrifugal 10min, discard the liquid residue DPH mark. Isotonic PBS buffer, washed three times with isotonic PBS buffer, the cell suspension was diluted to a 4mL, fluorescence polarization measurement immediately (Minghui et al., 2012).

Fluorescence polarization degree: This experiment produced Shimadzu RF-540 fluorescence spectrophotometer measured fluorescence polarization. Excitation wavelength of 362nm, emission wavelength of 432nm, slit 10nm, temperature 25°C, calculate the degree of polarization (P), membrane lipid fluidity (LFU) and micro-viscosity (η), respectively, according to the following formula (Yoshinori, 2011).

$$\mathbf{LFU=(0.5-P)/P^2,\eta=2P/(0.46-P),\ P=(I_v-I_h)/(I_v+I_h).}$$

I_v: the polarizer and the detector optical axis of the light intensity in the vertical direction.

I_h: axis of the polarizer in a vertical direction, the detector of the optical axis of the light intensity in the horizontal direction.

Cell erythrocytes shadow bubble

After administration 7 days, mouse eyeball blood, 3000rpm centrifuge 5 minutes, the supernatant was discarded, washed three times with pH7.4 PBS by centrifugation, and then resuspended in PBS at a 1:1 into erythrocytes, blood counts plate count. Add 10mmol/L Tris-HCl (1:20), while protease inhibitors (PMSF) within 1h, then centrifuged at 12000rpm 30min, the supernatant was discarded. 10mmol/L Tris-HCl, 12000rpm and washed 3 times to draw a white precipitate, which was suspended in a 1:1 solution of pH7.4 PBS. The final membrane protein concentration was 0.6mg/mL.

Ghost assay

Freshly isolated erythrocytes were washed with Tris-buffered isotonic saline (0.15M potassium chloride, 10mM Tris, pH7.4). The cells were then lysed and washed 3 times with 15 volumes of ice-cold hypotonic buffer (5mM Tris, 5mM potassium chloride, pH7.4). After centrifugation, the pellet was loaded with 1 ml calcein (1mg/ml) in ice-cold hypotonic buffer. To reseal the leaky ghosts, 0.1 volume of 1.5M potassium chloride, 50mM Tris, pH 7.4, was added to restore isotonicity, and the ghosts were incubated for 40 min at 37°C (Xu et al., 2009). *NADH-measured activity of cytochrome C oxidoreductase*

Zamudio method: take a film sample (liquid film) 0.6mL in the cuvette plus the reaction solution at a wavelength of 420nm at the mixing ratio of color, to add to NADH as a starting point, the recording starts within 6 minutes of the reaction OD values(Cardoso et al.,2004).

Ghost blocking degree

Ghost blocking degree(GBD) according to the following formula: $GBD=(OD_1-OD_2)/OD_1\times100\%$.

OD_1: liquid with NADH,

OD_2: liquid without NADH.

Erythrocyte membrane sialic acid tumor-bearing mice

Take the test, diluted with water to a protein concentration of about 1ml each containing 0.2~0.4mg, as the test solution. Take sialic acid reference solution, water, and the test solution in 10ml glass test tube, mix, then add between 5-methyl -hydroquinone solution 4ml each tube, cover, boil 15minutes in boiling water (water bath above the liquid surface face about 2cm), water cooled to room temperature, 3000rpm, centrifuged 10min, the supernatant at 560nm wavelength UV-visible spectrophotometry absorbance of blank pipe read zero absorbance of each tube, calculate sialic acid content(Stevenson et al., 2004).

Statistical analysis

The data was analyzed using SPSS16.0 package. The relationship between membrane lipid fluidity and Ghost blocking degree expression levels was analyzed using Variance analysis and statistical method. Differences with $p<0.01$ were considered statistically significant.

Results

Membrane fluidity is one of the basic characteristics of the membrane, the necessary conditions for carrying out

activities in accordance with the membrane structure and function of the plasma membrane, or cell membrane fluidity is important in maintaining normal cell physiological function (Bayer et al., 2000). The results showed that tumor-bearing mice erythrocyte membrane fluidity than normal erythrocyte membrane fluidity significantly reduced SNL each dose group could significantly restore S_{180} and H_{22} tumor-bearing mice erythrocyte membrane fluidity. Compared with the negative control group, SNL each dose group could reduce the role of S_{180} and H_{22} tumor-bearing mice *in vivo* red cell membrane micro-viscosity, tumor-bearing mice significantly increased erythrocyte membrane fluidity. SNL possible tumor-bearing mice by increasing red cell membrane fluidity, tumor-bearing mice restore normal function of red cell membrane.

Table 1. Membrane Fluidity of the Tumor S_{180} Cell Expression

Grouping	*p*	ŋ	LFU
Normal group	0.2177	1.7975	5.9609
Negative control	0.2415△	2.2096△	4.435△
Positive control	0.2259**	1.9303**	5.3715**
SNL (31.25mg/kg)	0.2369	2.1243	4.6919
SNL (62.5mg/kg)	0.2328**	2.0485**	4.9329**
SNL (125mg/kg)	0.2201**	1.9844**	5.1618**

**p<0.01 vs control group; △p<0.01 vs normal group

Table 2. Membrane Fluidity of the Tumor H_{22} Cell Expression

Grouping	*p*	ŋ	LFU
Normal control	0.2119	1.7089	6.4136
Negative control	0.2470△	2.3268△	4.1772△
Positive control	0.2147**	1.7657**	6.3614**
SNL(31.25mg/kg)	0.2383	2.1501	4.6104
SNL(62.5mg/kg)	0.2348*	2.0881*	4.8243*
SNL(125mg/kg)	0.2271**	1.9527**	5.3092**

*p<0.05; **p<0.01 vs control group; △p<0.01 vs normal group

Table 3. Erythrocyte Membrane S_{180} and H_{22} Blocking Degree

Grouping	blocking degree (%S_{180})	blocking degree (%H_{22})
Normal control	64.53	63.25
Negative control	19.09△	11.23△
Positive control	58.69**	52.95**
SNL(31.25mg/kg)	27.08	16.59
SNL(62.5mg/kg)	37.734*	29.22*
SNL(125mg/kg)	55.01**	30.52*

*p<0.05; **p<0.01; vs control group; △p<0.01 vs normal group

Table 4. Sialic Acid of Tumor S_{180} and H_{22}

Grouping	SA (%S_{180})	SA (%H_{22})
Normal control	2.2592	0.1769
Negative control	1.3181△	0.0342△
Positive control	2.0454*	0.1648**
SNL(31.25mg/kg)	1.7575	0.0617*
SNL(62.5mg/kg)	2.0227*	0.1146**
SNL(125mg/kg)	1.9545*	0.1620**

*p<0.05; **p<0.01 vs control group; △p<0.01 vs normal group

Effect of Solanum nigrum polysaccharide on erythrocyte membrane S180 and H22 blocking degree

SNL on S180 tumor-bearing mice closure of the red cell membrane significantly increased the role of high-dose 125mg/kg blocking degree can be increased to 55.01%, a significant difference (p<0.01) between the negative control group. SNL high-dose groups in H_{22} tumor-bearing mice body blocking degree of red cell membrane were increased, and the difference was significant (p<0.05). Transplanted tumors in two red cell membrane fluidity compared tumor weight erythrocyte membrane sealing ability, only about one-third of normal red blood cells in mice, so in the dual role of transplanted tumors and SNL, the red cell membrane structure and function will change, which will affect the red cell membrane again ability closed, the closure was reduced summary SNL restore or enhance the ability of tumor-bearing mice resealed red cell membrane, increasing the stability of the red cell membrane, improve the structure and function of the red cell membrane, this change may related to its anti-tumor effect. glycolipids on the cell membrane, the cell membrane to participate in many biochemical phenomenon of red, such as cell differentiation, cell migration of malignant cell recognition (Shannon et al., 2009). Reduce the red cell surface SA cancer patients, promote red blood cell aging, fragility, cell disintegration easy rupture, affecting the normal physiological function of red blood cells (Lu et al., 2012). Experimental results show that the negative control group of mice erythrocyte membrane SA content was significantly lower compared with normal group, SA content of tumor-bearing mice decreased erythrocyte membrane. Compared with the negative control group, SNL each dose group can significantly improve the S180 tumor-bearing mice red cell surface sialic acid content, including high-dose group SNL and SNL dose group

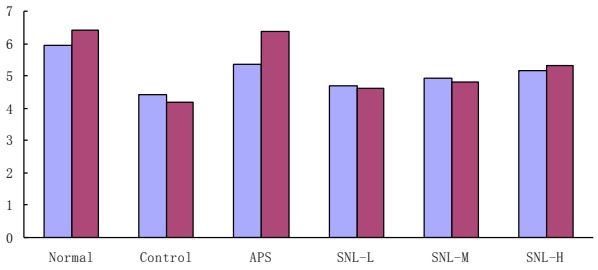

Figure 1. Effect of Total Alkaloid of Solanum Nigrum Polysaccharides on the Blocking Degree og Cell Membrane of Tumor S_{180} and H_{22}.

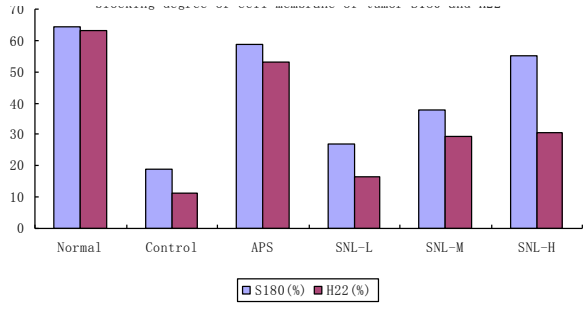

Figure 2. Effect of Total Alkaloid of Solaum Nigrum Polysaccharides on Blocking Degree og Cell Membrane of tumor S_{180} and H_{22}.

had significant difference ($p<0.05$), SNL each dose group enhanced H_{22} tumor-bearing mice can increase red cell surface sialic acid content. In summary SNL can significantly improve the sialic acid content of red blood cell membrane, thereby enhancing the body's red blood cells of tumor-bearing mice to identify, adhesion and transport function, prevent loss of sialic acid from the surface of the red cell membrane, restoring tumor-bearing mice red cell membrane function to achieve anti-tumor effect (Son et al., 2003).

Discussion

Cell membrane of the basic activities are conducted in the flow state, and many factors can influence the membrane fluidity, some drugs is to play to the effect by changing the membrane fluidity of the research results show that solanum nigrum polysaccharide on S_{180} tumor cells inhibition effect is partly from its function of cell membrane. This and have solanum nigrum polysaccharides on S_{180} tumor cells growth inhibition and enhance the immunity of the experimental results are consistent (Qinjian et al., 2014). Compared to two kinds of mice red cell membrane sealing capacity recovery results show that the solanum nigrum polysaccharide can effectively restore a tumor-burdened red cell membrane in mice, Through the increase of red cell membrane fluidity and rapid sealing capacity. Tumor immune escape one of the reasons is caused due to the effect of masking of the antigen molecules in SA Tumor cells acidification increase saliva, membrane SA, is conducive to the infiltration and metastasis Through two tumor-burdened comparative study found that in the mice (Jean et al., 2014). Solanum nigrum polysaccharide immune stimulation, can effectively improve tumor-burdened sialic acid content on the red cell membrane of mice, red on S_{180} mice erythrocytes effect more obvious, also have certain effect on H_{22} mice, but effect is not significant.

By comparing the results found that a tumor-burdened activity of red cell membrane of mice under the action of solanum nigrum polysaccharide has a partial recovery, but the variation in content of sialic acid cannot clearly show their influence on red cell membrane, membrane protein expression related to main factors affecting the membrane is the main function (Yubin et al., 2014), so we will further related on the red cell membrane protein expression of further experimental analysis.

Red cell membrane is a barrier to life activities of the cell protective effect, change and maintain the normal physiological function of the cell membrane is closely related to the characteristics of the drug to act on the cells through the cell membrane must first change will inevitably affect cell membrane function internal physiological functions the change, so from the perspective of the red cell membrane function were studied very meaningful. Alterations in the adhesive and rheological properties of red cells are of particular importance (Narla et al., 2012). Red blood cells are essential for the biological function of the body cells, an important way to transport oxygen and metabolites in addition (Doctor et al., 2012), the role of erythrocytes immunosorbent also is anti-tumor (Sabrina et al., 2011). RBC adhering tumor cells, enhancing immune cell function, but also by the role of peroxidase on the role of tumor cells with effector cells, blood cells can prevent tumor metastasis, for the clinical treatment of cancer patients are closely related. The data show that SNL cotreatment reversed or mitigated effects on each endpoint, in part, by two possible mechanisms; the SNL may be increasing the activity of the immune enhancing factor, or, an anti-oxidant effect of SNL might help to protect membrane structures and increase stability on the erythrocyte (Zhao et al., 2014). Determine the effect of drugs on erythrocyte membrane function, providing a practical basis for screening anticancer drugs, and to learn whether the drug functions by affecting the red blood cell membrane to reach the antitumor effect, inhibiting tumor cell proliferation or metastasis mechanism of scientific research methods and theoretical basis. Red cell immune function is made of red blood cells of the immune system as well as an important prerequisite, but the impact on red cell immune function of red cell membrane still many issues that need further research, basic research both in experimental or clinical applications, have important significance. Through the perspective of red cell membrane function studies on red cell immune-depth research and development of anti-tumor immunotherapy in future will also bring the heart of the revolution.

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