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Effects of gypenosides on mouse splenic lymphocyte transformation and DNA polymerase Ⅱ activity *in vitro*[1]

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AIM: To study the effects of gypenosides (Gyp) on lymphocyte transformation and DNA polymerase Ⅱ activity. **METHODS**: Lymphocyte transformation response was induced by concanavalin A and lipopolysaccharides respectively. The activity of DNA polymerase Ⅱ and DNA synthesis were assayed with TTP and [^{3}H]TdR incorporation respectively in mixed lymphocyte culture test. **RESULTS**: Gyp 2.5 — 20 mg L^{-1} enhanced splenic T- and B- cell transformation, increased the DNA synthesis and potentiated the activity of DNA polymerase Ⅱ. Howerer, Gyp $>$ 40 mg L^{-1} showed contrary effects. **CONCLUSION**: Gyp regulated lymphocyte transformation and DNA synthesis by regulating DNA polymerase Ⅱ activity.

KEY WORDS *Gynostemma pentaphyllum*; saponins; DNA polymerase Ⅱ; lymphocyte transformation; mixed lymphocyte culture test

Gypenosides (Gyp) are the active ingredients of *Gynostemma pentephyllum* (Family Cucurbitaceae) with 82 saponins identified, of which the principal saponins C_3, C_4, C_8, and C_{12} are similar to ginsenosides R_b, R_1, R_d, and F_1, respectively in structure. Gyp could increase the splenic plaque forming cell (PFC) response to SRBC in cyclophosphamide-immunosuppressed mice. It improved the immune function in S180 tumor-bearing mice, intensified the PFC response to SRBC *in* corticosterone-treated rats[1], and increased IL-2 production of splenocytes in both normal and immunosuppressed mice caused by cyclophosphamide[2]. The present research was to study the effects of Gyp on lymphocyte transformation, DNA synthesis and the activity of DNA polymerase Ⅱ of splenic lymphocytes.

MATERIALS AND METHODS

Drugs and chemicals Gyp (purity 90.8 %) was kindly provided by Prof CHEN Xiu, Department of Pharmacology, Hunan Medical University.

Concanavalin A (Con A) and lipopolysaccharides (LPS), Sigma Chemical Co. [^{3}H]TdR (851 TBq mol^{-1}) and [^{3}H]TTP (1110 TBq mol^{-1}), Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. dATP, dCTP, dGTP and dithiothreitol (DTT), Boehringer Mannheim. Fish sperm DNA, Shanghai Institute of Biochemistry, Chinese Academy of Sciences. RPMI 1640, JR Scientific, Inc.

Mice Inbred C_{57}BL/6J and BALB/c mice, ♀, ♂, (20.2 ± s 1.3 g) were purchased from Department of Experimental Animal, Beijing Medical University.

Lymphocyte transformation response (LTR) LTR was assayed by method of [^{3}H]TdR incorporation[3]. Cultured splenocytes with Gyp (2.5 — 80 mg L^{-1}) were incubated with Con A (25 μg/well) or LPS (6.25 μg/well) for 48 h. Then, [^{3}H]TdR (14.8 kBq/well) was added and cells were harvested for dpm count with liquid scintillation counter. Results were expressed as the relative proliferation index (RPI):

RPI = dpm (drug) /dpm (control) × 100 %

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Mixed lymphocyte culture test A 96-well plate was used for cell culture. Each well contained $4 \cdot 10^5$ mixed splenocytes and drugs. Splenocytes were incubated in humidified atmosphere containing 5 % CO_2 at 37 ℃ for 3 d. Ten hours prior to termination, [^{3}H]TdR (14.8 kBq/well) was added. The RPI, a parameter of DNA synthesis, was calculated as above.

Enzyme preparation and assay Extraction and assay of DNA polymerase Ⅰ were carried out according to our previous method[4]. One unit of enzyme activity was defined as 1 μmol L^{-1} of TTP incorporated into acid-insoluble materials h^{-1} at 37 ℃.

Statistical analysis All data were expressed as $\bar{x} \pm s$, and the singnificance between groups was tested by ANOVA.

RESULTS

LTR Gyp <10 mg L^{-1} augmented the T- and B-cell transformation stimulated by Con A and LPS, respectively, as shown by an increased rate of [^{3}H]TdR uptake. However, Gyp >40 mg L^{-1} inhibited their transformation (Tab 1).

DNA synthesis and DNA polymerase Ⅱ activty of lymphocytes in MLC Gyp <20 mg L^{-1} enhanced DNA synthesis and potentiated the activity of DNA polymerase Ⅱ, while >40 mg L^{-1} had a contrary effect (Tab 2).

Tab 2. Effects of gypenosides on DNA synthesis and DNA polymerase Ⅱ activity of lymphocytes. $n=6$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Gypenosides/ mg L^{-1}	DNA synthesis [^{3}H]TdR incorporation/ dpm	RPI/%	Enzyme activity (U/10^6 cells)
0	28 180±2 435	100.0	5.76±0.60
2.5	33 210±1 939^b	117.8	
5.0	34 142±3 103^b	121.2	7.61±0.79^c
10.0	33 225±3 073^b	117.9	8.12±1.22^c
20.0	37 973±2 284^c	134.8	6.28±1.37^a
40.0	25 273±1 270^a	89.7	4.58±0.83^b
80.0	19 052±377^c	67.6	3.46±0.89^c

DISCUSSION

Gyp can increase antibody and IL-2 production of splenocyte, potentiate the activity of NK cells and enhance splenic lymphocyte proliferation induced by Con A and LPS *ex vivo*[1,2]. Gyp induced a simultaneous response of immune system and neuroendocrine system in rats *in vivo* as shown by an increase in the lymphocyte proliferation to Con A with a decrease of hypothalamic norepinephrine and plasma corticosterone, which suggests that the immunomodulation of Gyp

Tab 1. Effects of gypenosides on T- and B-cell transformation. $n=6$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Gypenosides/ mg L^{-1}	T-lymphocytes [^{3}H]TdR incorporation/ dpm	RPI/%	B-lymphocytes [^{3}H]TdR incorporation/ dpm	RPI/%
0	45 480±7 472	100.0	14 348±1 880	100.0
2.5	48 152±4 874^a	105.9	15 210±2 148^a	106.0
5.0	54 313±5 672^b	119.4	16 258±2 746^a	113.3
10.0	57 918±3 356^c	127.3	19 248±4 352^b	134.2
20.0	49 169±3 448^a	108.1	24 213±1 372^c	167.8
40.0	41 822±3 540^a	91.9	12 013±710	83.7
80.0	34 153±5 021^b	75.1	10 737±1 178^b	74.8

may be mediated through neuroendocirine-immune modulation network[2]. In this study, we observed that Gyp exerted a simultaneous effect on immune response and enzyme activity with methods of LTR and MLC *in vitro*. Gyp <20 mg L^{-1} could induce a concomitant increase in both the lymphocyte transformation to the mitogen (Con A or LPS) and the activity of DNA polymerase Ⅱ. However, Gyp >40 mg L^{-1} had a contrary effect. The immunomodulating effect of Gyp is very similar to that of the ginseng root (*Panax ginseng*) and *Ganoderma* polysaccharides (*Ganoderma lucidum*)[5].

Mixed lymphocyte culture test is a model for specific immune response in which T cells act as responders and B-cell or macrophages carrying alloantigens (MHC antigens) on their membranes serve as stimulators[6]. The T cells undergo a blastoid transformation, DNA synthesis and proliferation in the process. There are several enzymes responsible for the DNA synthesis of lymphocyte[7], of which DNA polymerase Ⅱ is assumed to be the most important one. Present results demonstrate that Gyp could promote the rate of [^{3}H]TdR uptake by lymphocyte and the enzyme activity in the system, indicating that the effects of Gyp on DNA synthesis were correlative with the enzyme activity. It was concluded that Gyp increased the DNA synthesis of splenocytes by enhancing DNA polymerase Ⅱ activity and promoted the transformation of immunocompetent cells, leading to potentiation of the specific immune response.

REFERENCES

1 Qian BC, Zang XX, Chen J, Xu HJ, Liu XL, Yuan YY, Peng XY. Influence of gypenosides on immunological function in rodents. Chin J Pharmacol Toxicol 1986; 1: 53—6.

2 Li L, Xing ST. Effect of gypenosides on lymphocyte proliferation and interleukin-2 production in spleen of mice. Pharmacol Clin Chin Mat Med 1992; 8: 26—9.

3 Ding Y, Xing ST, Zhou JH. Stimulation of ^{3}H-thymidine incorporation into T and B cells and inducement of interferon *in vitro* on mice by epimedium polysaccharide. Chin J Immunol 1985; 1 (6): 42—6.

4 Lei LS, Lin ZB. Effects of ganoderma polysaccharides on the activity of DNA polymerase α in spleen cells stimulated by alloantigens in mice *in vitro*. J Beijing Med Univ 1991; 23: 329—33.

5 Zhang YX, Zhou JH, Xing ST, Yuan SL, Ruan JX. Effects of gypenosides on splenocyte proliferation and relationships with hypothalamic and splenic norepinephrine and plasma corticosterone in rats. Chin J Pharmacol Toxicol 1992; 6: 254—8.

6 Lohrmann H-P, Novikovs L, Graw RG Jr. Stimulatory capacity of human T and B lymphocytes in the mixed leukocyte culture. Nature 1974; 250: 144—6.

7 Bertazzoni U, Stefanini M, Pedrali G, Giulotto E, Nuzzo F, Falaschi A, Spadari S. Variations of DNA polymerases-α and β during prolonged stimulation of human lymphocytes. Proc Natl Acad Sci USA 1976; 73: 785—9.

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绞股蓝总皂甙对体外培养小鼠脾淋巴细胞转化及 DNA 多聚酶Ⅱ活性的影响

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目的：探讨绞股蓝总皂甙对淋巴细胞转化和 NDA 多聚酶Ⅱ活性的影响.

方法：采用 C_{57}BL/6J 和 BALB/c 小鼠淋巴细胞混合培养及淋巴细胞转化实验，按[^{3}H]TdR 和 TTP 掺入法检测 DNA 合成和 DNA 多聚酶Ⅱ活性.

结果：小剂量 Gyp (2.5—20 mg L^{-1})促进淋巴细胞转化，并提高 DNA 多聚酶Ⅱ活性，促进淋巴细胞 DNA 合成，而大剂量 Gyp (>40 mg L^{-1}) 则呈相反作用.

结论：Gyp 能通过调节 DNA 多聚酶Ⅱ活性调控淋巴细胞转化.

关键词 绞股蓝；皂甙类；脱氧核糖核酸聚合酶Ⅱ类；淋巴细胞转化；混合型淋巴细胞培养试验

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