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Effect of sodium glycyrrhetinate on chemical peritonitis in rats

BAO Jin-Feng[1], WU Yong-Jie, YANG Yong-Jian[2], LI Xin-Fang[3] (*Department of Pharmacology, [2]Department of Pharmacognosy, Lanzhou Medical College, Lanzhou 730000, China*)

KEY WORDS glycyrrhetinic acid; peritonitis; neutrophils; superoxide dismutase; cyclic AMP; prostaglandin E; dexamethasone; carrageenan; arachidonic acid

AIM: To study the anti-inflammatory mechanisms of sodium glycyrrhetinate (SG). **METHODS:** Rat chemical peritonitis was used. The protein content and prostaglandin E_2 (PGE_2) content in exudate were measured by Folin-phenol assay and RIA, respectively. SOD activity in neutrophils (Neu) was determined by pyrogallol-NBT colorimetry. cAMP content in Neu was detected by competitive protein binding assay. **RESULTS:** In peritonitis caused by histamine, SG 10 － 20 mg · kg^{-1} im reduced exudate volume and Neu counts, and 5－20 mg · kg^{-1} im lowered the protein content in exudate. In peritonitis induced by carrageenan, SG 20 mg · kg^{-1} im reduced exudate volume, Neu counts, protein content and PGE_2 content in exudate, increased SOD activity in Neu, but did not affect β-glucuronidase release from Neu. In peritonitis induced by arachidonic acid, SG 20 mg · kg^{-1} im reduced Neu counts, protein content, and PGE_2 content in exudate, and attenuated the reduction of cAMP level in Neu. **CONCLUSION:** SG exerts its anti-inflammatory action by lowering permeability of capillaries in inflammatory site, inhibiting Neu emigration and PGE_2 biosynthesis, and scavenging oxygen free radicals.

Sodium glycyrrhetinate (SG) inhibited many kinds of experimental inflammations[1,2]. Our previous experiment demonstrated that SG reduced the generation of prostaglandin E (PGE) and lipid peroxides in inflamed tissues[2], scavenged oxygen free radicals from Neu[3], and antagonized the contracting action of inflammatory mediator histamine on isolated guinea-pig ileum[2]. In this study, chemical peritonitis models in rats were used to investigate its effect on inflammatory process so as to further clarify its action mechanism against inflammation.

MATERIALS AND METHODS

SG (purity >98 %) was synthesized by the Department of Chemistry, Lanzhou University. Dexamethasone (Dex) was obtained from Xinyi Pharmaceutical Factory, Shanghai. Arachidonic acid (Fluka, purity >99 %) 2 mg was dissolved in ethanol 50 μL and prepared as sodium salt with equimolar $NaHCO_3$. After removal of ethanol by evaporation at 78 ℃, arachidonic acid was diluted with D-Hanks' solution. Histamine (Shanghai Institute of Biochemistry, Chinese Academy of Sciences), and carrageenan (Liaoning Institute of Materia Medica, Shenyang) were dissolved in normal saline. Bovine SOD was produced by Xiahe Biological Preparation Factory, Gannan, China. β-Glucuronidase (β-Glu) reaction matrix was kindly provided by Prof CHENG Shi-Me (in Research Institute of Field Operation Surgery, the Third Military Medical College, Chongqing). [^{3}H]PGE_2 RIA kit was from the General Hospital of Chinese People's Liberation Army, Beijing, China. [^{3}H]cAMP RIA kit was obtained from Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing.

Wistar rats (♂, $n = 136$) weighing 202 ± s 20 g were provided by Experimental Animal Breeding Center, Lanzhou Medical College.

The preparation of rat peritonitis models Rat peritonitis was induced by injecting ip carrageenan 15 mg · kg^{-1}, histamine 30 mg · kg^{-1}, or arachidonic acid 4 mg · kg^{-1}. After 3 h, rats were decapitated and injected ip D-Hanks' solution 5 mL. After 2-min massage on abdomen, the abdomen was incised and all the diluted exudate in peritoneal cavity was drawn out. The exudate volume and leukocyte counts were measured. The exudate was centrifuged (2500 × g, 4 ℃, 15 min). The supernatant was used to determine the content of protein, PGE_2, and β-Glu, while the leukocyte pellet was used to analyze SOD activity and cAMP content. The cell viability was >98 % as assessed by trypan-blue exclusion. The neutrophils (Neu) as demonstrated by staining classification occupied 98 %. The therapeutic drugs or normal saline 20 mL · kg^{-1} in control group was injected im 30 min prior to inducing peritonitis.

[1] Now in *Nei Menggu Institute of Medicinal Industry, Huhehot 010050, China*.
[3] Correspondence to Prof LI Xin-Fang.
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Analysis methods The content of protein was measured by Folin-phenol assay[4], β-Glu content by colorimetry[5], SOD activity by pyrogallol-NBT colorimetry[6], PGE_2 by RIA[7] and cAMP content by competitive protein binding assay[8].

RESULTS

Histamine peritonitis Histamine ip caused enhancement of exudate volume, Neu counts, and protein content in exudate of rat peritonitis. SG 10 or 20 $mg \cdot kg^{-1}$ im reduced exudate volume and Neu counts. SG 5, 10, or 20 $mg \cdot kg^{-1}$ lowered protein content in exudate in a dose-dependent manner (Tab 1).

Carrageenan peritonitis After ip carrageenan, the exudate volume, Neu counts, and protein content in the exudate were greatly increased as compared with saline group. SG 20 $mg \cdot kg^{-1}$ or Dex reduced the exudate volume, Neu counts, and protein content (Tab 2).

PGE_2 and β-Glu contents were raised while SOD activity was reduced in the exudate. SG 20 $mg \cdot kg^{-1}$ or Dex 1.5 $mg \cdot kg^{-1}$ reduced PGE_2 content in exudate and increased SOD activity in Neu. However, SG failed to affect Neu β-Glu release in contrast with the inhibitory effect of Dex on Neu β-Glu release (Tab 2).

Tab 1. Effect of sodium glycyrrhetinate (SG) and dexamethasone (Dex) on peritoneal exudate, neutrophil emigration, and protein exudate in rat experimental peritonitis induced by histamine (His, 30 $mg \cdot kg^{-1}$). $n = 10$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **normal saline (NS) + His group.**

Drugs /$mg \cdot kg^{-1}$		Peritoneal exudate /mL	Neutrophils in exudate /$1 \times 10^9 \cdot L^{-1}$	Protein in exudate /$g \cdot L^{-1}$
NS + NS	–	4.23 ± 0.27[c]	7.1 ± 1.0[c]	5.2 ± 1.7[c]
NS + His	–	4.8 ± 0.3	11 ± 3	9.2 ± 1.7
SG + His	5	4.6 ± 0.4[a]	8 ± 4[a]	6.1 ± 2.5[b]
SG + His	10	4.40 ± 0.23[c]	5.5 ± 1.3[c]	5.0 ± 1.0[c]
SG + His	20	4.38 ± 0.25[c]	6.3 ± 1.7[c]	5.4 ± 1.5[c]
Dex + His	1.5	4.41 ± 0.26[c]	4.6 ± 1.4[c]	5.3 ± 1.9[c]

Arachidonic acid peritonitis After ip arachidonic acid, Neu counts, protein content, and PGE_2 content in the exudate were evidently increased, but cAMP in Neu was reduced. SG 20 $mg \cdot kg^{-1}$ decreased Neu counts, protein content, and PGE_2 content in exudate, but enhanced cAMP

Tab 2. Effect of sodium glycyrrhetinate (SG) and dexamethasone (Dex) on peritoneal exudate, neutrophil emigration, protein exudate, β-glucuronidase (β-Glu) release, PGE_2 generation and SOD activity in rat experimental peritonitis induced by carrageenan (Car, 15 $mg \cdot kg^{-1}$). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **normal saline (NS) + Car group.**

Drugs/ $mg \cdot kg^{-1}$		Rats	Peritoneal exudate Volume/ mL	Neutrophil/ $1 \times 10^9 \cdot L^{-1}$	Protein/ $g \cdot L^{-1}$	β-Glu/ Unit	PGE_2/ $nmol \cdot L^{-1}$	SOD in Neu kU/g protein
NS + NS	–	9	4.5 ± 0.3[c]	6.4 ± 1.5[c]	6.9 ± 1.8[c]	244 ± 49[c]	6.1 ± 3.0[c]	18 ± 3[b]
NS + Car	–	12	7.0 ± 1.2	14.6 ± 2.2	13.8 ± 3.0	1 270 ± 73	9.3 ± 1.1	14 ± 4
SG + Car	20	12	5.7 ± 0.6[c]	10.3 ± 1.8[c]	9.5 ± 2.0[c]	1 250 ± 66[a]	6.8 ± 0.8[c]	21 ± 6[b]
Dex + Car	1.5	11	5.3 ± 0.4[c]	9.7 ± 2.4[c]	8.7 ± 1.6[c]	844 ± 33[c]	7.6 ± 1.8[b]	22 ± 4[c]

Tab 3. Effect of sodium glycyrrhetinate (SG) on rat experimental peritonitis induced by arachidonic acid (AA, 4 $mg \cdot kg^{-1}$), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **normal saline (NS) + AA group.**

Drugs/ $mg \cdot kg^{-1}$		Rats	Peritoneal exudate Volume/ mL	Neutrophil/ $1 \times 10^9 \cdot L^{-1}$	Protein/ $g \cdot L^{-1}$	PGE_2/ $nmol \cdot L^{-1}$	cAMP in 10^7 Neu/ pmol
NS + NS	–	9	4.3 ± 0.5[a]	5.0 ± 2.1[c]	5.4 ± 1.3[c]	5.9 ± 1.5[c]	7.0 ± 2.4[c]
NS + AA	–	12	4.4 ± 0.4	9.7 ± 3.3	9.2 ± 2.0	7.2 ± 0.7	3.4 ± 1.6
SG + AA	20	11	4.22 ± 0.26[a]	4.8 ± 1.8[c]	5.9 ± 2.2[c]	5.8 ± 1.2[c]	6.6 ± 2.2[c]

concentration in Neu (Tab 3).

DISCUSSION

SG reduced exudate volume and protein content in rat chemical peritonitis, indicating that SG has the action of reducing capillary permeability in inflammatory sites and decreasing inflammatory exudation. Simultaneously, SG reduced Neu counts in exudate, suggesting that it is capable of suppressing Neu emigration to inflamed sites. However, SG did not inhibit β-Glu release out of Neu, implying that SG has no action of stablizing lysosome membrane.

Inflammatory factors were considered to activate phosphodiesterase which degrates cAMP in Neu and causes a reduction of cAMP level in Neu when inflammation. This results in the activation of Neu and mediates the generation and release of oxygen free radicals and PGE_2[9,10]. Glycyrrhetinic acid was shown to inhibit the activity of phosphodiesterase selectively[11]. Our experiment demonstrated that SG alleviated the reduction of cAMP level in Neu stimulated by arachidonic acid, and inhibited PGE_2 synthesis induced by arachidonic acid or carrageenan. These data support the conclusion that one of antiinflammatory mechanisms by SG is to inhibit the activation of metabolism pathway of arachidonic acid by elevating cAMP level within Neu and further to suppress the activation of Neu and subsequent PGE_2 biosynthesis.

Oxygen free radicals and PGE_2 play important roles in inflammatory damage of tissues and capillary wall. SG inhibited the generation of active oxygen species from Neu *in vitro*[3] and reduced PGE_2 biosynthesis in chemical paw edema of rats[2]. The present experiment showed that SG enhanced SOD activity in Neu and inhibited PGE_2 generation in rat peritonitis, suggesting that its action of scavenging oxygen free radicals and inhibiting PGE_2 biosynthesis may also contribute to its action of lowering capillary permeability and reducing inflammatory exudation. Because SG fully antagonized inflammatory exudation caused by histamine, it is rational to conclude that SG is also capable of reducing capillary permeability directly.

REFERENCES

1 Li XF, Meng FM, Pan FS, Lin W, Lan ZF, Wang FL, *et al*. Effects of 18 β-sodium glycyrrhetic acid (SGA) on inflammation and immune function. Chin Pharmacol Bull 1990; 6: 105－8.

2 Wu YJ, Li XF, He L, Liu L, Chang ZM. Anti-inflammatory mechanism of sodium glycyrrhetinate Chin Pharmacol Bull 1991; 7: 46－9.

3 Wu YJ, Li XF. Effects of sodium 18 β-glycyrrhetinic acid on the production of active oxygen species and the concentration of intracellular free Ca^{2+} in rat neutrophils. Acta Pharmacol Sin 1991; 12: 280－4.

4 Lowy OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265－75.

5 Fishman WH. Preparation and assay of substrates for β-glucuronidase. Methods in enzymology; vol Ⅲ. New York: Academic Press Inc. 1957: 55－7.

6 Lan KW, Huang JM, Xie ZR, Deng BF, Fan YZ, Liu ZF. Determination of superoxide dismutase-A comparison of pyrogallol-NBT colorimetry with chemiluminescence assay. Biochem Biophys 1988; 15: 138－40.

7 Wang Z, Cheng JX, Zhu GQ, Wang ZG. RIA for prostaglandins as well as their metabolites. In Xu SY, Bian RL, Chen X, editors. Experimental methology in Pharmacology 2nd ed. Beijing: People's Medical Publishing House, 1991; 402－15.

8 Zurier RB, Weissmann G, Hoffstein S, Kammerman S, Tai HH. Mechanisms of lysosomal enzyme release from human leukocytes: Ⅱ effects of cAMP and cGMP, autonomic agonists, and agents which affect microtuble function. J Clin Invest 1974; 53: 297－309

9 Deporter DA, Dunn CJ, Willoughby DA. Cyclic adenosine 3′, 5′-monophosphate and the mechanism of action of three common anti-inflammatory drugs. Br J Pharmacol 1979; 65: 163－5.

10 Naik SR. Increased cyclic AMP-phosphodiesterase activity during inflammation and its inhibition by anti-inflammatory drugs. Eur J Pharmacol 1984; 104: 253－9.

11 Amer MS, McKinney GR, Akcasu A. Effect of glycyrrhetinic acid on the cyclic nucleotide system of the rat stomach. Biochem Pharmacol 1974; 23: 3085－92.

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甘草次酸钠对大鼠化学性腹膜炎的影响

包金凤[1]，吴勇杰，杨永健[2]，李新芳[3]（兰州医学院药理学教研室，[2]生药学教研室，兰州 730000，中国）

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关键词 甘草次酸；腹膜炎；嗜中性白细胞；超氧化物歧化酶；环腺苷一磷酸；前列腺素 E；地塞米松；角叉菜胶；花生四烯酸

目的：研究甘草次酸钠(SG)的抗炎机制. **方法**：

制备大鼠腹膜炎模型，用放免法测 PGE_2 含量，Folin-酚试剂法测蛋白含量，邻苯三酚－NBT 法测 SOD 活性，竞争性蛋白结合法测 cAMP 含量. 结果：组胺致炎时，SG 10－20 $mg \cdot kg^{-1}$ im 减少渗液量；角叉菜胶致炎时，SG 20 $mg \cdot kg^{-1}$ im 使渗液量及其中 PGE_2 含量减少，SOD 活性增加；花生四烯酸致炎时，SG 20 $mg \cdot kg^{-1}$ im 抑制 Neu 中 cAMP 浓度下降，减少渗出液中 PGE_2 含量. SG 也使三种腹膜炎渗出液中 Neu 计数和蛋白含量减少. 结论：SG 通过抑制 Neu 游走及 PGE_2 合成、降低毛细血管通透性、清除氧自由基而发挥抗炎作用.

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Quercetin induced apoptosis in human leukemia HL-60 cells[1]

XIAO Dong, ZHU Shou-Peng[2], GU Zhen-Lun (*Department of Pharmacology*, [2]*Department of Radiotoxicology, Suzhou Medical College, Suzhou 215007, China*)

KEY WORDS quercetin; apoptosis; DNA damage; HL-60 cells; cultured tumor cells

AIM: To examine whether quercetin (Que) might induce apoptosis in human leukemia HL-60 cells. **METHODS:** DNA fragmentation was visualized by agarose gel electrophoresis. Inhibition of proliferation was measured with a colorimetric MTT-assay. The DNA degradation was determined using flow cytometry, and the microscopic changes were observed by an electron microscope. **RESULTS:** Que 15－120 $\mu mol \cdot L^{-1}$ elicited typical apoptosis morphological changes including condensed chromatin, nuclear fragmentation, and reduction in volume. DNA fragmentation and DNA degradation in a concentration-dependent manner in HL-60 cells. Que inhibited HL-60 cell proliferation. The values of IC_{50} and 95 % confidence limits were 43 (30－61) $\mu mol \cdot L^{-1}$ after 48-h treatment with Que. **CONCLUSION:** Que induced apoptosis in HL-60 cells.

Quercetin (Que, 3, 3′, 4′, 5, 7-pentahydroxy flavone) is a natural bioflavonoid exerting many biological effects[1-5]. Que exerts growth-inhibitory effects on human breast, ovarian, leukemia, and colon cancer cells[6-8]. It was suggested that Que might be the parent compound of a novel class of anticancer agents. To gain more information on the therapeutic potential of Que we were prompted to examine the apoptotic effect of Que in this study.

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MATERIALS AND METHODS

Reagents Que was purchased from Shanghai Second Chemical Reagent Factory (900905); 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) and RPMI 1640 were the products of Sigma.

Cell culture Human leukemic *HL-60* cell line was kindly provided by Assoc Prof CHEN Zi-Xin (Jiangsu Institute of Hematology), and was maintained in RPMI 1640 supplemented with 10 % fetal calf serum, benzylpenicillin/streptomycin 100 $kU \cdot L^{-1}$, and *L*-glutamine 2 $mmol \cdot L^{-1}$. Cells were kept in 5 % CO_2 + 95 % air at 37 ℃ and used when in exponential growth. Que was dissolved in Me_2SO and diluted in medium immediately before each experiment with the Me_2SO <0.1 %.

MTT-assay Inhibition of proliferation after incubation with Que was measured in a colorimetric MTT-assay[9]. Single-cell suspensions were prepared, cells were counted using a hemocytometer and then dispersed in replicate 96-well microtiter plates with 2×10^8 cells $\cdot L^{-1}$ (100 μL/well) in RPMI 1640 supplemented as above. Que 3.25－120 $\mu mol \cdot L^{-1}$ were added immediately after plating. The absorbance was measured on DG-3022A ELISA micro-plate Reader at 540 nm.

Flow cytometry[10] The DNA degradation which often precedes visibly detectable apoptosis was determined directly using flow cytometry. Cells were harvested and fixed. The distribution of DNA contents of the cells in the cell cycle was