引起的收缩

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目的：研究粉防己碱(Tet)对豚鼠离体气管/支气管的感觉神经C纤维兴奋的抑制作用. **方法**：记录电场刺激所致的C纤维兴奋所产生的标本收缩(phase II)张力，了解Tet的作用. **结果**：Tet 0.3—30 μmol·L^{-1}抑制phase II收缩，在气管/支气管上，Tet 1 μmol·L^{-1}的抑制率分别是：40±38 %和75±22 %；用氯苯那敏或阿托品作用后，Tet 1 μmol·L^{-1}的抑制率分别是70±16 %和64±16 %；Tet不抑制外源性P物质引起的标本收缩. **结论**：Tet 1 μmol·L^{-1}抑制豚鼠离体气道收缩的机理与其抑制感觉神经C纤维兴奋释放神经肽的作用有关.

关键词 粉防己碱；P物质；电刺激；气管；支气管；神经纤维

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Effect of schisanhenol on function and surface shape of rat neutrophils[1]

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AIM: To study the regulatory effect of Schisanhenol (Sal) on function of rat neutrophils. **RESULTS**: It was found that Sal (1, 10, and 100 μmol·L^{-1}) inhibited neutrophil activities such as chemotaxis, phagocytosis, and superoxide anion production *in vitro* in a concentration-dependent manner. Changes of surface morphology of neutrophils were observed by scanning electron microscopy, showing that the ruffles and pseudopods on neutrophil surface increased under the stimulation by chemotactic peptide *N*-formyl-Met-Leu-Phe (FMLP). When pretreated with Sal 100 μmol·L^{-1}, the ruffles and pseudopods disappeared and the surface became smooth. Sal 100 μmol·L^{-1} decreased the cytosolic calcium concentration of neutrophils and increased the intracellular cAMP level. **CONCLUSION**: These data suggested that Sal could inhibit the function of rat neutrophils through affecting the cytosolic free calcium and cAMP level besides its antioxidant activity.

KEY WORDS schisanhenol; neutrophils; chemotaxis; phagocytosis; calcium; cyclic AMP; cyclic GMP

Schisanhenol (Sal) isolated from *Schizandrae rubriflora* has many pharmacological actions, such as antioxidant activity, induction of liver microsomal cytochrome p-450, protective action against CCl_4-, alcohol-, and *D*-galactosamine-induced liver injury[1—3]. In the *D*-galactosamine-induced liver injury, there was infiltration of neutrophils and macrophages in the liver tissue[4]. The parallel occurrence of both neutrophil degradation and

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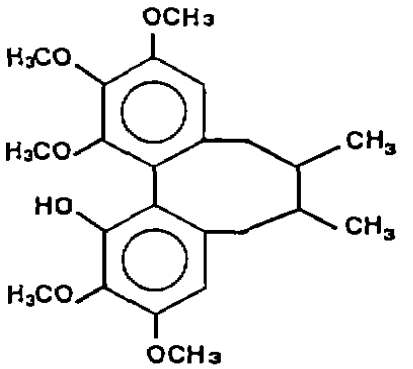
liver cell leakage in the galactosamine model were protected by turpentine pretreatment[5], suggesting that neutrophils play a pathogenic role in the liver injury. The activation and function of neutrophils have been considered to be closely related to respiratory burst and oxygen free radical generation. It is possible that the anti-oxidant activity of Sal is related to the mechanism of its protection against liver injury. Therefore, the effect of Sal on the function and regulatory mechanisms of rat neutrophils were studied *in vitro*.



Schisanhenol

MATERIALS AND METHODS

Reagents Sal was isolated from *Schizandrae rubriflora* by Professor CHEN Yan-Yong of our Institute. It is a white powder, *M*, 402, mp 126—129 ℃, $[\alpha]_D^{15}=+43.0°$ (C=0.125, ethanol), purity >99 %, and lipid-soluble. It was dissolved in Me_2SO. Fura-2/AM, glycogen, *N*-formyl-Met-Leu-Phe (FMLP) and latex beads (0.8 μm) were from Sigma. Nitroblue tetrazolium (NBT) was from Fluka. RPMI-1640 medium and fetal bovine serum (FBS) were from Gibco. The cAMP and cGMP RIA kits were from Department of Isotope, Institute of Atomic Energy, Chinese Academy of Sciences.

Preparation of rat neutrophils Male Wistar rats weighing 199±*s* 11 g (*n*=26) were used. Neutrophils were obtained from the peritoneal cavity 18 h after injected ip with 0.2 % glycogen. The neutrophils were washed with Dulbecco phosphate-buffered saline without calcium and magnesium (PBS(−)) twice and preincubated with various concentrations of Sal at 37 ℃ for 1 h. Then the neutrophils were washed with PBS(−) and resuspended in PBS containing Ca^{2+} 0.8, Mg^{2+} 0.5, and glucose 5.5 mmol·L^{-1} (PBS(+)). The viable cells amounted to >95 % examined by trypan blue dye staining method.

Chemotaxis assay Chemotaxis was performed by a modified agarose plate method[6]. The distances traveled by neutrophils toward chemotactic peptide FMLP (d_1, true chemotaxis) and medium (d_2, random migration) were measured with a microprojector. Chemotaxis of neutrophils was indicated by chemotactic index (d_1/d_2). Neutrophils were preincubated with Sal for 1 h in 37 ℃, and the chemotaxis toward 100 nmol·L^{-1} of FMLP was measured.

Phagocytosis assay[7] Latex particles were opsonized by incubating 0.2 mL of rat serum. The neutrophils (1×10^7 cells) in 0.1 mL of PBS(+) were incubated with 10^8 particles at 37 ℃ for 10 min. The average number of latex beads phagocytosed in 100 cells (phagocytic rate) and the number of cells containing at least one bead (phagocytic index) were counted under a light microscope. Neutrophils were preincubated with Sal for 1 h in 37 ℃, and phagocytosis on opsonized latex beads was measured.

Superoxide anion production Superoxide anion production was determined by NBT reduction test[8]. Formazan formation was measured using microplate method and detected at 570 nm using a Dynatech Laboratories MR700 Microplate Reader. Neutrophils were preincubated with Sal for 1 h in 37 ℃, and the superoxide anion production induced by FMLP was determined.

Scanning electron microscopy (SEM) examination The neutrophil suspension was incubated on a coverslip for 30 min and washed to remove the non-adherent cells. The adherent neutrophils were fixed in 0.25 % buffered glutaraldehyde (pH 7.4) for 2 h at room temperature (18—22 ℃), then washed and suspended in buffer, distilled and demineralized water in succession. Further preparation of samples for SEM has been described previously[9]. The specimens were examined with a Hitachi S-520 scanning electron microscope at 20 kV.

Measurement of cytosolic free calcium concentration The cytosolic free calcium concentration of neu-

trophils were measured by Fura-2 loading method [16]. The fluorescence of Fura-2 loaded neutrophils was measured using a Hitachi model F-4010 spectrofluorometer equipped with a magnetic stirrer and the cuvette incubator maintained at 37 ℃. The ratio of emitted fluorescence at 510 nm over the 340 and 380 nm excitation wavelength was used to indicate the change in cytosolic calcium[21]. Neutrophils were preincubated with Sal for 1 h in 37 ℃, and the cytosolic free calcium concentrations stimulated by FMLP were measured.

Measurement of intercellular cyclic nucleosides The concentration of intercellular cAMP and cGMP of rat neutrophils was determined by radioimmunoassay (RIA). After the addition of FMLP into the mixture of neutrophils, the incubations were terminated by placing them into an ice bath and the neutrophil membranes were destroyed by sonication. cAMP and cGMP in the supernatant of incubations were measured using the RIA kits.

RESULTS

Neutrophil chemotaxis The maximal effect of FMLP on chemotaxis was observed at 100 nmol·L^{-1}. Neutrophil chemotaxis toward FMLP 100 nmol·L^{-1} was inhibited by Sal in a concentration-dependent manner (Tab 1). The viable cells after being incubated with Sal amounted to >95 % examined by trypan blue dye staining method.

Neutrophil phagocytosis Pretreatment of the cells with Sal decreased the phagocytic rate in a concentration-dependent manner, but phagocytic index was decreased by Sal only at 100 μmol·L^{-1} (Tab 1).

Tab 1. Effect of Schisanhenol (Sal) on chemotaxis and phagocytosis of rat neutrophils. $n=3$ rats. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* each control.

Schisanhenol/ μmol·L^{-1}	Chemotactic Index	Phagocytic Rate/%	Phagocytic Index (beads/cell)
0	2.14±0.22	61.0±2.4	4.09±0.10
1	1.84±0.18^a	56.3±2.5^a	4.11±0.26^a
10	1.49±0.27^b	47.0±3.6^c	3.58±0.61^a
100	1.26±0.14^c	32.3±8.7^c	2.59±0.15^b

Superoxide anion production FMLP stimulated the neutrophils to generate superoxide anion, which was expressed as an increase of absorbance at 570 nm. The superoxide anion production of the neutrophils stimulated by different concentrations of FMLP was inhibited by Sal concentration-dependently. An inhibition was seen in Sal 10 and 100 μmol·L^{-1} (Tab 2).

Tab 2. Effect of Sal on superoxide anion production of rat neutrophils. $n=6$ rats. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* each control. $^dP<0.01$ *vs* control of FMLP(−).

Schisanhenol/ μmol·L^{-1}	Superoxide anion/Absorbance at 570 nm FMLP (−)	FMLP 10 nmol·L^{-1}	FMLP 100 nmol·L^{-1}
0	0.054±0.002	0.078±0.008^d	0.093±0.007^d
1	0.052±0.002^a	0.077±0.008^a	0.093±0.011^a
10	0.048±0.009^a	0.062±0.003^b	0.074±0.004^c
100	0.050±0.002^a	0.052±0.003^c	0.059±0.002^c

Morphology of neutrophils The ruffles and pseudopods on the surface were increased when stimulated with FMLP 100 nmol·L^{-1}, indicating a stimulation of neutrophil migration. After pretreatment with Sal 100 μmol·L^{-1}, the ruffles and pseudopods disappeared and the neutrophil surface became smooth (Fig 1, Plate 2).

Effect of Sal on cytosolic free calcium concentration of rat neutrophils In calcium (0.8 mmol·L^{-1})-containing medium, the rat neutrophils pretreated with Sal 100 μmol·L^{-1} showed a rise of cytosolic free calcium (from 28.3 to 69.1 mmol·L^{-1}) stimulated by different concentrations of FMLP. In calcium-free medium (containing edetic acid 0.5 mmol·L^{-1}), the intracellular calcium mobilization was also inhibited by Sal 100 μmol·L^{-1} (Tab 3).

Tab 3. Effect of Sal on cytosolic free calcium concentration of rat neutrophils. $n=6$ rats. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **each control.** $^fP<0.01$ *vs* **control of FMLP(−).**

Calcium in medium	Schisandrin/ μmol·L^{-1}	Cytosolic free calcium/nmol·L^{-1} FMLP (−)	FMLP 10 nmol·L^{-1}	FMLP 100 nmol·L^{-1}
+	0	45±5	81±7^f	151±17^f
+	1	43±4^a	85±12^a	145±14^a
+	10	45±7^a	74±6^a	135±11^a
+	100	43±12^a	59±13^b	103±11^b
−	0	28±2	45±5^f	69±12^f
−	1	34±8^a	51±7^a	70±11^a
−	10	22±2^a	31±8^b	49±6^c
−	100	20±3^b	30±6^b	28±6^c

Intracellular cAMP and cGMP level Decreased levels of cAMP (from 7.42±0.06 to 6.07±0.26 pmol/10^7 cells, $n=5$) and increased level of cGMP (from 0.49±0.03 to 0.72±0.18 pmol/10^7 cells, $n=5$) were shown when the neutrophils were stimulated by FMLP 100 nmol·L^{-1}. After pretreatment with Sal 100 μmol·L^{-1}, the intracellular cAMP level increased (6.90±0.43 pmol/10^7 cells, $n=5$. $P<0.05$). Sal had no effect on the cGMP level of neutrophils.

DISCUSSION

In this study, the anti-oxidant effect of Sal was also observed in rat neutrophils by using NBT reduction test. In addition, Sal not only had antioxidant activity, but also reduced the generation of reactive oxygen species (ROS) by inhibiting chemotaxis and phagocytosis of the neutrophils. Because phagocytosis of neutrophils stimulates the production of ROS including superoxide anion, hydrogen peroxide and hydroxyl radicals, which would cause tissue damage in some non-infective injury models. This result indicated that the hepato-protective effect of Sal might be partially mediated by its inhibitory effect on the neutrophil function as well as its anti-oxidant activity.

The migration and phagocytosis of neutrophils may have relation with the changes of surface morphology especially the appearance of pseudopods[12]. van Oss *et al* had demonstrated that glucose could inhibit the phagocytosis of neutrophils by causing a retraction of pseudopods and converting the cells into a spherical shape[13]. Our results showed that the ruffles and pseudopods on the surface of neutrophils were increased under the activation of FMLP, a chemotactic peptide. Sal reduced the appearance of ruffles and pseudopods on the surface of neutrophils, showing its inhibitory effect on the migration and phagocytosis of neutrophils.

The interaction of FMLP with its specific receptors on the surface of neutrophils was the initial event in neutrophil activation, was followed by a rise in cytosolic free calcium that was due in part to an increased influx of extracellular calcium and also to the mobilization of intracellular calcium[9,10]. Sal exhibited a down regulatory effect on cytosolic free calcium concentration by both the reduction of extracellular calcium influx and the intracellular calcium mobilization. The binding of FMLP to its receptor and formation of inositol phosphate were necessary while the cytosolic free calcium increased[13,14]. Therefore, the effect of Sal on FMLP binding and formation of inositol phosphate needs further study. In addition, the changes of neutrophils activity were also modulated by intracellular cyclic nucleotides. In our experiment, decreased level of cAMP and increased level of cGMP were demonstrated when neutrophils were stimulated by FMLP. Sal exerted a reducing effect on the intracellular cAMP level, although it did not affect the cGMP level. These data suggested that Sal could inhibit the function of rat neutrophils through affecting the cytosolic free calcium

and cAMP level besides its antioxidant activity.

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五味子酚对大鼠嗜中性白细胞功能与形态的作用

R965.2

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A **目的**：探讨五味子酚(Sal)对大鼠嗜中性白细胞(Neu)功能的调节作用。**结果**：Sal 1，10，100 μmol·L^{-1}剂量依赖性抑制 Neu 功能，Sal 100 μmol·L^{-1}使 Neu 表面伪足和皱褶消失，并可降低细胞内钙离子浓度、升高细胞内 cAMP 水平。**结论**：Sal 可通过影响细胞内钙离子浓度、cAMP 水平及细胞表面形态抑制 Neu 的功能。

关键词 五味子酚；嗜中性粒细胞；白细胞趋化性；吞噬作用；钙；环腺苷一磷酸；环鸟苷一磷酸