European Journal of Pharmacology 740 (2014) 421–427

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

Activity of antibacterial, antiviral, anti-inflammatory in compounds andrographolide salt

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ARTICLE INFO

Article history:
Received 2 April 2014
Received in revised form
24 June 2014
Accepted 26 June 2014
Available online 3 July 2014

Keywords:
Andrographolide sulfonic acid sodium salt
Antibacterial
Anti-inflammatory

ABSTRACT

Andrographolide sulfonic acid sodium salt (ASS) was synthesized to increase the the solubility of Andrographolide in aqueous solution. We have studied its pharmacological effect of antibiosis, anti-inflammatory and immunoregulation. Cylinder-plate method was used to study ASS's in vitro anti-bacterial activity, and its protection for mice infected by *Staphylococcus aureus* and *Shigella dysenteriae*. Various inflammation models, including the auricular edema induced by xylene in mice, CMC-Na induced air pounch model and the paw edema induced by albumen in rats were used to explore the characteristic of ASS's anti-inflammation effect. We built up the immune model by injecting chicken red cells in enter celiac of mice and study the effect of ASS on immunoregulation, taking andrographolide as the positive control. bacteriostasis in vivo and in vitro experiments show that ASS has a weak antibacterial effect and no bactericidal effect, but can reduce the mice mortality of *Staphylococcus aureus* infected. Anti-inflammatory experiments show that ASS can reduce the mouse ear swelling induced by xylene and rat paw swelling induced by egg albumin, and lessen leukocytes in air bag caused by CMCNa, and lower IL1 not ably in rat serum. Immune tests indicate that ASS can get spleen and thymus gain weight and increase rate of abdominal macrophage phagocytosis of mice. The result of bacteriostasis shows that ASS has weak in vitro antibacterial effect. ASS shows significant effects of anti-inflammation and improving immunity, thus enables the mice against bacteria better.

1. Introduction

Andrographolide is the main active ingredient in the common *Andrographis paniculata* (*Burm. f.*) *Nees* which is commonly used in chinese patent medicine against inflammatory (Lv et al., 2009; Yan et al., 2013; Yang et al., 2013). It can relieve internal fever, detoxicate, diminish inflammation, stop pain and has good curative effect to the upper respiratory tract infection and diarrhea by bacterial and virus, which makes it honored as the nature antibiotics. Andrographolide is a kind of diterpenes lactone and indissolvable in water(Thingale et al., 2014; Tsai et al., 2004), so only oral administration can be taken. We modify andrographolide into salt to improve its dissolvability, and confirm the clinical effective dose after various tests. The aims of the tests are: (1) to deduce in vitro bacteriostatic ability of ASS by bacteriostic test; (2) to assess the anti-inflammatory effect of ASS by anti-inflammatory test; (3) to investigate ASS improving immunity by immune test with mice. From all above we evaluate the clinical anti-inflammatory mechanism of ASS.

2. Materials and methods

2.1. Materials

ASS was purified by East China University of Science and Technology. Levofloxacin Hydrochloride injection (LOF) (No. 10111431) was produced by Yangzi River Pharmaceutical Co., Ltd. (Jiangsu, China). Aspirin (No. 1003182) was purchased from Shenwei PHARM Co., Ltd. Yanhuning injection (YHN) (No. 10040240) was purchased from Yaoyou Pharmaceutical Co., Ltd. (Chongqing,China). IL1 and IL2 (No. 201104) were purchased from R&D Co. Ltd. (USA). TNFα (No. 1011065) was purchased from Senxiong Biotech Co. Ltd. (Shanghai, China). Thymosin injection (No. 201002251) was purchased from Science Sun PHARM Co. Ltd.

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http://dx.doi.org/10.1016/j.ejphar.2014.06.053
0014-2999/

(Beijing, China). Andrographolide pills (No. 101004) were purchased from tianjing Tasly PHARM Co., Ltd. (Tianjin, China). Hydrocortisone (HYD) (No. 1003151) was purchased from Tianjin PHARM group Xinzheng Co., Ltd. (Henan, China). *Escherichia coli* (25922, ATCC), *Streptococcus pneumoniae* (49619, ATCC) and *Staphylococcus aureus* (29213, ATCC) are standard strains. *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Hemolytic streptococcus*, *Salmonella enteritidis*, *Shigella dysenteriae*, *Shigella sonnei* were separated by stuff of Microorganism Department in Hubei University of Chinese medicine and were identified by microorganism test and biochemistry identification.

2.2. Preparation of test samples

ASS got from East China University of Science and Technology was dissolved at relevant density before use.

2.3. Animals

Male Kunming(KM) mice weighing 1822 g and Wistar rats weighing 120 150 g were procured from The Center for Disease Prevention and Control in Hubei province, China (Reg. no. SCXK (Hubei) 20080005). They were housed at 22 ± 2 °C under a 12 h light/12 h dark cycle and with access to food and water ad libitum. The animals were acclimatized and habituated to the laboratory for at least a week before testing and were used only once throughout the experiments. The study has been carried out along the "principles of Laboratory Animal Care" (Xu et al., 2002). The experiment was performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, and approved by the Animal Experiment Ethical Committee of Hubei University of Chinese medicine.

2.4. Study about the in vitro bacteriostat test of ASS

2.4.1. Activation of the bacterium

Operated by the reference.(Hu et al., 2010; Kavali and Badami, 2000; Schillaci et al., 2005; Soriano and Greenwood, 1979)

2.4.2. Preparation of the bacterium suspension

We use the loop to 3–4 bacterium to the sterile nutrient broth fluid medium after The streak cultivation of the activated bacterium all above. After being incubated for 3 successive generations in 18–20 h at 37 °C, the bacterial colony was taken into common broth medium and made into suspension at a bacterium content of 1×10^8/ml, which was eventually diluted into the suspension at the density of 10^6/ml.

2.4.3. Preparation of the soup

Take 51.2 mg ASS in the sterile operation, dissolve it with 10 ml sterile wa ter and make it the soup that contains 5120 mg/L ASS. Dissolve YHN 0.512 g with 1000 ml sterile water. and make it the soup that contains 512 mg/L YHN. Dissolve Levofloxacin 0.125 g with 1000 ml sterile water and make it the soup that contain 125 mg/L Levofloxacin. All the soups above must be filtered to remove the bacterium.

2.4.4. Admeasurement of the ASS' minimal inhibitory concentration (MIC) double dilution method

Common broth medium was prepared by the prescription, and was split into several test tube with 1 ml, finally sterilized under the pressure 1.05 kg/cm^2 at 121 °C for reserve. Then we add the prepared ASS soup 0.2 ml into 1.8 ml broth medium, and get 1 ml broth medium diluted at the following rate: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256. Only 1 ml was retained in the last tube. 0.1 ml prepared bacterium suspension was added to each tube. And set three control groups: one group was treated with bacterium suspension but no drug as the positive control, and one group was treated with drug but no bacterium suspension as negative control. At last shake them up and put them in the incubator at 37 °C 18 h. Observe whether there is bacterium living and take the lowest drug concentration as the ASS's MIC. The result is showed in Table 1.

2.5. Antibacterial activity of ASS against Staphylococcus aureus/ Shigella dysenteiae (Intraabdominal infection) in vivo

2.5.1. Determination of infective dose

Before infection we have to determine the minimum lethal dose (MLD) of relevant strain, which can make all infected animals die within 12 days, as the infective dose. So pretests at the infective dose of test bacteria should be carried out. Dilute the fresh prepared bacteria with 15% dry yeast solution to the required concentration, such as 10^1, 10^2, 10^3, 10^4, etc. i.v. each dilution 0.5 ml and observe the animals' death. According to the pretests, MLD of *Staphylococcus aureus* is 2×10^8 and MLD of *Shigella dysenteriae* is 1.5×10^{10}.

2.5.2. Study for therapeutic test

8 groups (10 each) of mice were respectively administrated by intraperitoneal injection with high and low doses (125 and 31 mg/kg) of ASS, by gastric perfusion with high and low doses (125 and 31 mg/kg) of ASS, by i.v. with 67 mg/kg of hydrochloride levofloxacin, and by i.g. with 125 mg/kg of andrographolide Dripping Pill. Give Staphylococcus aureus (at the infective dose got in pretests) 0.5 ml to mice by intraperitoneal injection, except negative control

Table 1
Study on minimal inhibitory concentration (MIC) of ASS.

Strain	ASS (mg/L)										Control	Bacteria	LOF	YHN
	512	268	128	64	32	16	8	4	2	1				
Haemophilus influenzae	−	+	+	+	+	+	+	+	+	+	−	+	−	+
Staphylococcus aureus	−	−	−	+	+	+	+	+	+	+	−	+	−	−
Pseudomonas aeruginosa	−	−	−	−	+	+	+	+	+	+	−	+	−	−
Streptococcus pneumoniae	−	+	+	+	+	+	+	+	+	+	−	+	−	+
Hemolytic streptococcus	−	+	+	+	+	+	+	+	+	+	−	+	−	+
Salmonella enteritidis	−	+	+	+	+	+	+	+	+	+	−	+	−	+
Shigella dysenteriae	−	−	−	−	−	+	+	+	+	+	−	+	−	+
Shigella sonnei	−	+	+	+	+	+	+	+	+	+	−	+	−	+
Escherichia coli	−	−	−	−	−	+	+	+	+	+	−	+	−	+

Mark: "+" expressed bacterial growth. "−"showed no bacterial growth. Minimal inhibitory concentration was called MIC for short. Levofloxacin(LOF) and Yanhuning (YHN) were set as the positive control.