ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Suppl. 2, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

IN VITRO ANTI-INFLAMMATORY ACTIVITY OF 4-BENZYLPIPERIDINE

JAYASHREE V*, BAGYALAKSHMI S, MANJULA DEVI K, RICHARD DANIEL D

Department of Pharmacology, School of Pharmaceutical Sciences, Vels University, Pallavaram, Chennai, Tamil Nadu, India.
Email: mailtovjayashree@gmail.com

Received: 05 May 2016, Revised and Accepted: 30 June 2016

ABSTRACT

Objective: To study the *in vitro* anti-inflammatory activity of 4-benzylpiperidine.

Methods: This study was conducted to evaluate the *in vitro* anti-inflammatory activity of 4-benzylpiperidine using *in vitro* models such as inhibition of albumin denaturation and proteinase inhibitory activity.

Results: This study revealed the dose-dependent inhibition of protein denaturation and proteinase inhibitory activity by 4-benzylpiperidine.

Conclusion: In the present study, results indicate that the 4-benzylpiperidine possess anti-inflammatory properties. The drug inhibited the heat induced albumin denaturation and proteinase inhibitory activity. It shows dose-dependent significant activity when compared with a standard drug. Hence, this study gives an idea that the 4-benzylpiperidine can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation.

Keywords: Anti-inflammatory activity, 4-Benzylpiperidine, Protein denaturation, Proteinase inhibitory activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9s2.12623

INTRODUCTION

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling, and disturbed physiological functions. It is triggered by the release of chemical mediators from injured tissue and migrating cells. The inflammatory mediators are histamine, 5-HT (serotonin), bradykinin, prostaglandins E2 (e.g., PGE2), interleukins, substance P, nitrous oxide [1]. In inflammatory disorders, there is excessive activation of phagocytes, production of O_{2-}, OH radicals as well as non free radicals species (H_2O_2) [2], which can harm severely tissues either by powerful direct oxidizing action or indirect with hydrogen peroxide and –OH radical formed from O_2^- which initiates lipid peroxidation resulting in membrane destruction. Tissue damage then provokes inflammatory response by the production of mediators and chemotactic factors [3]. The reactive oxygen species are also known to activate matrix metello proteinase damage seen in various arthritic tissues [4]. Inflammation is a protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, cell migration, fluid extravasations, tissue breakdown, and repair [5]. If any form of injury to the human body can elicit a series of chemical changes in the injured area. Earlier it was believed that inflammation was contemplated as a single disease caused by disturbances of body fluids. According to the modern concept, inflammation is a healthy process resulting from some disturbance or disease [6].

Inflammation can be classified as either acute or chronic. An acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. Chronic inflammation can also lead to a host of diseases such as hay fever, periodontitis, rheumatoid arthritis (RA), atherosclerosis, and gallbladder carcinoma. It is for that reason that inflammation is normally closely regulated by the body [7]. The inflammatory disease is Alzheimer's, ankylosing spondylitis, arthritis (osteoarthritis, RA, psoriatic arthritis), asthma, atherosclerosis, Crohn's disease, colitis, dermgatitis, diverticulitis, fibromyalgia, hepatitis, irritable bowel syndrome, systemic lupus erythematous, nephritis, Parkinson's disease, and ulcerative colitis.

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system. The commonly used drugs for the treatment of inflammation are non-steroidal anti-inflammatory drugs (NSAIDs) and SAIDs. Which posses many adverse side effects especially gastric irritation leading to the formation of gastric ulcers [7].

Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid (AA) metabolism which produces PGs. PGs are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence for treating inflammatory diseases anti-inflammatory and analgesic agents are required. These points are to be utilization of plants possessing anti-inflammatory and analgesic properties. Now day's herbal drugs are routinely used for curing diseases rather than chemically derived drugs having side effects. The drugs used in inflammatory disorders may be either with analgesic and insignificant anti-inflammatory effects or with analgesic and mild to moderate anti-inflammatory activity [6].

The drugs used for inflammaton are NSAIDs such as aspirin, diclofenac, indomethacin, and ibuprofen [7]. SAIDs such as cortisone and hydrocortisone (e.g., glucocorticoids). They induce synthesis of a protein "lipocortin-1" which has the inhibitory effect on phospholipase A2 [7]. The NSAIDs are useful for the treatment of inflammation, pain, and fever. The mechanism of action is based on the inhibition of the enzyme COX, which catalyzes the metabolism AA to PGH2. PGH2 is further metabolized to prostanoids, PGs and thromboxane-A2. Various physiological effects of PGs include acute and chronic inflammatory reactions, blood pressure change, platelet aggregation, induction of labor and intensification of pain and fever [8]. They act by indirect

inhibition of the enzyme phospholipase A2 which activates synthesis of AA with subsequent formation of PGs.

4-benzylpiperidine is a drug and research chemical used in scientific studies. It acts as a monoamine releasing agent with 20-40 fold selectivity for releasing dopamine versus serotonin. It has a fast onset of action and a short duration [9]. It also functions as a monoamine oxidase inhibitor with a preference for MAO-A [10]. 4-benzylpiperidine is used to produce pimetine, an hypolipidemic agent [11]. It is a potential candidate for treatments for cocaine dependence. It has been used to treat various ailments such as joint pain, arthritis, and fever and also used to treat Parkinson's disease by increasing the dopamine synthesis. This drug is used in the treatment of Alzheimer and neuronal vascular diseases. This drug also has various other properties such as antiplatelet, anti-arrythmic, and antioxidant. 4-benzylpiperidine acts as a dopamine-selective releaser, which means that it increases the amount of dopamine available in the synapse between nerve cells. Dopamine release is accompanied by euphoria, increases in motivation and energy as well as physical feelings of pleasure. However, drugs that act as dopamine releasers tend to have withdrawal symptoms and also tend to be addictive. This is because dopamine release in the brain is known to stimulate the reward-motivation pathway in the brain which reinforces whatever behavior stimulated the pathway. Therefore, if a drug, such as 4-benzylpiperdine, stimulates the reward-motivation pathway, then it may cause the person to be addicted to the drug if it was taken consecutively for a long period of time [9].

This study was conducted to evaluate the *in vitro* anti-inflammatory activity of 4-benzylpiperidine using *in vitro* models such as inhibition of albumin denaturation, proteinase inhibitory action.

METHODS

Drugs and chemicals

4-benzylpiperidine was procured from Sigma-Aldrich, Chennai, India. All other chemicals used were of analytical grade obtained commercially.

Assessment of *in vitro* anti-inflammatory activity

Inhibition of albumin denaturation [12-14]

- Control solution (5 ml): 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (pH 6.4), and 2 ml distilled water
- Standard solution (5 ml): 0.2 ml of egg albumin, 2.8 ml of phosphate buffer, and 2 ml of various concentrations of the standard drug (aspirin) (100-1000 µg/ml)
- Test solution (5 ml): 0.2 ml of egg albumin, 2.8 ml of phosphate buffer, and 2 ml of various concentrations of test sample (100-1000 µg/ml).

All of the above solutions were adjusted to pH 6.4 using a small amount of 1 N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using ultravioletvisible spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula.

Percentage inhibition = (Abs control − Abs sample) × 100/Abs control

Proteinase inhibitory activity

The test was performed according to Sakat [14] and Oyedepo and Femurewa [15] with minor modifications. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100-1000 µg/ml). The mixture was incubated for an additional 20 minutes 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed as triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control − Abs sample) × 100/Abs control

Statistical analysis

All experiments were carried out in triplicate, and the results are expressed as a mean ± standard error of mean. The significance between the results was assessed using the Student's t-test and significance was accepted for $p<0.01$ and 0.05.

RESULTS

Albumin denaturation method

The inhibitory effect of different concentration of 4-benzylpiperidine on protein denaturation as shown in Table 1. 4-benzylpiperidine at a concentration range of 100, 200, 400, 800, 1000 µg/ml and standard (aspirin) 100, 200, 400, 800, 1000 µg/ml showed significant inhibition of denaturation of egg albumin in concentration dependent manner. In this study, 4-benzylpiperidine showed maximum inhibition, 73.80% at 1000 µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 75.52% at the concentration of 1000 µg/ml.

Proteinase inhibitory action

The inhibitory effect of different concentration of 4-benzylpiperidine on proteinase inhibitory action as shown in Table 2. 4-benzylpiperidine at a concentration range of 100, 200, 400, 800, 1000 µg/ml and standard (aspirin) 100, 200, 400, 800, 1000 µg/ml showed significant inhibition of proteinase inhibitory action in concentration dependent manner. In another study, it was reported that *Enicostemma axillare* methanol extract showed maximum inhibition of 53% at 500 µg/ml and aspirin showed 55% at 100 µg/ml [16]. In this study, 4-benzylpiperidine exhibited significant antiproteinase activity at different concentrations as shown in Table 2. It showed maximum inhibition of 57.77% at 1000 µg/ml and aspirin showed maximum inhibition of 77.77% at 1000 µg/ml.

Table 1: *In vitro* anti-inflammatory activity of 4-benzylpiperidine and aspirin by inhibition of albumin denaturation method

Treatments	Concentration (µg/ml)	Absorbance (660 nm)	Inhibition %
Control	-	0.42±0.04	-
4-benzylpiperidine	100	0.24±0.03	47.85
	200	0.20±0.03	52.38
	400	0.17±0.01	59.52
	800	0.14±0.05	66.66
	1000	0.11±0.02	73.80
Aspirin (standard)	100	0.19±0.01	54.76
	200	0.17±0.02	59.52
	400	0.14±0.05	66.19
	800	0.12±0.01	69.09
	1000	0.10±0.06	75.52

Each value represents the mean±standard deviation

Table 2: *In vitro* anti-inflammatory activity of 4-benzylpiperidine and aspirin by proteinase inhibitory activity method

Treatments	Concentration (µg/ml)	Absorbance (660 nm)	Inhibition %
Control	-	0.45±0.09	-
4-benzylpiperidine	100	0.37±0.06	17.70
	200	0.34±0.01	24.44
	400	0.29±0.02	35.50
	800	0.22±0.06	51.11
	1000	0.19±0.01	57.77
Aspirin (standard)	100	0.21±0.06	53.33
	200	0.17±0.01	62.22
	400	0.15±0.03	66.66
	800	0.12±0.02	73.33
	1000	0.10±0.03	77.77

Each value represents the mean±standard deviation

DISCUSSION

Albumin denaturation method

Denaturation of proteins is a well-documented cause of inflammation. Protein denaturation is a process in which protein lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base a concentration inorganic salt, an organic solvent or heat most biological protein lose their biological function when denatured. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs), etc., have shown dose-dependent ability to thermally-induced protein denaturation [17,18]. Thus in our study, 4-benzylpiperdine significantly inhibit the protein denaturation when compared with standard drug aspirin.

Proteinase inhibitory action

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and a significant level of protection was provided by proteinase inhibitors [19]. In this study, the 4-benzylpiperidine exhibited significant antiproteinase activity when compared with standard drug aspirin.

CONCLUSION

On the basis of above experimental results from the present study, it was concluded that the 4-benzylpiperidine possess anti-inflammatory activity. The drug inhibits the heat induced albumin denaturation and proteinase activity. It shows dose dependent significant activity when compared with the standard drug. Hence, this study gives an idea that the c4-benzylpiperidine can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation.

ACKNOWLEDGMENT

The authors are grateful to Vels University for providing generous support and lab facilities.

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