International Immunopharmacology 20 (2014) 229–237

Contents lists available at ScienceDirect

International Immunopharmacology

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

Synergistic interaction between choline and aspirin against acute inflammation induced by carrageenan and lipopolysaccharide

Zhi-Yuan Pan [a], Hai Wang [a,b,*]

[a] Cardiovascular Drug Research Center, Institute of Health and Environmental Medicine, Academy of Military Medical Sciences, Beijing 100850, PR China
[b] Thadweik Academy of Medicine, Beijing 100039, PR China

ARTICLE INFO

Article history:
Received 26 January 2014
Received in revised form 19 February 2014
Accepted 5 March 2014
Available online 19 March 2014

Keywords:
Choline
Aspirin
Alpha 7 nicotinic acetylcholine receptor
Carrageenan
Lipopolysaccharide
Synergism

ABSTRACT

The simultaneous use of drugs with different mechanisms of anti-inflammatory action is a strategy for achieving effective control of inflammation while minimizing dose-related side effects. Choline was described to potentiate the antinociceptive action of aspirin at small doses in several inflammatory pain models. However, these findings are only limited to alleviating pain, more associated data are required to confirm the effectiveness of the combined choline and aspirin therapy against inflammatory disorders. Moreover, no report is available regarding the mechanism responsible for their synergism. Here, we first investigated the anti-inflammatory activity and pharmacological mechanisms of co-administration of choline and aspirin in 2 commonly studied inflammation models, carrageenan-induced paw edema and lipopolysaccharide (LPS)-induced sepsis in mice. Isobolographic analysis revealed that combined choline and aspirin administration exhibited a strong synergistic interaction in reducing carrageenan-mediated edema, and the estimated combination index values at 50%, 75%, and 90% effective dose (ED_{50}, ED_{75}, and ED_{90}) were 0.25, 0.32, and 0.44. Drug co-administration also afforded synergistic protection against LPS-induced sepsis and mortality, since aspirin or choline alone was inadequate to improve survival. The effects of choline–aspirin co-administration were blocked by methyllycaconitine, suggesting that activation of alpha 7 nicotinic acetylcholine receptor participates in the interaction between choline and aspirin. Furthermore, co-administration of choline and aspirin was more likely to inhibit the production of pro-inflammatory mediators induced by LPS. Our results indicated that combined choline and aspirin therapy represented a significant synergistic interaction in attenuating acute inflammatory response. This preclinical relevant evidence provides a promising approach to treat inflammation-based diseases such as arthritis and sepsis.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

A number of debilitating inflammatory diseases, such as arthritis, ulcerative colitis, and sepsis, severely endanger health and life [1,2]. The limitations of currently available anti-inflammatory drugs against such diseases are well recognized, and more effective pharmacological interventions are urgently needed [2–4].

Aspirin, one of the traditional non-steroidal anti-inflammatory drugs targeting cyclooxygenase, is the most frequently prescribed drugs for relieving pain and attenuating inflammatory symptoms. However, aspirin medication in an anti-inflammatory dosage is known to increase the risk of gastrointestinal bleeding and perforation, which severely restricts its clinical application [5]. Nonetheless, it has been recently reported that aspirin not only promotes the formation of novel lipid mediators to play a potent anti-inflammatory action [6,7], but also displays some intriguing traits to modulate the immune response during autoimmune-based inflammatory diseases [8]. Moreover, clinical evidence indicated that the use of aspirin was associated with reduced medial tibial cartilage loss in patients with arthritis [9,10] and lower hospitalization rate in patients with sepsis [11], indicating that aspirin will be more widely prescribed to treat inflammation-based diseases in the future. Thus, it is of great value to study how to make aspirin retain its potent anti-inflammatory efficacy with lower doses, thereby avoiding the adverse effects usually acquired through high dose aspirin treatment.

In general, concurrent use of drugs with different mechanisms of action is a useful pharmacological approach for achieving effective control of inflammation while minimizing dose-related side effects [12,13]. Therefore, the key question is to find an anti-inflammatory candidate well suited to combine with aspirin.

The modern view holds that activation of alpha 7 nicotinic acetylcholine receptor by pharmacological interventions is a prospective

Abbreviations: CI, combination index; ED_{50}, 50% effective dose; ELISA, enzyme linked immunosorbent assay; HMGB1, high mobility group box 1; IFN-γ, interferon-gamma; IL, interleukin; LTB_4, leukotrienes B_4; LPS, lipopolysaccharide; MLA, methyllycaconitine; PGE_2, prostaglandin E_2; PGI_2, prostaglandin I_2; TNF-α, tumor necrosis factor-alpha; TXA_2, thromboxane A_2.

* Corresponding author. Tel.: +86 10 6818 6828; fax: +86 10 6693 1557.
E-mail address: wh9588@sina.com (H. Wang).

http://dx.doi.org/10.1016/j.intimp.2014.03.004
1567-5769/© 2014 Elsevier B.V. All rights reserved.

strategy for the treatment of inflammatory disorders [1,14]. A promising candidate in this area is choline, a natural specific alpha 7 nicotinic receptor agonist [15] that has been reported to have therapeutic effects on inflammatory pain and experimental sepsis in animals, due to its marked inhibition on the synthesis and release of tumor necrosis factor-alpha (TNF-α) and high mobility group box 1 (HMGB1) [16–20] Interestingly, our previous observations showed that co-administration of choline and aspirin exhibited synergistic antinociceptive effects in several experimental inflammatory pain models in mice [19,20]. Such meaningful results suggested that choline might be able to combine with aspirin as a novel anti-inflammatory regimen. However, these findings are only limited to relieving pain, and more associated evidences are required to confirm the availability and effectiveness of combined aspirin and choline therapy for the treatment of inflammation-based diseases. Moreover, to our knowledge, no reports are available regarding the underlying mechanisms for the anti-inflammatory synergy of choline and aspirin.

In the present study, we hypothesized that choline and aspirin have synergistic anti-inflammatory actions in carrageenan-mediated paw edema and LPS-induced uncontrolled systemic inflammatory response in mice. We also investigated the pharmacological mechanisms for co-administration of choline and aspirin.

2. Materials and methods

2.1. Reagents

Lipopolysaccharides (LPS) from *Escherichia coli* E (0127:B8), λ-carrageenan, choline choride, and methyllycaconitine citrate (MLA) were purchased from Sigma-Aldrich (St. Louis, MO, USA); aspirin (acetylsalicylic acid) was from Acros Organics (New Jersey, USA). All drugs were dissolved in physiological saline (0.9% sodium chloride). The commercial enzyme linked immunosorbent assay (ELISA) kits for TNF-α, interferon-gamma (IFN-γ), interleukin-1β (IL-1β), IL-6, IL-10, IL-12, high mobility group box 1 (HMGB1) were obtained from Boster Biological Technology (Wuhan, China); the commercial radioimmunoassay kits for prostaglandins E_2 (PGE_2), 6-keto-PGF1α (a stable metabolite of prostacyclins I_2, PGI_2), thromboxane B_2 (a stable metabolite of thromboxane A_2, TXA_2), and leukotrienes B_4 (LTB_4) were provided by Eastern Asia Radioimmunity Research Institute (Beijing, China).

2.2. Animals

Kunming mice were obtained from the Experimental Animal Center of the Academy of Military Medical Sciences (Beijing, China). All animal care and experimental procedures were in accordance with the Declaration of the National Institutes of Health Guide and Use of Laboratory Animals and with the approval of the local animal care and use committee. All mice were housed in animal rooms at 24 ± 1°C under a 12-h light/dark cycle with free access to water and food.

2.3. Carrageenan-induced paw edema in mice and drug treatments

Eight-week-old male mice were randomly divided into groups (n = 10 per group). The carrageenan-induced paw edema was carried out as described previously [21]. Briefly, the paw was marked in order to immerge it always at the same extent in the measurement chamber. The raw data were obtained by using a paw volume measuring instrument according to the manufacturer's instructions (YLS-7B from Huai Bei Zheng Hua Biologic Apparatus Facilities LTD. CO., Anhui, China). After the initial paw volume (basal) has been obtained, each mouse received intraplantar injection of 30 μl of λ-carrageenan 1% (w/v) in saline in left hind paw. The paw volume after carrageenan injection was measured at 1 hour intervals for 6 h, respectively. The swelling rate (the increased percentage of paw volume, %) was calculated using Eq. (1).

$$\text{The swelling } rate\ (\%) = \frac{\text{Paw volume at each time point-the initial paw volume}}{\text{The initial paw volume}} \times 100 \quad (1)$$

Choline in doses of 12.5, 25, 50, and 80 mg/kg was intraperitoneally administered 3 h prior to carrageenan injection, whereas aspirin in doses of 11.25, 22.5, 45, 100, and 200 mg/kg was intraperitoneally administered 1 h before carrageenan injection, respectively. When the drugs were used in combination, choline and aspirin were co-administered at a constant 1.11:1 dose ratio (choline + aspirin: 3.125 mg/kg + 2.8 mg/kg, 6.25 mg/kg + 5.625 mg/kg, 12.5 mg/kg + 11.25 mg/kg, 25 mg/kg + 22.5 mg/kg, 50 mg/kg + 45 mg/kg). Physiological saline served as the vehicle control. The swelling rate of the saline group was set as 100%, and the normalized inhibitory effects (subtracted 100% to the normalized swelling rate) after drug treatments at 3-h post carrageenan injection were calculated and used to evaluate the potential interaction between choline and aspirin by isobolographic analysis. In the pharmacological antagonism experiments, MLA was intraperitoneally administered 30 min before choline treatment.

2.4. LPS-induced uncontrolled systemic inflammatory response in mice and drug treatments

2.4.1. Establishment of LPS-induced mortality

Male mice weighing 20–24 g were randomly divided into groups (n = 10 per group). They were intravenously injected (i.v.) with physiological saline or different doses of LPS at 10, 20, 40, 50 mg/kg. Survival was monitored for 72 h.

2.4.2. Effects of choline on LPS-induced mortality

Mice in each group (n = 10 per group) received intraperitoneal treatment with saline or choline (10, 20, 40, 50, 60, 70, and 80 mg/kg) 1 h prior to LPS challenge (50 mg/kg, i.v.).

2.4.3. Effects of aspirin on LPS-induced mortality

Mice in each group (n = 10 per group) intraperitoneally received saline or aspirin (10, 20, 40, 50, 60, and 100 mg/kg) 30 min prior to LPS challenge (50 mg/kg, i.v.).

2.4.4. Effects of co-administration with choline and aspirin on LPS-induced mortality

Mice in each group (n = 10 per group) were intraperitoneally pretreated with aspirin alone (20 mg/kg), choline alone (40 mg/kg), and in combination (choline 40 mg/kg + aspirin 20 mg/kg) before LPS challenge (50 mg/kg, i.v.). The saline group was set as control, and survival was monitored for 72 h. In another independent experiment, in the presence of fixed dose aspirin (20 mg/kg), we co-administered choline in doses of 10, 20, 40, and 60 mg/kg, and their effects on survival were also monitored for 72 h after LPS exposure (50 mg/kg, i.v.).

2.4.5. Pharmacological antagonism of the effects of choline and choline–aspirin on LPS-induced mortality

MLA (2 mg/kg) was intraperitoneally administered at 30 min before choline (60 mg/kg) or choline–aspirin (choline 40 mg/kg + aspirin 20 mg/kg) treatments, and then mice received LPS (50 mg/kg, i.v.) injection. The effects of MLA 2 mg/kg on LPS-induced mortality were also investigated. Saline group was set as control, and survival was monitored for 72 h.

Download English Version:

https://daneshyari.com/en/article/5832849

Download Persian Version:

https://daneshyari.com/article/5832849

Daneshyari.com