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Anti-inflammatory effects of orally ingested lactoferrin and glycine in different zymosan-induced inflammation models: Evidence for synergistic activity ☆

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Abstract

There is a growing awareness of the interaction of food constituents with the immune system. The present study aims to evaluate the anti-inflammatory effects of two of these nutritional components (glycine and bovine-lactoferrin (b-LF)) using two different mouse models.

In a zymosan-induced ear–skin inflammation model both components decreased the inflammatory response locally (ear swelling and inflammatory cytokine concentration in the ears) and systemically (number of TNF-α producing spleen cells). Glycine effects (20, 50 or 100 mg/mouse/day) were concentration dependent. B-LF (0.1 or 1 mg/mouse/day) inhibited the inflammatory response although higher doses (5 and 25 mg/mouse/day) were not effective. A combination of b-LF 0.1 mg/mouse/day and glycine 20 or 50 mg/mouse/day counteracted the zymosan-induced ear swelling synergistically and enhanced the decrease in the number of TNF-α producing spleen cells of the individual components.

In a zymosan-induced acute arthritis model glycine (50 mg/mouse/day) inhibited joint swelling, inflammatory cell infiltration and cartilage proteoglycan depletion. A b-LF dose of 5 mg/mouse/day reduced the zymosan-induced joint swelling without modulating inflammatory cell infiltration and cartilage proteoglycan depletion.

The present study indicates that the anti-inflammatory effects of glycine are independent of the used models. B-LF displays a reversed concentration dependency and the activity is model dependent. A combination of glycine and lactoferrin demonstrated a synergistic anti-inflammatory effect on zymosan-induced skin inflammation and an enhanced decrease in the number of TNF-α producing spleen cells compared to the effect of the single components. Therefore, this nutritional concept might be a new option for the treatment of chronic inflammatory diseases.

Keywords: Glycine; Lactoferrin; Inflammation; TNF-α; Zymosan

1. Introduction

There is a growing awareness of the interaction of food constituents with the immune system [1]. The present study aims to evaluate the anti-inflammatory effects

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of two of these nutritional components, i.e. the amino acid glycine and the iron-binding protein lactoferrin [2,3].

The simple nonessential amino acid glycine is an inhibitory neurotransmitter in the central nervous system that acts via a glycine-gated chloride channel (GlyR) [4]. Apart from the nervous tissue, glycine has been presumed to be biological neutral for a long time. In the past years however, evidence accumulated indicating that glycine comprises anti-inflammatory and immunomodulatory activities, at least in part, via activation of the GlyR. The existence of a GlyR has been demonstrated on a wide variety of cells including different cell types involved in immune responses, such as macrophages, monocytes, neutrophils and T lymphocytes [3,5,6]. Activation of the GlyR blunts calcium ion influxes in these cells via a chloride induced hyperpolarization of the membrane [7]. Counteraction of the calcium ion influxes, which could be induced by many different stimuli, sabotages various downstream events including the production of cytokines and other inflammatory mediators. It has been demonstrated that glycine largely prevents the endotoxin-induced TNF-α production by Kupffer cells and alveolar macrophages [5,8]. In addition, glycine reduces the lipopolysaccharide (LPS)-induced TNF-α and IL-1β expression while it stimulates the IL-10 expression in monocytes [9].

Lactoferrin (LF) is a widespread iron-binding protein and member of the transferrin family. It is produced by exocrine glands and might be released by degranulating neutrophils at the side of infection and inflammation. Iron binding is, without any doubt, a key property of LF that accounts for many of its biological roles in host defense such as bacteriostasis and protection against oxygen radicals catalyzed by free iron. Other direct effects of LF on host defense include the binding to bacteria, fungi and parasites. In addition, it has been demonstrated that LF plays a role in modulating immune responses by its ability to interact with target molecules and cells. Anti-inflammatory effects of LF have been shown by the inhibition of pro-inflammatory cytokine production [10–12] and the up regulation of anti-inflammatory cytokines [13]. On the other hand, LF may enhance directly or indirectly the immune response (*in vitro* and *in vivo*) by regulating the proliferation, differentiation and activation of both T and B cells [14,15].

Both glycine as well as LF can modulate innate immune reactions which might offer a new opportunity for the treatment of chronic inflammatory diseases. The present study was designed to evaluate the anti-inflammatory properties in more detail. The objective was bipartite. 1) Literature describes an immune stimulatory as well as an immune inhibitory potential for LF. Therefore, the first goal of the study was to evaluate the immunomodulatory activities of orally ingested glycine and b-LF in different models of inflammation. The models used are the zymosan-induced ear–skin inflammation model and the zymosan-induced acute arthritis model. 2) The interaction between different pharmaceutical immunomodulatory medications has been studied extensively. However, the possibility that different food components might boost or counteract their individual effect on the immune system is rarely examined. The second part of the study addresses the question whether the immunomodulatory effects of glycine and LF interact with each other. For this latter goal the ear–skin inflammation model was used. The results indicated that both glycine as well as b-LF were able to significantly inhibit inflammatory responses. Furthermore, the combination of the two nutritional components showed a synergistic anti-inflammatory effect which opens new avenues for the treatment of chronic inflammatory diseases.

2. Materials and methods

All experimental procedures using laboratory animals were approved by an independent animal experiments committee (DEC Consult, Bilthoven, The Netherlands). The b-LF, with an iron saturation of 16%, was obtained from DMV (DMV International, Veghel, The Netherlands).

2.1. Induction of ear–skin inflammation

Male Balb/C mice (Charles River, Maastricht, The Netherlands), aged 14 weeks at the start of the experiment were acclimatized to the animal housing starting one-week prior to the start of the experiment. All animals had free access to a standard rodent diet and tap water. Different amounts of glycine (0, 20, 50 or 100 mg/day/mouse), b-LF (0, 0.1, 1, 5 or 25 mg/day/mouse), or a combination of glycine and b-LF (0.1 mg b-LF combined with 20 or 50 mg glycine/mouse/day) were administered daily for three constitutive days (day 1 till 3) by gavage. Tap water (vehicle) was applied in the same volume, 200 μl, to control mice. Inflammation was induced at day two, 3 h after administration of the supplements, by injecting 25 μl zymosan (0.5% suspended in PBS) or PBS (sham), intradermally in both ears [16,17]. Ear thickness was measured prior to and 3, 6 and 24 h after zymosan injection using an engineers micrometer (Mitutoyo Dinamic, Veenendaal, The Netherlands). After the last ear thickness measurement, mice were bled under terminal anesthesia (isoflurane/N_2O/O_2) and sacrificed. Both ears and the spleen were collected for histology, inflammatory cytokine detection and spleen cell isolation respectively.

2.2. Histological processing and analysis of ears

Ears were dissected, fixed in 4% formaldehyde in PBS for 16 h at 4 °C, dehydrated and embedded in paraffin. Semi-serial