Cytokine 90 (2017) 31–37

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Buthionine sulfoximine, a glutathione depletor, attenuates endotoxic fever and reduces IL-1β and IL-6 level in rats

Sylwia Wrotek [a,*], Krzysztof Domagalski [b], Tomasz Jędrzejewski [a], Eliza Dec [a], Wiesław Kozak [a]

[a] Department of Immunology, Nicolaus Copernicus University, Ul. Lwowska 1, 87-100 Torun, Poland
[b] Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Wilenska 4, 87-100 Torun, Poland

ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form 12 October 2016
Accepted 12 October 2016

Keywords:
BSO
Fever
Inflammation
IL-1β
IL-6

ABSTRACT

Purpose: The aim of our study was to investigate the effect of buthionine sulfoximine (BSO) - a glutathione depletor - on a course of endotoxic fever and IL-1β and IL-6 production.
Material and methods: Male Wistar rats were subjected to intraperitoneal injection of lipopolysaccharide (LPS) from *E. coli* (50 μg/kg, *ip*) to provoke fever. The level of spleen glutathione, plasma interleukin (IL)-1β, IL-6, and deep body temperature (Tb) were measured.
Results: The LPS administration provoked fever (the average Tb was 38.14 ± 0.05 °C in NaCl/LPS-treated rats vs 37.10 ± 0.03 °C in control, not-treated rats; $p < 0.001$). We observed that LPS injection induced a decrease in spleen glutathione level (7.67 ± 0.92 nM/g vs 13.27 ± 0.47 nM/g in not-treated rats; $p < 0.001$). Furthermore, the injection of LPS provoked an elevation of plasma IL-1β and IL-6 concentration (from values below the lowest detectable standard in not-treated animals to 199.99 ± 34.89 pg/mL and 7500 ± 542.21 pg/mL, respectively; $p < 0.001$). Pretreatment with BSO enhanced glutathione decrease in LPS-treated rats (5.05 ± 0.49 nM/g), and significantly affected fever (maximal Tb was 37.81 ± 0.07°C in BSO/LPS-treated rats vs 38.76 ± 0.11 °C in NaCl/LPS-treated rats). BSO 4 h after LPS injection decreased IL-1β and IL-6 gene expression (about 1.5 fold, and 2 fold, respectively). In a consequence we observed a decrease in plasma IL-6 concentration (4 h after LPS injection plasma IL-6 was 4167.17 ± 956.54 pg/mL in BSO/LPS-treated rats vs 7500 ± 542.21 pg/mL in NaCl/LPS-treated rats; $p < 0.001$), and later IL-1β (7 h after LPS injection the IL-1β concentration was not detected).
Conclusion: Based on these data, we conclude that BSO, in addition to well-known application as an inhibitor of glutathione synthesis, is an antipyretic agent which reduces both IL-1β and IL-6 concentration.

1. Introduction

Fever is a common response to infection and inflammation. The current concept of fever physiology suggests that the febrile response is orchestrated by a number of host cell-derived molecules. Among these factors are cytokines, such as interleukin (IL)-1β, IL-6, tumour necrosis factor-α (TNF-α), and interferon (IFN)-γ, collectively termed endogenous pyrogens [1,2].

Generally, infectious fever is considered to be beneficial for healing process. Fever enhances numerous functions of the immune system: it intensifies dendritic cell and T cell activity, or neutrophil migration. As a result, more immune cells are ripened and ready for the battle [3–5]. These findings explain why for many centuries fever has been regarded as a powerful mechanism of fighting with all kind of diseases, including cancer [6].

Glutathione is a tripeptide with multiple functions in living organisms [7]. At the cellular level, glutathione acts as a water-soluble antioxidant and is directly involved in specific detoxification reactions that protect the body from various dangerous substances [8]. It is essential for some functions of the immune system, including T lymphocyte proliferation [9,10], phagocytic activity of polymorphonuclear neutrophils [11], and dendritic cell action [12]. Accumulating evidence suggests that glutathione affects secretion of various cytokines, including those associated with fever and inflammation [13]. In accordance, in our previous paper we reported that rats treated with phorone, a compound that decreases glutathione via enzymatic conjugation with glutathione-transferases, react with attenuated fever to pyrogenic dose of endotoxin [14].

In addition to above mentioned properties, it has been observed that intentional glutathione reduction sensitizes cancer cells to chemotherapy and irradiation [15–17]. For this reason, glutathione

* Corresponding author.
E-mail addresses: wrotek@umk.pl (S. Wrotek), krydom@umk.pl (K. Domagalski), tomaszj@umk.pl (T. Jędrzejewski), ejc.dec@gamil.com (E. Dec), wkozak@umk.pl (W. Kozak).

http://dx.doi.org/10.1016/j.cyto.2016.10.005
1043-4666/

has drew the attention of pharmacologists, as a possible target for medical intervention against cancer progression and chemoresistance. In this context, buthionine sulfoximine (BSO) is considered to be the most popular glutathione-depleting agent studied in both preclinical and early clinical trials [18,19]. BSO is a specific, irreversible inhibitor of γ-glutamyl-cysteine (γ-GCS) synthetase, a key enzyme in glutathione synthesis. Currently, BSO is the most frequently used drug in oncology in order to decrease glutathione concentration [20–25]. It had been found that BSO may sensitize biliary tract cancer cells to standard first-line chemotherapeutic agents (cisplatin and gemcitabine), and may sensitize cells to irradiation [26,27]. In phase I study it was revealed that continuous infusion of BSO was relatively non-toxic and resulted in the depletion of tumour glutathione in patients with advanced cancers such as: ovarian, lung, breast, colon, and melanoma [19,28,29]. It has been demonstrated that a combination of azathioprine with BSO was useful for localized treatment of human hepatocellular carcinoma [30]. Furthermore, BSO combined with melphalan [19,29] is undergoing a clinical evaluation in children with neuroblastoma [25]. Moreover, a clinical trial in patients with persistent or recurrent stage III malignant melanoma has been carried out (NCT00661336).

In our previous studies [14] we have found that rats treated with phorone, which is a glutathione depletor, responded with diminished fever to pyrogenic dose of endotoxin. BSO is also an agent which decreases glutathione level, however in contrast to phorone, this compound has been investigated in pre-clinical trials to sensitize cancer cells to chemotherapy and irradiation. In the present studies we hypothesized that BSO might possess similar antipyretic properties. Indeed, we have found that BSO treatment inhibits significantly feverish response and reduces IL-1β and IL-6 level in the LPS-injected rats.

2. Materials and methods

A total of 80 specific, pathogen-free male Wistar rats weighing 200–250 g were used throughout the experimentation. The exact number of animals in each experiment is provided in figures description. Rats were obtained from Experimental and Clinical Medical Institute Warsaw (Poland) and were housed in individual plastic cages and placed in a temperature/light-controlled chamber set at 22 ± 1 °C, 12:12 h light:dark cycle, with light on at 7:00. Humidity was maintained at 50% during the experiments. Rodent laboratory food and drinking water were provided *ad libitum*. One week after shipping, the rats were implanted with biotelemetry devices to monitor deep body temperature. Rats showing a regular and stable 24-h body mass gaining were taken to the experiments. All experimental procedures were approved by the Local Bioethical Committee for Animal Care (permission No 8/2011 and 7/2013).

2.1. Body temperature measurement

Deep body temperature (Tb) of the rats was measured using battery-operated telemetry transmitters (model TA-F40, Data Sciences International, USA) implanted intra-abdominally under sterile conditions. Before the implantation, the rats were anaesthetized with a mixture of ketamine (87 mg/kg) (Biowet, Poland) and xylazine (13 mg/kg) (ScanVet, Poland) injected intramuscularly. Then, following shaving and sterilization of a small abdomen surgical area, an incision was made in the skin and muscles of the abdomen, and a miniature temperature-sensitive telemetry device was introduced into the peritoneal cavity. The muscle level of the abdomen and the skin were separately sutured closed. All experiments started 10 days after recovery from the surgery.

2.2. Induction of LPS fever in the telemetry implanted rats

Lipopolysaccharide (LPS) extracted from *Escherichia coli* 0111: B4 (Sigma-Aldrich, USA, CAT NO L2630) was dissolved in sterile 0.9% sodium chloride (saline). Before injection, the stock solution of LPS (2.5 mg/mL) was warmed to 37 °C, diluted in a warm sterile saline to the desired concentration, and injected intraperitoneally (*ip*) at a dose of 50 μg/kg, as described previously [14,31]. Control rats were injected *ip* with an equivalent volume of pyrogen-free saline.

2.3. BSO preparation and administration

D,L Buthionine-[*S*,R]-sulfoximine (BSO), a well-known glutathione depleting agent [32,33] was purchased from Toronto Research Chemicals, Inc. (Canada). It was dissolved in saline and injected twice *ip* at a dose of 4 mM/kg 24 h (h) and then 2 h prior to LPS challenge. Not-treated rats (NT) were used as a control. Before injection, both BSO solution and saline were warmed to 37 °C. The effect of BSO on the normal circadian rhythm in Tb of rats was also evaluated and compared to the not-treated free-running rats.

To perform all injections described in this section, as well as in the previous one, the rats were briefly restrained and then placed in their home cages immediately afterwards.

2.4. Spleen glutathione assay

Spleen samples were dissected out from anesthetized Wistar rats 4 h after LPS injection. Next, they were rinsed twice with phosphate buffered saline (PBS, pH 7.4) and homogenized on ice in 1 mL of 5% trichloroacetic acid (PoCh, Poland). Total glutathione contents were determined using Glutathione Assay Kit (Sigma-Aldrich, USA) according to the manufacturer's protocol. Samples were assayed in triplicate. Colorimetric changes in the assays were detected using plate reader (Bio-Tek Instruments, USA).

2.5. Plasma IL-1β and IL-6 assay

Blood samples for cytokine analysis were collected from rats described in the previous section by cardiac puncture 4 h and 7 h after LPS injection. Blood was drawn into EDTA tubes and plasma was separated by centrifugation (20 min at 1000*g*), and stored at −20 °C until assay.

Plasma IL-1β and IL-6 levels were determined using a plate reader (Bio-Tek Instruments, USA) and highly sensitive colorimetric assay kits from R&D Systems Inc. (USA) according to the manufacturer's instruction. Samples were assayed in duplicate.

2.6. Rat peripheral blood mononuclear cells (PBMCs) isolation

Blood was sterilely collected from anesthetized rats by cardiac puncture into the solution of Na_2EDTA (Sigma Chemicals, USA). Peripheral blood mononuclear cells (PBMCs) isolation was performed according to density gradient centrifugation method, as we described previously [34]. Finally, PBMCs were stored at −80 °C in RLT buffer (Qiagen, Japan) until isolation.

2.7. RNA isolation

Total RNA from the PBMCs lysates was extracted using Blood/Cell RNA Mini Kit (Syngen, USA) according to the manufacturer's instructions. Residual genomic DNA was removed by DNase I enzyme treatment during isolation. The amount and quality of RNA were determined by spectrophotometry using a NanoDrop ND-1000 (NanoDrop Technologies, USA) and by electrophoresis