

Research Article



The Anti-inflammatory Action of Glutamine in Comparison with Diclofenac and Dexamethasone in Rats

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ABSTRACT

The aim of this study is to evaluate the possible anti-inflammatory action of glutamine in comparison with two known anti-inflammatory drugs. The study designed to investigate the anti-inflammatory action of glutamine (on experimental rat's models) by inducing acute and sub chronic inflammation through measuring some of inflammatory parameters (Serum levels of tumour necrosis factor alpha (TNF- α), interleukin 1 Beta (IL-1 β), IL-6, IL-10 and CRP). Results of this study showed significant anti-inflammatory effect of glutamine comparable to that of diclofenac and dexamethasone.

Keywords: Glutamine, anti-inflammatory, diclofenac, dexamethasone.

INTRODUCTION

Inflammation is a protective strategy for the host, evolved in higher organisms in response to detrimental insults such as microbial infection, tissue injury and other noxious conditions. It is an essential immune response by the host that enables the removal of harmful stimuli as well as the healing of damaged tissue; one of the main aims of inflammation is to reinstate cellular homeostasis in response to any damaging condition.¹ Glutamine (Gln) is the most abundant free amino acid in human muscle and plasma, the human normal plasma glutamine concentration is fluctuating around 550-750 $\mu\text{mol/L}$.² It is usually found in high concentration in skeletal muscle that represent up to 60% of total body glutamine, from where it is released into blood stream and transported to variety body tissue that need it, and also found in the lungs, liver, brain, and stomach tissue in less concentration.³ Gln is one of the conditionally essential AA that must be supplemented during situations such as critical illness meaning that in hyper catabolic or stress conditions such as trauma, burns, surgery, the body suffers depletion in the circulating levels of Glutamine.⁴

Humans obtain glutamine through catabolism of proteins in foods they eat. Such as, beef, chicken, fish, dairy products, eggs, vegetables like beans, beets, cabbage, spinach, carrots, parsley, vegetable juices and also in wheat, papaya, celery, and kale.⁵ Gln has taken at a dose of up to 10 g/day. While, in situations when this basic supply is insufficient, such as in critical illness, and where tissue is being built or repaired, like growth of infants, or healing from traumatic wounds or severe illness, Gln should be part of any clinical nutrition regimen, Gln should be given to provide sufficient level, up to a maximum total dose of 30 g/day.⁶

The physiological role of glutamine

It is serving as a source of fuel for the cells such as enterocytes, renal epithelial cells, hepatocytes, neurons, immune cells, β -cells of pancreas.³ Glutamine is essential for the growth, survival and physiological health of actively dividing cells such as enterocytes, fibroblasts and lymphocytes.⁷ Glutamine metabolism plays multiple roles in: A-nitrogen balance, B-regulation of glucose metabolism, C- Acid base homeostasis. It is quantitatively the most important donor of ammonia in kidney and liver, it plays a role in maintaining the acid-base balance of body fluids, such as in alkalosis with elevated ammonia level is associated with increased production of glutamine, while during acidosis glutamine is broken down to glutamate and ammonia serving to elevate plasma pH (10–12). It is a major transporter of nitrogen from the sites of synthesis (skeletal muscle, liver and lung) to the sites of utilization (kidney, intestine, neuron and immune cells) and serves as a nontoxic ammonia shuttle in the body.⁸ Glutamine is utilized at a high rate in rapidly dividing immune cells and promotes many functional activities of immune cells such as T-cell proliferation, B-cell differentiation, phagocytosis, antigen presentation, cytokine production and neutrophil superoxide production.⁹ It is also an important osmolyte for cell volume control and shown to increase hepatocyte cell volume by eliciting anabolic process.¹⁰ It is the precursor for synthesis of some peptides, amino sugars, purines, pyrimidines, nucleic acids and other nitrogenous compounds in the cells. Glutamine could protect the liver function after chemotherapy through increasing the glutathione biosynthesis and preserving the glutathione stores of hepatic tissue.¹¹ Several metabolic products derived from glutamine also include neurotransmitter, proline and hexosamines.¹² In the Irritable Bowel



Syndrome, the enterocytes of the small intestines are the body's largest consumers of glutamine, accounting for about 40-50% of glutamine consumption.¹³ and several studies have shown that glutamine, when used as an oral rinse, can help to reduce cancer chemotherapy-induced mouth sores.¹⁴ Thus, Glutamine had to be present in 10- to 100-fold excess of any other amino acid in culture and could not be replaced by glutamic acid or glucose.¹⁵

The aim of this study

To evaluate the possible anti-inflammatory action of glutamine in comparison with two known anti-inflammatory drugs.

MATERIALS AND METHODS

Forty-eight male waster rats weighing (180-250) gram were brought from the Animal House of the College of Pharmacy /Baghdad University. The animals were maintained on normal conditions of temperature, humidity and light/dark cycle. They were fed standard rodent pellet diet and have free access to water.

Study of the anti-inflammatory activity of Glutamine in experimental animal models of acute inflammation

The animals used in this study divided into four groups each group with 6 rats as follows: Group I: Six male rats received intraperitoneally, single dose of Normal saline (2ml/kg), the group served as negative control. Group II: Six male rats received single dose of the standard drug dexamethasone in a dose of 5mg /kg, the group served as positive control (steroidal anti-inflammatory drug). Group III: six male rats received single dose of the standard drug diclofenac sodium in a dose of 10mg /kg, the group served as positive control (non-steroidal anti-inflammatory drug). Group IV: six male rats received 1000mg/kg, dose of Glutamine. All drugs were administered intraperitoneally; and the inflammation was induced by injecting 0.1ml of fresh egg albumin into the sub planter surface of the right hind paw, thirty minutes post treatment.¹⁶

Study of the anti-inflammatory activity of Glutamine in experimental animal models of sub-chronic inflammation

Twenty-four male waster rats weighing (180-250) gram were used in this study. The animals used in this study classified into four groups each group with 6 rats as follows: Group I: Six male rats received single intraperitoneally dose of Normal Saline (2 ml/kg), the group served as negative control. Group II: Six male rats received single dose of the standard drug dexamethasone in a dose of 5mg /kg, the group served as positive control (steroidal anti-inflammatory drug). Group III: six male rats received single dose of the standard drug diclofenac sodium in a dose of 10mg /kg, the group served as positive control (non-steroidal anti-inflammatory drug). Group IV: six male rats received 1000mg/kg dose of Glutamine. All drugs were administered intraperitoneally 30 minutes before injection of 0.1 ml of 2% formalin to

induce sub-chronic inflammation. At the end of experiment of both models of inflammation (acute and sub-chronic inflammation) which was after four hours in acute inflammation study and after 7 days in sub-chronic inflammation study, blood samples were collected by intracardiac puncturing under light diethyl ether anesthesia and collected in gel test tubes, allowed for clotting, then centrifuged for 20 minutes at 3500 (r.p.m). Serum was separated and stored into Eppendorf tubes at – 20 °C to be used for the determination of the levels of the inflammatory mediator's interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 1 beta (IL-1 β), Tumor Necrosis Factor Alpha (TNF-α), and C-Reactive Protein (CRP) by ELISA technique. All the results were expressed as mean± Std. The significance of difference between the control and treated groups were determined using unpaired student's *t*-test. *P*-values<0.05 were considered significant.

RESULTS AND DISCUSSION

The results of this study showed a significant elevation (*P*<0.05) of the cytokines level in the group I (the negative control group) tables 1 and 2. This result is in agreement with that mentioned in other studies.¹⁷⁻¹⁹ Meanwhile the results of group II (dexamethasone treatment 5mg/kg) and group III (diclofenac treatment 10mg/kg) showed significant reduction (*P*<0.05) in the cytokines levels in comparison with control group, this result was also noticed by other studies.^{20,21}

In this study, the anti-inflammatory effect of Glutamine 1000mg/kg (IP) was assessed and compared with those of diclofenac 10mg/kg and dexamethasone 5mg/kg (IP).

The glutamine supplied group showed a significant (*P*<0.05) reduction in CRP serum levels in sub-chronic and acute phase, this comes in agreement with Paul (2003).²², and a significant reduction (*P*<0.05) in TNF-α serum levels as mentioned by Zhihui Lin *et al* (2013).²³, Glutamine was shown to reduce the production of the pro-inflammatory cytokines IL-6, and enhance the production of the anti-inflammatory cytokine, IL-10 as in the study of M Coëffier *et al* (2003).²⁴ results also showed a significant reduction (*P*<0.05) in IL-1 serum levels which in agreement with results of Xiang Zhong *et al* study (2012).²⁵, also we noticed a significant reduction (*P*<0.05) in TNF-α serum levels that as mentioned by Micah Zuhl *et al* study (2014) that show acute and chronic oral glutamine supplementation up regulated HSP70 expression and also decreased TNF-α release by inhibits the NF-κB pro-inflammatory pathway in human peripheral blood mononuclear cells.²⁶ all these results are in agreement with other studies that states the anti-inflammatory effect of glutamine; glutamine supplementation reduces the rate of infection, inflammation, length of hospital stay, and mortality, and improves gut barrier function and immune function.²⁷ there is evidence that glutamine-starving cells show an increased susceptibility to cell stress and apoptosis, as well as a reduced responsiveness to pro-inflammatory



stimuli. Several studies suggest that glutamine supplementation has beneficial effects on the clinical outcome of critically ill patients. These results may be explained by the glutamine's influences on the inflammatory response, oxidative stress, apoptosis

modulation, and the integrity of gut barrier. High-dose parenteral (>0.50 g/kg/day) glutamine appears to present the greatest potential for benefit in critically ill patients.²⁸ The mechanism of these

Table 1: The anti-inflammatory effect of dexamethasone, diclofenac sodium, and glutamine on serum inflammatory mediators on groups of acute inflammation

Treatment groups	Serum levels of TNF- α	Serum level of IL-1 β	Serum level of IL-10	Serum level of IL-6	Serum level of CRP
Group I Normal Saline	117.76 \pm 28.31	381.66 \pm 208.59	22.31 \pm 3.83	141.28 \pm 46.41	36.80 \pm 11.92
Group II Dexamethasone	63.18 \pm 34.25 *	178.66 \pm 65.12 *	34.75 \pm 8.82*	86.65 \pm 21.62*	14.86 \pm 4.68*
Group III Diclofenac sodium	52.71 \pm 23.43 *	180.16 \pm 38.26 *	40.28 \pm 10.19*	89.41 \pm 14.35*	16.50 \pm 3.29*
Group IV Glutamine	46.31 \pm 35.37 *	196.33 \pm 77.43 *	35.86 \pm 10.07*	79.01 \pm 4.76*	18.55 \pm 8.47*

Mean \pm Std. Deviation; *Represent significant (*P* value less than 0.05) with control group

Table 2: The anti-inflammatory effect of dexamethasone, diclofenac sodium, and glutamine on serum inflammatory mediators on groups of sub-chronic inflammation

Treatment groups	Serum levels of TNF- α	Serum level of IL-1 β	Serum level of IL-10	Serum level of IL-6	Serum level of CRP
Group I Normal Saline	110.11 \pm 20.93	688.33 \pm 318.38	27.71 \pm 3.81	123.56 \pm 18.51	36.93 \pm 14.20
Group II Dexamethasone	42.55 \pm 13.24 *	296.16 \pm 144.28 *	38.95 \pm 8.59*	52.00 \pm 27.26*	24.96 \pm 6.00
Group III Diclofenac sodium	49.60 \pm 21.06 *	273.0 \pm 117.22 *	40.11 \pm 7.65*	78.25 \pm 19.13*	21.66 \pm 4.83*
Group IV Glutamine	33.40 \pm 15.18 *	261.0 \pm 89.90 *	36.20 \pm 9.65*	78.86 \pm 16.93*	19.61 \pm 4.14*

Mean \pm Std. Deviation; *Represent significant (*P* value less than 0.05) with control group

protective effects includes attenuated pro inflammatory cytokine expression, improved gut barrier function, enhanced ability to mount a stress response, improved immune cell function, and decreased mortality.²² Glutamine mechanism is through activation of the heat shock response, which serves dual roles: stabilization of the intestinal epithelial cell tight junctions preventing toxin leakage and suppression of the NF- κ B inflammatory pathway in peripheral leukocytes.²⁶

CONCLUSION

The present study was conclude the administration of glutamine as supplement produce remarkable anti-inflammatory activity in animal models of acute and sub-chronic inflammation in comparison with steroidal (dexamethasone) and non-steroidal (diclofenac sodium) anti-inflammatory drugs with less side effects, lower cost and compliance as daily supplement at a dose of approximately (0.5gm/kg/day) without any side effects,

which will use to improve many inflammatory and auto immune diseases such as rheumatoid arthritis.

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