Eur J Nutr 39: 103–105 (2000)
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ORIGINAL CONTRIBUTION

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Lymphocyte proliferation is possible with low concentrations of glycyl-glutamine

Received: 11 November 1999
Accepted: 14 April 2000

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Summary The positive effect of glutamine on lymphocyte proliferation has previously been described. Its dipeptide glycyl-glutamine (GlyGln) is more stable than pure glutamine in aqueous solutions. The aim of our study was to investigate the relationship between lymphocyte proliferation and varying concentrations of glycyl-glutamine in vitro.

Isolated human lymphocytes were stimulated with the mitogens phytohaemagglutinin (PHA), Concanavalin A (ConA), pokeweed mitogen (PWM), and *Staphylococcus aureus* (SAC). Glycyl-glutamine was added to yield final concentrations of 0–2 mmol/l.

Overall, minimal concentrations of 0.01 mmol/l glycyl-glutamine were sufficient to enhance lymphocyte proliferation over baseline (glutamine-free) levels. No difference was found between concentrations in the "physiological" range of 0.4 mmol/l and very low concentrations (0.04–0.1 mmol/l) with SAC, ConA and PWM. Increasing the concentration beyond 0.4 mmol/l (up to 2.0 mmol/l) offered further gain with PHA-stimulation only.

Lymphocyte proliferation under in vitro polyclonal stimulation is maintained even at very low concentrations of glycyl-glutamine. Raising the concentration above the equivalent of physiological levels does not seem to provide further benefit.

Key words Lymphocyte proliferation – glycyl-glutamine

Introduction

Glutamine is the most abundant free amino-acid in human plasma with physiological concentrations of 0.54–0.66 mmol/l (1). Several studies indicate its crucial role in host defence mechanisms. It augments lymphocyte proliferation in vivo and in vitro (2–5) as well as gut integrity (6–8). However, the parenteral administration of glutamine is limited by its instability in aqueous solutions. It is readily hydrolysed into toxic metabolites, such as ammonia and pyroglutamic acid. Glutamine containing dipeptides, such as glycyl-glutamine or alanyl-glutamine, are preferred for intravenous use as they are stable in aqueous solutions and rapidly hydrolysed following infusion (9, 10). The minimal concentration required to avoid significant loss of lymphocyte function has not been established. The aim of our study was to investigate the relationship between mitogen induced lymphocyte proliferation and glycyl-glutamine concentration in vitro.

Methods

Cell preparation: Informed consent was obtained from 12 healthy donors (8 female, 4 male) with a mean age of 27.8 years (range 22–36). Lymphocytes were isolated from heparinized whole blood (8 female, 4 male) by gradient centrifugation (20 min, 1300 g, 20°C) on Ficoll-Isopaque (Pharmacia, Freiburg, Germany). Lymphocytes were suspended in glutamine-free RPMI 1640 medium at a con-

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centration of 2 × 106/ml. Cell viability was assessed by trypan blue exclusion and only preparations with a viability of over 80 % prior to incubation were used.

The mitogens phytohaemagglutinin (PHA) 1µg/ml, Concanavalin A (ConA) 1µg/ml, pokeweed mitogen (PWM) 10µg/ml, and *Staphylococcus aureus* (SAC) 1:1000 were pipetted in 96-well tissue plates (Limbro, Meckenheim, Germany). Final concentrations were achieved by adding RPMI medium (Biochrom, Berlin, Germany) enriched with Streptomycin-Penicillin (Flow, Meckenheim, Germany) (5000 U/ml). Glycyl-glutamine (Pfrimmer, Erlangen, Germany) was added to each well to reach final concentrations between 0–2 mmol/l. No fetal calf serum (FCS) was added as it contains about 0.6 mmol/l glutamine.

After 72 hours of incubation, 20µl tritiated thymidine (^{3}H-thymidine; 0.5µCi), diluted in RPMI, was added to the cell cultures and incubated for another 3 hours. The contents of the dishes were harvested after lysis of cells. Cellular DNA was filtered and transferred into test tubes containing scintillation liquid (Beckmann, Munich, Germany). ^{3}H-thymidine was measured by β-counter (Beckmann, Munich, Germany), results were given as counts per minute. All assays were performed in triplicate.

Mann-Whitney-U test was used for statistical analysis, p values < 0.05 were considered significant.

Results

^{3}H-thymidine incorporation was higher upon stimulation with PHA or ConA than with PWM or SAC. Increasing glycyl-glutamine concentration from 0.4 mmol/l to 2.0 mmol/l did not increase lymphocyte proliferation on stimulation with ConA, PWM and SAC. In contrast, a significant increase by more than 20 % was found with PHA (Fig. 1).

Lymphocyte proliferation was mimimal in glycyl-glutamine free medium. With PHA, PWM and ConA concentrations as low as 0.01 mmol/l offered significantly higher proliferation rates. SAC stimulation resulted in a more linear increase (Fig. 2). When compared to proliferation rates seen at 0.4 mmol/l, significant losses were seen with glycyl-glutamine concentrations dropping below 0.1 mmol/l (SAC), 0.04 mmol/l (PWM, PHA) or 0.05 mmol/l (ConA), respectively. No incorporation of ^{3}H-thymidine was found in the absence of mitogens regardless of glycyl-glutamine concentration.

Discussion

There is growing interest in the use of dipeptides as a source of glutamine in clinical nutrition (11, 5, 12). Dipeptides containing glutamine can be almost completely hydrolysed, resulting in a prompt release of their constituent amino acids (9). The equivalence of free glutamine and its dipeptides was demonstrated by a study showing the growth of a hematopoietic cell line to be equally well sustained by either source of glutamine (13). We have previously extended these findings to lymphocyte proliferation in vitro (14). Lymphocyte proliferation rates were comparable with equimolar concentration of glycyl-glutamine and glutamine, which was observed in both healthy and HIV-infected lymphocyte populations (14).

In this study, surprisingly small amounts of glycyl-glutamine were found to be adequate to enhance lymphocyte proliferation. Function is maintained at the same level seen

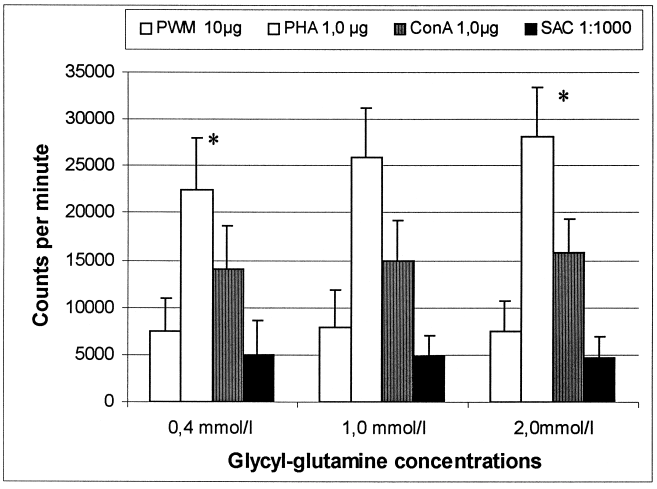

Fig. 1 Effect of various glycyl-glutamine concentrations on lymphocyte proliferation under stimulation with PHA, ConA, SAC, PWM. Mean values of 12 individuals and standard deviation, each assay performed in triplicate. Measured as ^{3}H-thymidine incorporation in counts per minute. (*=p< 0.05 values at 2.0 mmol compared to 0.4 mmol/l).

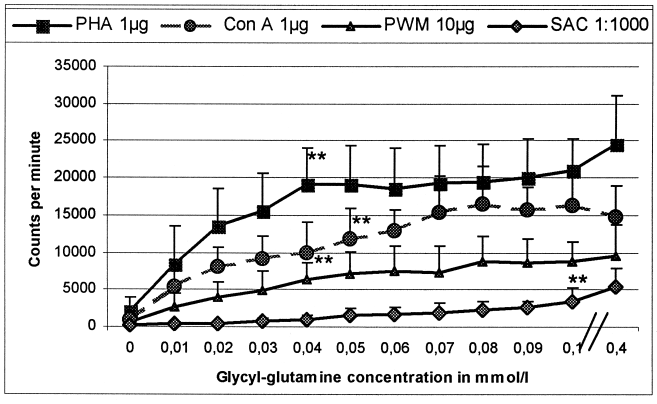

Fig. 2 Effect of glycyl-glutamine in very low concentrations on mitogen induced lymphocyte proliferation (PHA, ConA, PWM and SAC). Mean values of 6 individuals and standard deviation, each assay performed in triplicate. Measured as ^{3}H-thymidine incorporation. (** = lowest glycyl-glutamine concentration yielding lymphocyte proliferation not significantly different from 0.4 mmol/l).

with a "physiological" glutamine concentration of 0.4 mmol/l by glycyl-glutamine concentrations as low as 0.04 mmol/l – 0.1 mmol/l. We conclude that glycyl-glutamine supports lymphocyte proliferation in vitro, and concentrations well below plasma glutamine levels seen in catabolic patients (5, 15) are adequate.

Additional effects by concentrations of glycyl-glutamine higher than the "physiological" 0.4 mmol/l were detected with one of the mitogens only (PHA). The influence of different mitogens on glycyl-glutamine induced lymphocyte proliferation was not uniform. Proliferation rates were higher upon PHA- and ConA- stimulation than upon stimulation by PWM or SAC. The former are T-cell stimuli, whereas SAC induces proliferation of B-cells and PWM of both T- and B-cells. This might indicate that the T-cell system is more responsive to glutamine or glycyl-glutamine effects than the B-cell system. This is in line with recent studies which demonstrated a significant increase in total lymphocytes and in particular T-lymphocytes in patients receiving glutamine supplemented total parenteral nutrition (TPN) after bone marrow transplantation (4) or colorectal surgery (16). Further, the application of even high doses of glycyl-glutamine has been demonstrated to be safe (15). Supplementing TPN with a glutamine dipeptide similar to glycyl-glutamine (alanine-glutamine) resulted in shorter inpatient periods following major abdominal surgery and more rapid lymphocyte recovery in the glutamine dipeptide group (5), although plasma glutamine levels differed only mildly (543±29 mmol/l vs. 0.427±27 mmol/l in controls). These results suggested that the use of glycyl-glutamine in parenteral nutrition may be beneficial for lymphocytes even when plasma glutamine concentrations are not markedly decreased. This contrasts with our finding that concentrations as low as 0.1 mmol/l are adequate to sustain lymphocyte proliferation. It appears that effects other than those measured here account for these findings. Glutamine is known to exert various physiological effects in vivo apart from lymphocyte proliferation, notably on gut integrity.

Future studies should investigate the effects of glycyl-glutamine on cells from immunocompetent patients with plasma glutamine deficiency. It may also be necessary to relate findings to intracellular glutamine levels, as these may be of greater importance for lymphocyte proliferation than extracellular levels.

Acknowledgment This study was generously supported by the Kinder AIDS-Hilfe Deutschland.

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