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REVIEW

Glutamine as indispensable nutrient in oncology: experimental and clinical evidence

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Abstract
Background In hypermetabolic situations, glutamine is intensively used by rapidly dividing cells such as enterocytes, lymphocytes, and fibroblasts as nitrogen source and/or alternative energy fuel. It is hypothesized that in cancer patients the increased glutamine demands of the host increase the capacity of endogenous production resulting in a strong glutamine deprivation with detrimental effects on organ functions. In long-term periods of cancer cachexia, an adequate nutrition support including glutamine can essentially contribute to cover glutamine needs and, thus, to spare energy reserves of the host and to retard severe complications such as multi-organ failure. Due to the early in vitro knowledge that cancer cells preferably consume glutamine, oncologists often refuse to supply glutamine to the tumor-bearing host to avoid any potential risk. An objective evaluation whether supplemental glutamine supports tumor growth in vivo is, however, still lacking.
Aim of the study The present review evaluates in vivo experimental and clinical data with respect to potential effects of glutamine administration in tumor-bearing hosts and draws conclusions for the use of glutamine supplements in clinical oncology.
Methods Experimental and clinical intervention studies were identified in a systematic review of MEDLINE Database (last entry: June 2008) using key search terms and review articles. These studies were supplemented with reports identified through manual searches and other studies previously known by the authors.
Results Numerous experimental studies (rat/mouse model) show that oral/enteral or intravenous glutamine supports metabolism of the tumor-bearing host and can ameliorate gastrointestinal toxicity of therapeutical measures. Within the last two decades, 36 (24 oral/enteral, 12 parenteral) clinical studies evaluating the tolerance, safety and effects of glutamine in various patient groups have been published. In the great majority of these clinical studies, glutamine supplementation in cancer patients improves host metabolism and clinical situation without increasing tumor growth. Potential mechanisms of glutamine effects include maintenance of mucosal integrity, improved immune competence, inhibition of cell proliferation, increased apoptosis rate, increased synthesis of glutathione, induction of heat shock protein synthesis, and increased synthesis of glucagons-like peptides.
Conclusions In various clinical situations, appropriate exogenous glutamine supply is safe and can beneficially contribute to diminish risks of high-dose chemotherapy and radiation. In addition, there is some evidence that adequate glutamine availability can beneficially affect outcome, especially in patients undergoing bone marrow transplantation.

Keywords Glutamine · Oncology · Experimental studies · Clinical studies · Cellular mechanisms

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Introduction

The amino acid glutamine is frequently used by rapidly dividing cells such as enterocytes, lymphocytes, and fibroblasts as nitrogen source and/or energy fuel [61]. Physiologically, metabolic needs are predominantly covered by endogenous synthesis and subsequent release from skeletal muscle and, to a minor effect, from the lung [61]. In healthy adults, nutritive intake plays a minor role [60].

Historically, the knowledge that tumors readily consume glutamine dates back to 1935. Eagle could demonstrate that the proliferation of cultured malignant cervical cells (HeLa cells) is greatest at glutamine concentrations $\geq$1 mmol/L reflecting a continuous demand for glutamine in the absence of the normal in vivo supply [29]. More recently, it has been shown in various cell lines of tumor and non-tumor origin that cell growth is a function of glutamine availability [20, 83].

By mobilizing and augmenting circulating glutamine [1], tumor cells induce a net glutamine flux from host to tumor. In patients with progressive cancer, the liver switches from glutamine balance to glutamine release to provide additional circulating glutamine for the growing malignancy [17]. In addition, skeletal muscle glutamine release into the blood stream further increased resulting in a considerable decrease of intramuscular glutamine concentrations [16]. The resulting host glutamine deprivation leads to adverse effects on host immune status, gastrointestinal mucosal integrity, and host protein and energy metabolism [63]. It has been suggested that the disruption of interorgan glutamine flux due to progressive tumor growth may essentially contribute to host cachexia [51]. Metabolic impairments due to lack of available glutamine include insulin resistance, hyperlipidaemia, loss of adipose tissue, muscle wasting, increased hepatic production of acute-phase proteins, and reduced gut-barrier function [4, 6, 22, 24, 25, 62, 79, 97, 107, 108].

Various randomized controlled trial (RCT) studies in severely ill, non-cancer patients have shown that an enteral and/or parenteral glutamine (dipeptide) supply can prevent glutamine deprivation thereby improving nitrogen metabolism, maintaining gastrointestinal integrity and function, beneficially influencing immune response, and improving patient outcome [35, 110]. It is, thus, consequent to hypothesize that also in long-term periods of cancer cachexia with dramatically increased glutamine consumption an adequate nutrition support including glutamine can essentially contribute to prevent an overall glutamine deprivation, to spare energy reserves of the body and to retard severe complications such as multi-organ failure [4, 6, 51, 79, 109]. Within the last years, concerns have been, however, raised whether the administration of glutamine not only supports host metabolism but might also stimulate tumor growth.

Due to the early in vitro knowledge that cancer cells preferably consume glutamine [20, 29, 83], oncologists often refuse to supply glutamine to the tumor-bearing host to avoid any potential risk. An objective evaluation whether supplemental glutamine supports tumor growth in vivo has not been made. Moreover, potential beneficial effects for the cancer patient are not considered.

The goals of the present review are, thus, to evaluate experimental and in vivo clinical data with respect to potential effects of glutamine administration in tumor-bearing hosts and to draw conclusions for the use of glutamine supplements in clinical oncology.

Methods

Review process

Publications were identified in a systematic review of MEDLINE Database (last data entry: June 2008) using "glutamine and cancer" as keywords. Eligible experimental and clinical intervention studies were those that examined the influence of glutamine or glutamine dipeptide administration (second level keywords: oral, enteral, parenteral) on selected biomarkers and, in the case of trials in cancer patients, on clinical endpoints. These studies were supplemented with reports identified through manual searches and other studies previously known by the authors.

Results

Experimental studies with supplemental glutamine

In 1988, Fox et al. [33] demonstrated that a glutamine-supplemented enteral diet (2% glutamine) in a rat model of lethal enterocolitis exerted beneficial effects on weight loss, nitrogen retention, mucosal morphology and function as well as on survival time and mortality compared to a isonitrogenous control (equivalent amounts of glycine). Since the severity of enterocolitis is a dose-limiting factor for chemotherapy treatment, the findings from this landmark study gave rise to a series of experiments with sarcoma-bearing rats with methotrexate (MTX)-induced enterocolitis. Provision of similar amounts of glutamine ameliorated host toxicity while enhancing tumor volume loss as compared to a placebo group receiving glycine supplementation [52, 80]. The enhanced tumoricidal effectiveness of MTX in glutamine-fed animals was explained by an increase in the intratumoral concentration

of MTX, mediated via polyglutamation impairing the efflux and improving retention of MTX from the tumor [53, 81]. Simultaneously, gut concentrations of polyglutamated MTX decreased with supplemental glutamine [81]. Other groups could confirm these results in various rat models of colonic or whole body irradiation: enteral or parenteral application of glutamine after radiation has been shown to decrease intestinal injury [82], beneficially affect the repair of the intestinal wall [27], and improve colon anastomotic healing while not affecting bacterial translocation [30]. Even a preventive provision of glutamine before abdominal radiation in rats has been shown to exert a radioprotective effect on the small bowel mucosa [54]. In cats, however, oral glutamine does not preserve intestinal function in MTX-induced enterocolitis [68]. Interestingly, Shou et al. [88] could demonstrate that provision of a polypeptide diet prior to MTX treatment is superior to an elemental diet supplemented with free glutamine (2%) with respect to survival. Probably, the polypeptide diet contained more glutamine (bound to peptides) than the supplemented elemental diet.

The first study assessing the effects of glutamine supplementation on tumor growth and tumor cell proliferation in an in vivo rat model was published in 1990 [55]. Feeding an elemental diet supplemented with 0.39 g glutamine/60 mL diet (30% of total dietary nitrogen) was effective in repleting muscle glutamine stores while having no negative effect on tumor DNA content, glutaminase activity, or weight. Long-term dietary glutamine supplementation in rats with mammary tumors exerted a trophic effect on the small intestinal mucosa whereas no stimulation of tumor growth kinetics or formation of metastases occurred [7]. Glutamine gavage and pair-fed food (total dosage 1 g glutamine/kg day) even reduced tumor growth by 40% which was associated with a 30% increase in natural killer (NK) cell activity [31]. Oral glutamine was also shown to suppress the growth of inoculated hepatoma in mice in vivo as well as in vitro [67]. In Ehrlich ascites tumor (EAT) cell-bearing mice fed a glutamine-enriched diet (30% of total nitrogen from glutamine) the growth rate of tumor was not affected [14, 70].

Similar results with respect to body weight, protein synthesis rates, and tumor growth have been obtained when glutamine was given as part of total parenteral nutrition (TPN). In rats with small and large sarcoma, glutamine-supplemented TPN (20% of total nitrogen load; 0.3 g glutamine/day) did not affect tumor glutathione (GSH) levels but increased gut mucosal GSH levels significantly. While glutamine did not affect tumor weight per se in this trial, it could not be excluded that the actual amount of tumor cells within a tumor may be increased with glutamine supplementation [5]. After 5-fluorouracil (5-FU) administration (50% LD_{50}) in rats, TPN with the dipeptide alanyl-glutamine (Ala-Gln) increased plasma glutamine and GSH levels but did not improve mortality rate [114]. In rats with ascites hepatoma, TPN with glutamine (1.5 g Ala-Gln/100 mL) decreased loss of body weight associated with mitomycin treatment and enhanced muscle fractional synthetic rate in both untreated and treated rats. Tumor protein synthesis was not altered and whole body protein breakdown was decreased with glutamine [47]. In hepatoma-bearing rats, glutamine-supplemented TPN did not affect tumor weight, protein and DNA synthesis rates. In addition, the tumor-induced GSH deficiency of the jejunal mucosa could be prevented with parenteral glutamine supplementation indicating a beneficial role for glutamine in repleting host tissue GSH stores [39, 112]. In a hamster mucositis model, oral glutamine or Ala-Gln reduced macroscopic and histological parameters and maintained serum glutamine and GSH levels [64].

Clinical intervention studies

Oral/enteral glutamine supplementation

Within the last two decades, 24 clinical studies evaluating the tolerance, safety, and effects of oral/enteral glutamine in various patient groups have been published (Table 1).

In children and adults experiencing stomatitis after chemotherapy [3, 93] as well as in head and neck cancer patients on radiation therapy [38], oral glutamine applied as "swish and swallow" therapy has been shown to decrease the severity and duration of stomatitis. In a large randomized, placebo-controlled clinical trial in 70 adequately nourished (mean BMI 22.8 and 23.2 in verum and control groups, respectively) patients with advanced colorectal cancer receiving 5-fluorouracil/folinic acid (5-FU/FA) chemotherapy, oral glutamine supplementation (18 g/day) was effective in reducing impairments of intestinal absorption and permeability [23].

In patients with esophageal cancer on radiochemotherapy, the reduction in the lymphocyte count could be prevented, lymphocyte mitogenic function enhanced and gut permeability attenuated with high-dose oral glutamine supplementation (30 g glutamine/day) [111, 113]. Similar doses of oral glutamine were also shown to be effective in increasing serum concentrations of glutamine, decreasing intestinal permeability, and lowering the incidence of grade 2–4 mucositis during chemotherapy [18, 43, 104].

A large randomized, double-blind, placebo-controlled trial of oral glutamine supplementation ("swish and swallow" of 1 g/m^2 4 times a day) in 193 autologous and allogeneic BMT patients was performed by Anderson et al. [2]: Compared to controls receiving glycine, less mouth pain was observed. More importantly, at day 28, survival of allogeneic patients was improved. Trends toward decreased

Table 1 Clinical studies with oral glutamine supplementation

Study	Date	Study design	*n*	Patients	Regimen	Outcome
Jebb [42]	1994	RCT	28	GI cancer on 5-FU chemotherapy	16 g/day Gln vs. placebo	Good tolerance No effect on mucositis
Skubitz [93]	1996	RCT	14	Stomatitis after chemotherapy	4 g Gln swish and swallow, 2×/day	Decreased severity and number of days of mucositis
Bozzetti [11]	1997	RCT	65	Advance breast cancer on doxifluridine chemotherapy	10 g Gln vs. maltodextrin, 3×/day	No impact on incidence of diarrhea, severity and duration of tumor growth and response rate
Anderson [3]	1998	RCT	24	Children/adults on cytotoxic chemotherapy	2 g/m^2 Gln swish and swallow vs. Gly, 2×/day	Amelioration of stomatitis, lesser duration and severity of mouth pain
Anderson [2]	1998	RCT	193	Autologous and allogeneic BMT	1 g/m^2 Gln swish and swallow vs. Gly, 4×/day	Less mouth pain with Gln in autologous BMT, yet not in allogeneic BMT Day 28 survival of allogeneic BMT patients with Gln improved No toxicity of Gln observed
Yoshida [113]	1998	RCT	13	Esophageal cancer on radiochemotherapy	30 g/day Gln over 28 days	Reduction in the lymphocyte count prevented and gut permeability attenuated
Schloerb [87]	1999	RCT	66	Hematologic and solid malignancies on chemotherapy and allogeneic or autologous BMT	10 g Gln vs. Gly, 3×/day; when necessary TPN with Gln/standard TPN	No effect on hospital stay, TPN days, neutrophils recovery, pos. blood cultures, mucositis and diarrhea. Possible limited benefit on need for TPN and long-term survival
Coghlin Dickson [19]	2000	RCT	58	Autologous and allogeneic BMT on chemotherapy	30 g/day Gln during CT	Trends toward decreased median LOS and number of days on TPN, yet no stat. Significance, no support for benefit of oral Gln
Huang [38]	2000	RCT	17	Head and neck cancer on radiation therapy	2 g/m^2 Gln swish therapy, 4×/day	Shorter duration and severity of oral mucositis
Vahdat [102]	2001	Controlled clinical trial	33/12	Patients with stage IV breast cancer on high-dose CT with paclitaxel	10 g Gln, 3×/day	Reduction in the severity of peripheral neuropathy, no effect on response rate
Daniele [23]	2001	RCT	70	Patients with advanced/metastatic colorectal cancer on chemotherapy with FU/FA	18 g Gln/d for 15 days	Reduced degree of impairments of intestinal absorption and intestinal permeability: First randomized trial showing effects of oral gln in protecting the intestinal mucosa from CT-induced damage
Yoshida [111]	2001	RCT	13	Patients with esophageal cancer receiving radiochemotherapy	30 g Gln/day over 4 weeks	Enhanced lymphocyte mitogenic function, reduced gut permeability
Kozelsky [59]	2003	RCT, phase III	129	Pelvic radiation therapy	4 g Gln, 2×/day for 2 weeks after RT	No effect on toxicity, quality of life and incidence of grade 3 diarrhea
Jacobson [40]	2003	RCT, crossover design	36	Patients who had experienced myalgias/arthralgias related to paclitaxel CT	10 g Gln, 3×/day for 5 days	No effect of Gln on the development and severity of myalgias/arthralgias Good tolerance

Table 1 continued

Study	Date	Study design	*n*	Patients	Regimen	Outcome
Ward [105]	2003	Phase I clinical dose-finding trial	13	13 pediatric malignancy patients	0.35, 0.5, and 0.65 g Gln/kg b.w. day, bolus	No untoward plasma Gln and ammonia levels up to 0.65 g/kg b.w. day
Pan [73]	2005	Phase II, open-labeled, multicenter trial	40	Advanced colorectal cancer, chemotherapy (IFL-regimen)	10 g Gln every 8 h during CT, 1–6 cycles	No effect on either efficacy or toxicity of CT
Stubblefield [95]	2005	Non-randomized, controlled trial	46	High-dose paclitaxel chemotherapy	10 orally 3 times/day for 4 days	Less weakness, less loss of vibratory sensation, less toe numbness
Jiang [43]	2006	RCT	39	GI cancer, postop., intensive chemotherapy	30 g Gln/day	Increased serum concentrations of gln Decreased intestinal permeability
Li [65]	2006	RCT	60	Breast cancer, chemotherapy	Gln ≥12 days	Higher plasma Gln level Amelioration of the CT-induced increase in intestinal permeability No significant positive effects on stomatitis, diarrhea and the antitumor effect of CT
Okur [72]	2006	Controlled clinical trial	21	Children with solid tumors on chemotherapy	4 g/m^2 Gln day for 5 days	Improvement of some nutritional and immunological parameters, reduced antibiotic necessity
Choi [18]	2007	Controlled clinical trial	51	Advanced or metastatic cancer, receiving FU/LV chemotherapy	30 g Gln/day for 15 days	Lower intestinal permeability score with Gln vs. controls Lower incidence of grade 2–4 mucositis
Jazieh [41]	2007	Phase I clinical trial of escalating doses	15	Locally advanced lung cancer, chemoradiation therapy	10 g Gln/day during CT/RT	Serious toxicities not prevented by Gln
Wang [104]	2007	RCT	86	Metastatic colorectal cancer, chemotherapy (oxaliplatin)	15 g Gln, 2×/day for 7 days every 2 weeks during CT	Lower incidence of oxaliplatin-induced mucositis No effect on response to CT and on survival
Peterson [75]	2007	RCT phase III, multicenter	326	Patients with WHO grade ≥2 mucositis	Gln (Saforis)	Reduced incidence of ≥grade 2 and 3 mucositis, safe and effective

RCT randomized controlled trial, *LOS* length of hospital stay

length of hospital stay and median numbers of days on TPN in autologous and allogeneic BMT patients on chemotherapy with oral glutamine supplements (30 g/day) were observed; however, these results missed statistical significance [19].

Only limited benefit of oral glutamine supplements (30 g/day), replaced by parenteral glutamine in case TPN was required, was reported by Schloerb and Skikne [87]: While no beneficial effects were seen on hospital stay, days on TPN, neutrophils recovery, positive blood cultures, mucositis and diarrhea, possible benefit rested in the reduced need for TPN as well as in improved long-term survival.

In a recent randomized, placebo-controlled phase III multicenter trial, efficacy and safety of an orally applied glutamine powder (Saforis®) for the prevention and treatment of oral mucositis were studied in 326 breast cancer patients on chemotherapy [75]. Compared with placebo, glutamine significantly reduced the incidence of clinically relevant and severe mucositis in this group of patients. Saforis® is an oral suspension which is administered via a new drug delivery system (UpTec™) enabling a more rapid and effective provision of free glutamine directly into chemotherapy or radiotherapy damaged cells of the oral mucosa. Indeed, the uptake of free glutamine from conventional preparations into mucosal cells may be limited.

Within a RCT study in 60 breast cancer patients under chemotherapy, Li et al. [65] found higher plasma levels of glutamine and an amelioration of the chemotherapy-induced increase in intestinal permeability; a positive effect, however, on the incidence of stomatitis and diarrhea was not observed. In patients with gastrointestinal cancer on FU chemotherapy, application of 16 g glutamine/day was not effective in preventing mucositis as compared to a placebo [42] and oral glutamine supplementation during and after pelvic radiation therapy did not affect treatment-related toxicity, quality of life, and incidence of grade 3 diarrhea [59].

Peripheral neuropathy, myalgias, and arthralgias are frequent dose-limiting side effects of paclitaxel, an anti-neoplastic agent used extensively in the treatment of breast cancer. The symptoms of peripheral neuropathy such as numbness, tingling, paresthesis, dysesthesia, pain or weakness can interfere with the usual activities of daily living and, thus, be a significant source of distress to patients [95]. In patients with stage IV breast cancer on high-dose chemotherapy with paclitaxel, supplementation with 3 times 10 g of oral glutamine/day was effective in reducing the severity of treatment-induced peripheral neuropathy [102]. With this regimen, patients experienced less weakness, less loss of vibratory sensations, and less toe numbness [95]. As compared to a placebo, oral glutamine was, however, not effective in preventing or alleviating the severity of paclitaxel-induced myalgias or arthralgias [40].

In advanced breast cancer patients, high-dose oral glutamine (30 g/day) showed no effects on the incidence of diarrhea as well as on tumor response to chemotherapy [11]. Similarly, supplemental glutamine did not affect either efficacy or toxicity of chemotherapy in patients with advanced colorectal cancer [73]. A small controlled clinical trial in children with solid cancers on chemotherapy suggests that the application of oral glutamine might be associated with improvements in some nutritional and immunological parameters as well as reduced antibiotic necessity during chemotherapy treatment [72].

Parenteral glutamine/glutamine peptide supplementation

Up to now, only 12 studies have evaluated the effects of glutamine-containing TPN in cancer patients (Table 2).

The first prospective, randomized, double-blind trial of glutamine-supplemented TPN in bone marrow transplant patients was published by Ziegler et al. [115] in 1992. Forty-five patients who had undergone allogeneic BMT were randomized to receive either glutamine-containing TPN (0.57 g/kg b.w. day) or standard TPN for 4 weeks post-transplantation. Glutamine-supplemented patients had an improved nitrogen balance, diminished incidence of clinical infections, lower rates of microbial colonization, and a shorter length of hospital stay. In a similar RCT in 29 patients with hematologic malignancies and solid tumors undergoing allogeneic or autologous BMT, Schloerb and Amare [86] found a significantly shortened hospital stay, but no change in infection rate with glutamine-supplemented TPN. Glutamine-containing TPN was shown to be safe and effective in consecutive randomized clinical trials in patients with hematologic malignancies undergoing high-dose chemotherapy and autologous peripheral blood stem cell transplantation (aPBSCT), lymphocyte recovery after aPBSCT was improved with glutamine [76].

A broad application of glutamine-containing parenteral solutions has been hampered by the poor water solubility and instability of free glutamine during heat sterilization. Consequently, the introduction of glutamine dipeptides (Ala-Gln; glycyl-glutamine, Gly-Gln) as highly soluble and stable glutamine sources in commercial parenteral solutions [35] encouraged further clinical studies in cancer patients. During high-dose cytotoxic chemotherapy, daily infusion of 50 g Gly-Gln preserved protein C and albumin levels and, thus, protected hepatic function in patients with general adequate nutritional status [12]. Safety and tolerance of TPN containing high doses (40 g/day) of Ala-Gln could be demonstrated in hematologic patients undergoing chemotherapy; yet, the authors did not observe any significant beneficial effects in this group of patients [103].

Table 2 Clinical studies with parenteral glutamine/glutamine dipeptide supplementation

Study	Date	Study design	*n*	Patients	Regimen	Outcome
Ziegler [115]	1992	RCT	45	Patients with hematologic malignancies and allogeneic BMT	TPN supplemented with Gln (0.57 g/kg b.w. day) vs. standard TPN	Improved nitrogen balance Diminished incidence of clinical infections Lower rate of microbial colonization Shortened LOS
Schloerb [86]	1993	RCT	29	Patients with hematologic malignancies and solid tumors with allogeneic and autologous BMT	TPN supplemented with (0.57 g/kg b.w. day) vs. standard TPN	Decreased total body water Decreased LOS (5.8 days) after BMT
van Zaanen [103]	1994	RCT	15	Hematologic patients, chemotherapy	TPN supplemented with Ala-Gln (40 g/day) for 18–20 days	Safe, no dipeptide-related toxicity at the high dose No significant beneficial effects
Brown [12]	1998	RCT	34	Patients undergoing BMT on high-dose cytotoxic therapy	50 g/day i.v. Gly-Gln vs. placebo (amino acid mixture) until discharge from BMT unit	Preserved protein C and albumin levels → protected hepatic function
Decker-Baumann [26]	1999	RCT	24	Patients with metastatic colorectal carcinoma receiving 5-FU/CF chemotherapy	i.v. Gly-Gln	Reduced mucositis and ulcerations of gastric and duodenal mucosa, preserved villous height
Pytlik [78]	2002	RCT	40	Patients with hematological and solid tumors and multiple sclerosis treated by high-dose Ct and autologous SCT	30 g/day i.v. Ala-Gln vs. isonitrogenous placebo (AA-solution)	Gln supplementation did not improve nutritional parameters
Piccirillo [76]	2003	2 consecutive RCT	27/21	Hematologic malignancies, high-dose chemotherapy and autologous peripheral SCT	TPN supplemented with free Gln (20 g/day and 13.46 g/day, resp.)	Safe and effective Improved lymphocyte recovery after aPBSCT
Scheid [85]	2004	RCT	54	Acute myeloid leukemia, myelosuppressive CT without BMT	PN supplemented with Gly-gln (20 g) during CT cycles	Significantly faster neutrophils recovery in Gln patients than in controls No impact on neutropenic fever or recovery of CD4+ or CD8+ lymphocytes or monocyte activation
Sykorova [96]	2005	RCT, comparative study design	44	Autologous transplant patients with hematologic malignancies	Prophylactic PN with or without 0.5 g/kg b.w. day Ala-Gln vs. PN given ad hoc	No beneficial effects of Gln peptide supplementation Worse long-term outcome with Gln peptide
Cerchietti [15]	2006	RCT	32	Head and neck cancer, chemoradiotherapy	i.v. Ala-Gln (0.3–0.4 g/kg b.w. day) during CT days	Effective in the prevention of severe mucositis
Oguz [71]	2007	Prospective, randomized, controlled trial RCT	109	Surgery for colorectal cancer and EN	1 g/kg b.w. day parenteral Ala-Gln (1 g/kg b.w. day) together with EN, ≥5 days pre- and postop.	Decreased postop. complications (wound-infection abscess formation, wound dehiscence) Shorter hospital stay
da Gama Torres [21]	2008	RCT	53	Leukemia, Allo-SCT	i.v. Ala-Gln (0.3–0.4 g/kg b.w. day) during 6 days after transplantation	Increased survival on D+180 and D+100 No effect on intestinal permeability

RCT randomized controlled trial, *LOS* length of hospital stay

In patients with metastatic colorectal carcinoma receiving 5-FU/CF chemotherapy, parenteral supplementation with Gly-Gln was effective in reducing mucositis and ulcerations of the gastric and duodenal mucosa and preserved villous height [26]. Gly-Gln-containing TPN was also effective in enhancing neutrophil recovery in patients with acute myeloid leukemia receiving myelosuppressive chemotherapy. Yet, an impact on neutropenic fever or recovery of CD4+ or CD8+ lymphocytes or monocyte activation was not observed [85]. With the use of Ala-Gln-supplemented TPN, the development of severe mucositis could be prevented in head and neck cancer patients receiving chemoradiotherapy [15] and supplemental parenteral Ala-Gln in combination with enteral nutrition decreased postoperative complications and shortened hospital stay in surgical patients with colorectal cancer [71]. Nutritional status (BMI, SGA, serum albumin, and protein levels) were similar in both glutamine and verum groups. In generally well-nourished patients with hematological and solid tumors treated by high-dose chemotherapy and autologous BMT [78] as well as in autologous transplant patients with hematologic malignancies [96], beneficial effects of parenteral glutamine supplementation could not be confirmed; in the latter group of patients, glutamine has even been connected with apparently worse long-term outcome.

Currently, the efficacy of a glutamine dipeptide (Ala-Gln; 0.3–0.4 g/kg b.w.)-supplemented parenteral nutrition to increase short-term survival after Allo-SCT could be demonstrated in a randomized study in 53 leukemia patients suggesting an early immunomodulatory role of glutamine [21].

Discussion

Numerous in vitro studies brought evidence forward that rapidly dividing tumor cells need glutamine as energy/nitrogen source (ATP production) and as an effective modulator of cell metabolism (regulator of protein synthesis and amino acid transport) [94, 101, 106]. Obviously, human cancer cell lines exhibited a five- to tenfold faster rate of glutamine consumption than non-malignant cells [10, 32, 57]. It can be interpreted that available glutamine predicts a more aggressive tumor behavior and raise the possibility that nutritional supplementation with glutamine might stimulate tumor growth. On the other hand, *glutamine deprivation* in human breast carcinoma cell lines has been shown to induce the secretion of the pro-angiogenic and pro-metastatic cytokines vascular endothelial growth factor (VGEF) and IL-8 upregulated mainly by hypoxia [9]. Furthermore, NFκB activation in response to glutamine deprivation may promote angiogenesis, survival, and motility of cancer cells within a nutrient-poor solid tumor [9]. These contradictory results of in vitro studies clearly indicate that reliable information about effects of supplemental glutamine can only be made on the basis of in vivo studies.

If glutamine is not available from exogenous sources, tumor cells can manipulate host metabolism to endogenously cover the needs. Any measures to "artificially" establish a situation of glutamine depletion, thus, cannot stop or even retard tumor growth. On the other hand, the host can develop serious glutamine depletion which is associated with impaired physiological functions like disturbed mucosal integrity and diminished immune competence. Following this line of reasoning, any nutritional efforts to provide sufficient exogenous glutamine might improve the general metabolic situation of the patient and, thus, life quality.

Obviously, substantial experimental evidence is available supporting this hypothesis. Supplemental oral, enteral, or parenteral glutamine with generally high dosages protected the host tissues, such as the intestinal mucosa and the liver, from severe harm during antitumor treatment. In the great majority of the studies, tumor growth and tumor protein synthesis remains unaltered. In more than 20 controlled clinical trials, the effects of oral glutamine supplementation on the adverse side effects of anti-cancer treatment have been evaluated (Table 1). Dosage, time, and frequency of glutamine supply as well as primary goals vary considerably. Generally, oral/enteral and parenteral glutamine/glutamine dipeptide supply is safe and well tolerated. However, only in some studies with oral glutamine, integrity and function of target organs such as intestine were preserved and the severity of treatment-induced complication was reduced compared to control treatment. Even with high glutamine dosages (up to 30 g/day) neither the toxicity of chemotherapy nor the complication rates were affected in some patient groups. It is, however, to mention that there were no negative effects on the efficacy of antitumor treatment.

A clear and convincing explanation for these inconsistent results is not available. Unfortunately, only in five clinical studies, reliable information on the nutritional status of the patients were reported [12, 23, 71, 78, 113]. Metabolic and nutritional parameters were not different between verum and control groups; moreover, BMI and/or body weight were generally within the normal range. Consequently, an impact of the nutritional status on the effect of glutamine supplementation can be ruled out. Since in most of the studies cited no or insufficient information with respect to the general handling of glutamine supplements (preparation, storage) is provided, it cannot be ruled out that relative instable glutamine [35] was partly decomposed leading to a lower intake than originally

planned. An unsolved question is still the glutamine amount needed: up to now, we can only estimate the glutamine demand of rapidly consuming cells.

Interestingly, the use of parenteral glutamine/glutamine dipeptides was more effective than oral/enteral application (Table 2). In various patient groups, i.v. glutamine beneficially affects intestinal morphology and function as well as neutrophile recovery. In patients undergoing bone marrow transplantation, several major randomized, controlled clinical trials carried out during the 1990s suggest that glutamine supplementation might beneficially affect outcome in this group of most severely ill patients. Indeed, parenteral application of freshly prepared free glutamine solutions or commercially available dipeptide products ensures a 100% availability of the substrate. Moreover, potential host targets such as immune competent cells can directly use glutamine for protein synthesis, formation of GSH, or for energy production [61]. Probably, i.v. application is generally more effective in supporting host metabolism.

Recently, the mechanisms by which glutamine may potentially impact clinical outcome have come into the focus of experimental work. Potential mechanisms for the beneficial effects of glutamine supplementation in the tumor-bearing state are depicted in Fig. 1 [adopted from 109].

Programmed cell death is an evolutionary conserved biochemical pathway resulting in a characteristic morphological cell death termed *apoptosis*. Liu et al. [67] explored the effects of glutamine on growth and apoptosis of hepatoma cells in vitro and in vivo. In hepatoma cell culture treated with different concentrations of glutamine solution, cell proliferation was inhibited and cells were induced to apoptosis, which was dependent on glutamine concentration. The authors concluded from their experiments that hepatoma cell apoptosis and tumor growth inhibition by glutamine may be associated with increased GSH activity and its intervention in hepatoma cell proliferation and simultaneous release of NO.

Since glutamate itself can only be hardly transported via cell membranes, glutamine is an essential precursor of glutamate for the *synthesis of GSH*. GSH is a tripeptide protecting cells from oxidative stress [17]. In EAT, cell-bearing mice fed a glutamine-enriched diet, glutamine synthetase activity was downregulated and glutaminase activity upregulated with glutamine [14]. The resulting increase in cytosolic glutamate concentration led to a selective depletion of GSH in tumor mitochondria via a glutamate-induced inhibition of glutamate transport from cytosol into the mitochondria. The mitochondrial GSH depletion rendered tumor cells more susceptible to oxidative stress-induced mediators, such as TNF-α-induced cytotoxicity. The TNF-α-induced production of reactive oxygen intermediates further depleted mitochondrial GSH, thus activating the molecular mechanisms of apoptotic cell death [70]. The underlying mechanisms may provide the basis to identify critical molecules, such as Bcl-2 and Mn-SOD antisense oligodeoxynucleotides that can be sequentially targeted to facilitate elimination of highly resistant metastatic cells in combination with a glutamine-enriched

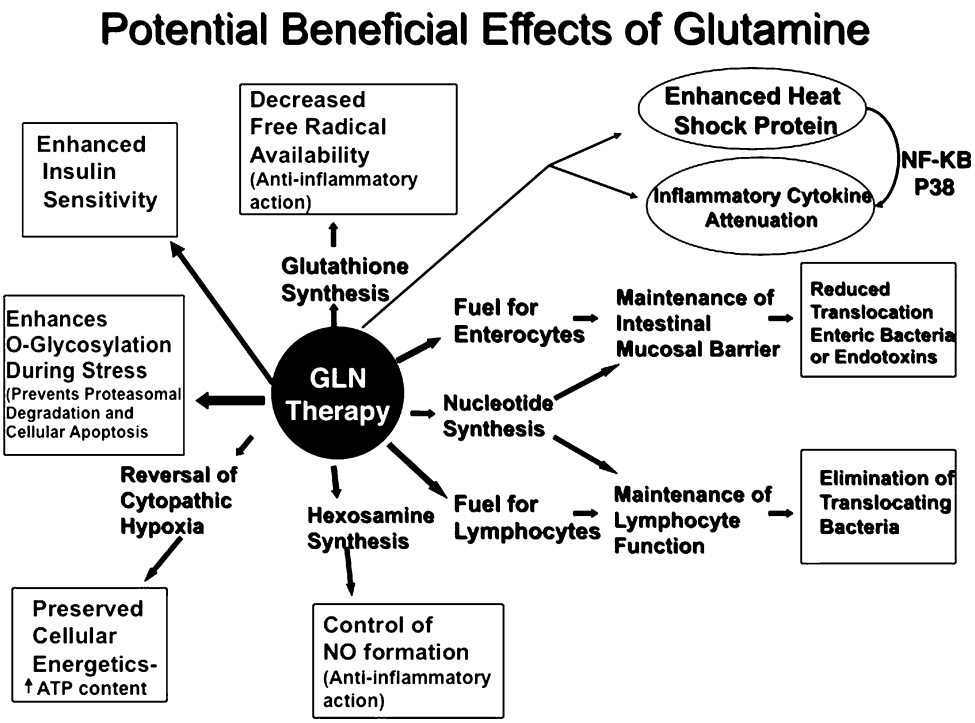

Fig. 1 Potential beneficial effects of glutamine

diet [8]. Dietary glutamine suppressed mammary carcinogenesis by activation of apoptosis in tumor cells through GSH downregulation in a 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced breast cancer model [98–100]. Protein levels and gene expression of Bax, a member of the Bcl-2 family which can induce apoptosis and caspase-3 which is the main apoptosis-executing enzyme, were increased with glutamine while mRNA and protein levels were decreased for Bcl-2, a proto-oncogene whose product is known to play a role in promoting cell survival and inhibition of apoptosis. Oral glutamine has been shown to be effective in preventing tumor formation in a DMBA-induced breast cancer model in the rat, possibly via restoration of normal gut GSH production [44, 48, 49]. Indeed, increased GSH levels in blood, breast tissue, and gut mucosal were observed in DMBA rats treated with glutamine, while blood levels of insulin-like growth factor (IGF)-1 and transforming growth factor (TGF)-β-1 were lowered in a range that is considered clinically significant. Yet, the authors failed to show a correlation between the increase in mucosal GSH production and the decrease in cytokine levels [45].

Interestingly, glutamine administration beneficially influences GSH levels in host tissues but does not influence GSH in tumor cells. A possible explanation for the dichotomy in GSH metabolism in tumor and host tissues has been provided by Klimberg and McClellan [51] and is based on the fact that tumor cells have a relatively more acidic intracellular environment compared to normal cells. As shown, glutamine is able to bypass the acidotic block in the GSH recycling enzyme, oxoprolinase in the host, but not in tumor cells. Uptake and metabolism of extracellular GSH molecules require the oxidation of an intracellular GSH molecule. In cells receiving radio- or chemotherapy, intracellular GSH regeneration is slowed due to inhibition of the pH-sensitive enzyme oxoprolinase in the resulting acidotic environment, leading to intracellular GSH depletion in the tumor. In normal cells, glutamine can upregulate the enzymes γ-glutamyl transferase and glutaminase, thus providing additional glutamate to bypass the blocked oxoprolinase. In the tumor, however, these enzymes are not upregulated by supplemental glutamine. If no radiation or chemotherapy is given, tumor GSH remains unchanged, while host GSH stores increase and tumor growth decreases, possibly through GSH-mediated upregulation of the immune system.

Another mechanism discussed is the role of glutamine in the *host immune response to tumors*. NK cells, a subpopulation of cytotoxic lymphocytes present in normal individuals, are capable of spontaneous cytolytic activity against a variety of tumor cells and have been shown to be important in the control of both induction and progression of cancer. NK cells depend upon an adequate supply of glutamine for proliferation [51, 56, 109]. Tumor progression is associated with a depression in the activity of NK cells due to a decrease in glutamine and GSH concentrations in these cells [69]. Consequently, supplemental glutamine slows tumor growth by upregulating the immune system [51]. In vitro, the dependence of glutamine and GSH on IL-2 augmentation of NK cytotoxicity could be demonstrated [66]. Klimberg et al. [50], in a rat breast cancer model, were able to show that the enhanced NK cell activity seen with oral glutamine in the tumor-bearing host was associated with a GSH-mediated suppression of prostaglandin (PG)E_2 synthesis.

There is growing body of evidence that the protective effect of glutamine on the gastrointestinal tract might be attributable to the induction of heat shock proteins (HSPs) synthesis. HSPs are a part of the natural defence response to a variety of cell stressors [84]. In vitro, enhanced expression of HSP (in particular HSP 70) has been shown to be responsible for glutamine-mediated cellular protection after inflammatory cytokine-induced cellular injury [74, 84, 92]. A study in mice with a specific deletion in HSP 70 expression has confirmed the hypothesis that HSP 70 expression is required for the beneficial effects of glutamine on survival, tissue injury, and the inflammatory response after global inflammatory injury [90]. As shown in an actual study in a septic mice model, this glutamine effect on HSP production might be mediated via *O*-glycosylation and subsequent phosphorylation of key transcription factors (HSF-1, Sp1) in the HSP 70 pathway [91].

Cytopathic hypoxia refers to the failure of ATP generation and mitochondrial function following major illness. Specific to cellular mitochondrial function, glutamine preserves tissue metabolic function (ATP/ADP levels, NAD levels) in the face of sepsis, shock, and I/R injury. This effect appears to be linked to enhanced HSP expression [89, 90].

Probably, glutamine supplements can contribute to weaken the *immunosuppressive effect of chemotherapeutic L-asparaginase*, an enzyme that breaks down the amino acids, asparagine and glutamine. It is used as a chemotherapeutic agent in the treatment of acute lymphoblastic leukemia. Yet, many patients fail to successfully complete asparaginase treatment due to allergic reactions or cytotoxic complications such as liver dysfunction, pancreatitis, and immunosuppression [13]. The immunosuppressive properties of asparaginase are based on the inhibition of antibody-precursor cells in the bone marrow and of both cell-mediated and humoral immune response [34, 36]. In addition, asparaginase depletes circulating and intracellular glutamine and this glutamine depletion has been suggested to be the primary immunosuppressive agent [46]. Bunpo et al. [13] were able to demonstrate that an unlimited supply of glutamine, provided as Ala-Gln, may benefit the

functioning of peripheral effector T-cells and/or promote leukocyte migration or adhesion during asparaginase treatment in mice. In this study, Ala-Gln supplementation was partially successful at mitigating losses in $CD4^+$ and $CD8^+$ cells and in cells expressing CD11b/Mac-1, the latter playing important roles in cellular adhesion, phagocytosis, and extravasation, in chemotaxis and neutrophil respiratory burst, as well as in the binding of diverse ligands. Further studies evaluating the beneficial effects of Ala-Gln supplementation in patients during asparaginase chemotherapy are awaited.

Recent experimental and clinical data suggest that glutamine administration might work through its *secretagogue effect on enteroendocrine L cells* producing glucagon-like peptide-1 (GLP-1) and GLP-2. The actions of GLP-1 include the control of satiety, gastrointestinal motility, islet hormone secretion, and the regulation of β-cell proliferation and survival [28]. A glutamine-induced increase in circulating GLP-1 has been shown to enhance insulin secretion and glucose tolerance in obese rats [77] and in humans with diabetes and obesity [37]. The principal action of GLP-2 is to stimulate the proliferation and repair of the intestinal epithelium. Because GLP-1 is coproduced with GLP-1 at a 1:1 molar ratio, it is likely that the protective effects of glutamine on the gut mucosa under cancer therapy are also attributable to the release of GLP-2. Despite its actions in cell proliferation, however, it has been demonstrated that GLP-2 does not modify intestinal tumor cell growth or survival [58].

Conclusions

In summary, a huge body of evidence from experimental as well as from clinical studies confirms that glutamine supplementation in cancer patients improves host metabolism and clinical situation without increasing tumor growth. In vitro results that available glutamine predicts a more aggressive tumor behavior could not be confirmed in vivo.

Oral and especially parenteral glutamine supplementation in comparably high dosages generally enhances the tumoricidal effectivity of chemotherapy while reducing toxicity to host tissues. Underlying mechanisms include glutamine-induced changes in GSH mechanism, modulation of apoptotic regulators, effects on the host immune response to tumors, and the induction of HSPs. Additional beneficial effects of glutamine administration in cancer might derive from the amelioration of glucose intolerance through incretin secretion. Improved glucose utilization might in turn protect muscle tissue from tumor-induced atrophy. However, studies in this respect are warranted.

In clinical setting, sufficient administration of glutamine within a targeted nutrition therapy is a safe and well-tolerated measure to improve life quality of cancer patients. In various clinical situations, appropriate exogenous glutamine supply can beneficially contribute to diminish risks of high-dose chemotherapy and radiation. In addition, there is some evidence that adequate glutamine availability can beneficially affect outcome, especially in patients undergoing bone marrow transplantation.

Conflict of interest statement None.

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