Amino Acids (2014) 46:1059–1067
DOI 10.1007/s00726-014-1670-x

ORIGINAL ARTICLE

Enteral glutamine infusion modulates ubiquitination of heat shock proteins, Grp-75 and Apg-2, in the human duodenal mucosa

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Received: 15 November 2013 / Accepted: 8 January 2014 / Published online: 22 January 2014

Abstract Glutamine, the most abundant amino acid in the human body, plays several important roles in the intestine. Previous studies showed that glutamine may affect protein expression by regulating ubiquitin–proteasome system. We thus aimed to evaluate the effects of glutamine on ubiquitinated proteins in human duodenal mucosa. Five healthy male volunteers were included and received during 5 h, on two occasions and in a random order, either an enteral infusion of maltodextrins alone (0.25 g kg^{-1} h^{-1}, control), mimicking carbohydrate-fed state, or maltodextrins with glutamine (0.117 g kg^{-1} h^{-1}, glutamine). Endoscopic duodenal biopsies were then taken. Total cellular protein extracts were separated by 2D gel electrophoresis and analyzed by an immunodetection using anti-ubiquitin antibody. Differentially ubiquitinated proteins were then identified by liquid chromatography–electrospray ionization MS/MS. Five proteins were differentially ubiquitinated between control and glutamine conditions. Among these proteins, we identified two chaperone proteins, Grp75 and hsp74. Grp75 was less ubiquitinated after glutamine infusion compared with control. In contrast, hsp74, also called Apg-2, was more ubiquitinated after glutamine. In conclusion, we provide evidence that glutamine may regulate ubiquitination processes of specific proteins, i.e., Grp75 and Apg-2. Grp75 has protective and anti-inflammatory properties, while Apg-2 indirectly regulates stress-induced cell survival and proliferation through interaction with ZO-1. Further studies should confirm these results in stress conditions.

Keywords Glutamine · Ubiquitination · Proteasome · Intestine · Human · Heat shock protein

Introduction

Gut homeostasis plays a major role in health and well-being. In the intestinal mucosa, protein turnover

This trial was registered at clinical trials.gov as NCT01254110.

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approaches 50 % per day in humans (Nakshabendi et al. 1996; Coeffier et al. 2003) and results from a balance between protein synthesis and degradation. An alteration of this balance may contribute to intestinal or systemic disease pathophysiology by regulating gut barrier for instance. The ubiquitin–proteasome system (UPS), a major pathway of intracellular protein degradation, is involved in the regulation of many cellular processes in the intestine, i.e., cell proliferation, apoptosis, inflammatory response and antigen presentation (Visekruna et al. 2006; Leblond et al. 2006; Coeffier et al. 2010a; Monaco and Nandi 1995). Proteasome activities are regulated by pathophysiological and nutritional conditions in the gut (Coeffier et al. 2010a; Visekruna et al. 2006; Bertrand et al. 2013a). However, two major steps compose UPS-mediated degradation, i.e., the ubiquitination and the degradation by 26S proteasome, and the specificity of protein degradation is achieved by the ubiquitination step that tags proteins with a small protein called ubiquitin. Ubiquitination results from the activation of ubiquitin residues by an E1 enzyme also called ubiquitin-activating enzyme (Glickman and Ciechanover 2002). Then, an ubiquitin-conjugating enzyme (E2 enzyme) transfers activated ubiquitin to a complex made up of an E3 enzyme. Ubiquitinated proteins lead to different futures according to the type of ubiquitin linkage or of the length of ubiquitin chain (Clague and Urbe 2010). Polyubiquitinated proteins are degraded by the 26S proteasome (Monaco and Nandi 1995; Glickman and Ciechanover 2002). We recently reported that inflammation altered the intestinal pattern of ubiquitinated proteins or ubiquitome in experimental models (Bertrand et al. 2013b), but its modulation by nutrients remains unknown.

The effects of amino acids on protein metabolism have been extensively tested in vitro and in vivo, particularly on protein synthesis (Bertrand et al. 2013a). Glutamine, the preferential substrate of rapidly dividing cells, i.e., enterocytes and immune cells, is associated with less infectious complications in critically ill patients (Dechelotte et al. 2006), that could be partly due to maintenance of gut barrier function (De-Souza and Greene 2005). Glutamine increased protein synthesis in intestinal cells in vitro (Boukhettala et al. 2012) or in hypercatabolic dogs (Humbert et al. 2002), but not in malnourished rats (Tannus et al. 2009). In healthy humans, we previously reported that glutamine stimulated duodenal protein synthesis in fasted (Coeffier et al. 2003), but not in fed state (Coeffier et al. 2013). However, the effects of glutamine on protein degradation and particularly on proteasome-mediated degradation in intestine have been poorly documented. In intestinal epithelial cell line HCT-8, anti-inflammatory effects of glutamine were associated with a decrease of the ubiquitination of IκBα, the inhibitor of NF-κB, without affecting proteasome activities (Hubert-Buron et al. 2006). In Caco-2 cells, glutamine increased NF-κB proteasomal degradation (Lesueur et al. 2012). Glutamine also reduced ubiquitin mRNA in duodenal mucosa of piglets (Adegoke et al. 2003) or humans (Coeffier et al. 2003). In this latter study, proteasome activities were not affected by glutamine. All these data suggest that glutamine is able to regulate intestinal functions or cellular processes through its effects on UPS, but the regulated proteins remains unknown.

To better understand the beneficial effects of glutamine in some stressed or critically ill conditions, we aimed to assess the effects of enteral glutamine on the ubiquitinated protein pattern in the duodenal mucosa in healthy volunteers.

Subjects and methods

Clinical protocol and ethical authorizations

The current study was performed in accordance with the guidelines of the Center for Clinical Investigations, after approval by the local ethics committee (North-west I, France). Five healthy male volunteers gave their written informed consent for this study. The subjects were in good general health and had no hepatic, renal, or cardiac dysfunction or any medical or surgical digestive history. The volunteers had a mean (±SEM) age of 26.8 ± 10.8 years and a mean body mass index of 22.56 ± 1.63 kg/m^2 (Table 1).

During the 3 days before the experimental trial, all subjects consumed a controlled diet providing 30 kcal and 0.9 g protein kg^{-1} day^{-1}. On the morning of the study after an overnight fasting, the subjects received over 5 h, on two occasions, and in a random order by a nasogastric feeding tube either maltodextrins (control condition: 0.25 g kg^{-1} h^{-1}; Lactalis Nutrition Santé, Torcé, France) or maltodextrins plus glutamine (glutamine condition: 0.117 g free L-glutamine kg^{-1} h^{-1}; Cooper, Melun, France). The infusion rate was 3.5 mL kg^{-1} h^{-1}. The dose of glutamine was similar to that used in our previous studies (Coeffier et al. 2003, 2008). To avoid a comparison of glutamine supply to a fasted status, we compared maltodextrins supplemented with glutamine with maltodextrins alone. Thirty minutes after the end of infusion, endoscopic biopsy samples were collected from the duodenal mucosa, immediately snap-frozen in liquid nitrogen, and stored at −80 °C until analysis. In the present study, we compared the effects of glutamine supplementation to maltodextrins alone to mimic a fed state in the control condition, because we previously observed that short-term food restriction did not alter protein synthesis but modified proteins degradation in jejunal mucosa in rats (Boukhettala et al. 2009).

Table 1 Volunteer characteristics

Volunteers/gender		Age (years)	Body weight (kg)	Height (cm)	BMI* (kg/m^2)
1	M	46	73.5	171	25.1
2	M	21	70.4	180	21.7
3	M	24	73	180	22.5
4	M	21	72.5	187	20.7
5	M	22	78	185	22.8
Mean ± SEM		26.8 ± 10.8	73.48 ± 2.78	180.6 ± 6.18	22.56 ± 1.63

* *BMI* body mass index; BMI = [weight (kg)]/[height (m)]^2

Protein extraction and 2DE-separation

Protein extraction and 2DE-separation were performed as previously described (Goichon et al. 2011). Briefly, endoscopic samples were homogenized in ice-cold lysis buffer containing 7M urea, 2M thiourea, 4 % (w:v) CHAPS, 50 mM dithiothreitol, 0.5 % (v:v) IPG buffer pH 3–10 NL (GE Healthcare, Orsay, France), and 1 % protease inhibitor cocktail (P2714; Sigma Aldrich), 0.1 % phosphatases inhibitor cocktail 2 (P5726, Sigma Aldrich) and proteasome inhibitor MG132 at 10 μM. The resulting samples were placed on ice for 30 min and then centrifuged at 12,000×*g* for 20 min at 4 °C. The supernatant fluid was collected, and protein concentrations were measured with the 2D Quant kit (GE Healthcare). Total proteins (350 μg) were resolved in the first dimension by isoelectric focusing for a total of 31,500 V-h by using the IPGphor system (GE Healthcare). After focusing, IPG strips were equilibrated for 20 min in the equilibration buffer [6 M urea, 30 % (v:v) glycerol, 2 % (w:v) sodium dodecyl sulfate, 50 mM Tris–HCl pH 8.8] containing 2 % (w:v) dithiothreitol and then alkylated for an additional 20 min in the equilibration buffer containing 4 % (w:v) iodoacetamide and 0.25 % (w:v) bromophenol blue. IPG strips were then affixed onto 12 % polyacrylamide gels (20 cm × 18 cm × 1 mm) for SDS-PAGE (O'Farrell 1975). The second dimension was performed in the Ettan Daltsix vertical system (GE Healthcare) at 10 °C and 12 mA/gel, 2 W/gel, 150 V during 16–17 h. To ensure reproducibility of gels, five gels of each group (control and glutamine) were prepared and analyzed.

Immunoblotting and quantification of proteins

Following 2DE, proteins were transferred onto Hybond-ECL nitrocellulose membrane (GE Healthcare) by using a liquid transfer method (Transblot Cell, Biorad) and a constant voltage (100 V) for 1h15. After transfer, membranes were blocked with 5 % (w:v) bovine serum albumin (fraction V, Eurobio) in [TBS (10 mM Tris, pH 8; 150 mM NaCl) plus 0.05 % (v:v) Tween 20 = TBS-T]. After washes with TBS-T, membranes were incubated overnight at 4 °C with rabbit polyclonal anti-ubiquitin (1:500, SantaCruz Biotechnology, Le Perray en Yvelines, France), then washed again and incubated with swine anti-rabbit IgG HRP conjugated (1:5,000, Dako, Trappes, France) as previously described (Bertrand et al. 2013b). Immunocomplexes were revealed by using the ECL detection system (GE Healthcare). Immunoblots were scanned with ImageScanner II (GE Healthcare) previously calibrated by using a greyscale marker (Kodak) and digitalized with Labscan 6.00 software (GE Healthcare). Differential analysis was performed by using ImageMaster 2D Platinum v 5.0 software (GE Healthcare) for spot detection, quantification, matching, and comparative analysis. The most representative membrane (gel migration, spot definition, and spot number) of each set was used to test the influence of inflammatory state on protein ubiquitination. Two classes of gels were defined, namely control and glutamine. Differential image analysis and groupwise statistical comparisons were then performed on groups of spots determined from 10 representative gels, 5 in control conditions and 5 in glutamine conditions. The expression level was determined by the relative volume of each spot in the gel and expressed as % volume, calculated as spot volume/Σvolumes of all spots resolved in the gel. This normalized spot volume takes into account variations due to protein loading and staining by considering the total volume over all the spots present in the gel. Variations in abundance were calculated as the ratio of average values of % volume for a group of spots between the two classes. Only spots with a % volume variation ratio ≥ 1.5 were considered relevant. The absence of a spot within a gel indicated that no detectable expression could be reported for the related protein under the selected experimental condition. The corresponding *P* values were determined by using paired Student's *t* test (significance level $P < 0.05$) after spot % volume log-transformation. The corresponding prerequisites for this statistical test were carefully checked.

Protein identification by liquid chromatography–electrospray ionization MS/MS

Spots of interest were manually excised from two new CBB-stained 2D gels (one control and one glutamine gel),

and in-gel digestion of proteins was performed as previously described (Bertrand et al. 2013b). Briefly, gel fragments were washed 3 times for 20 min in destain solution containing 50 mM ammonium bicarbonate and 50 % (v:v) methanol. The spots were then air-dried for 60 min, which was followed by in-gel digestion overnight in 30 µL of a digestion buffer containing 50 mM ammonium bicarbonate and 6 ng/µL sequencing-grade bovine trypsin (Roche Diagnostics). The digestion mixture was extracted with 50 % (v:v) acetonitrile and 5 % (v:v) formic acid. Speed-vac-dried peptide extracts were resuspended in 10 µL of 5 % (v:v) acetonitrile/0.1 % (v:v) formic acid and then analyzed with a nano-LC1200 system coupled to a Q-TOF 6520 mass spectrometer equipped with a nanospray source and an HPLC-chip cube interface (Agilent Technologies).

For protein identification, MS/MS peak lists were extracted and compared with the UniProtKB/Swiss-Prot protein database by using the MASCOT Daemon version 2.2.2 (Matrix Science) search engine. The searches were performed with the following specific parameters: enzyme specificity, trypsin; one missed cleavage permitted; no fixed modifications; variable modifications, methionine oxidation, cysteine carbamidomethylation, serine, tyrosine, and threonine phosphorylation; monoisotopic; peptide charge, 2+ and 3+; mass tolerance for precursor ions, 20 ppm; mass tolerance for fragment ions, 0.06 Da; ESI-QUAD-TOF as instrument; taxonomy, human; database, UniProtKB/Swiss-Prot v55.6. Protein hits were automatically validated if they satisfied the following criterion: identification ≥2 top-ranking peptides (bold and red), each with a MASCOT score of >32 ($P < 0.01$).

Immunoprecipitation of ubiquitinated proteins and western blotting

The regulation of the expression of Grp75 and hsp74 was confirmed with immunoprecipitation experiment followed by western blotting or by western blotting, respectively. Immunoprecipitation was performed with 10 µm cutoff ultrafiltration spin-columns (Pierce/Thermo Scientific, Rockford, IL, USA) and 50 µL (50 % slurry) protein G-agarose beads (Calbiochem, EMD Chemicals, San Diego, USA). First, total protein samples were incubated overnight with antibody directed against protein of interest (monoclonal mouse anti-Grp75, SantaCruz Biotechnology). Then, samples were incubated overnight with beads at 4 °C in a tube rotator. Beads were washed two times with 400 µL of ice-cold PBS. Proteins of interest were finally eluted two times with 50 µL of 5 M urea solution. Eluted samples were loaded on SDS-PAGE gels as previously described. Proteins were transferred on nitrocellulose membranes. After transfer, membranes were soaked in TBS-T/BSA solution [5 % (w:v) BSA] for 1 h at least at room temperature. Then, blots were incubated overnight at 4 °C in TBS-T/BSA with anti-ubiquitin antibody (1:500, SantaCruz Biotechnology) or polyclonal rabbit anti-hsp74 (SantaCruz Biotechnology) or anti-polyubiquitin antibody (Affiniti). Membranes were washed 3 times for 10 min with TBS-T, incubated with swine anti-rabbit IgG HRP conjugated (1:5,000, Dako, Trappes, France) in TBS-T/BSA for 1 h at room temperature, and then washed 3 times in TBS-T. Immunocomplexes were revealed by using the ECL detection system (GE Healthcare). To determine protein abundance, band intensities were quantified by using an ImageScanner II densitometer (GE Healthcare) and the ImageQuant TL analysis software (GE Healthcare).

Statistical analysis

The results are expressed as mean ± SEM and were compared by using GraphPad Prism 5.0 (GraphPad Software Inc). Paired Student's test was used. $P < 0.05$ was considered significant.

Results

Glutamine infusion modifies ubiquitinated protein profile

Glutamine significantly increased the number of spots on nitrocellulose membranes suggesting an increase of ubiquitinated proteins. Indeed, approximately 212 spots were detected in control condition compared with 271 spots in glutamine condition ($P < 0.05$; Fig. 1). These ubiquitinated proteins were comprised within a pI range of 3–10 and a relative molecular mass range within 30–150 kDa (Fig. 2a).

After image analysis, only five spots were differentially (at least 1.5-fold change) and significantly (two-tailed paired Student's t test, $P < 0.05$) affected by glutamine (Table 2). Two and three spots were less and more present after glutamine, respectively (Fig. 2b).

Identification of differentially ubiquitinated proteins

These spots of interest were then manually picked on new 2D Coomassie-stained gels (Fig. 3) and trypsin digestion was performed. Spectral analysis by informatics revealed proteins identification. Among downregulated ubiquitinated proteins, we identified Glucose-related peptide 95 kDa (Grp-75, spot no 1, Fig. 2), a chaperone which belongs to the Heat Shock Proteins (Hsp) family and encoded by the hspa9 gene and Serum Albumin (Albu, spot no 5, Fig. 2). Interestingly, spot for Grp-75 was not found in any volunteers after glutamine condition.

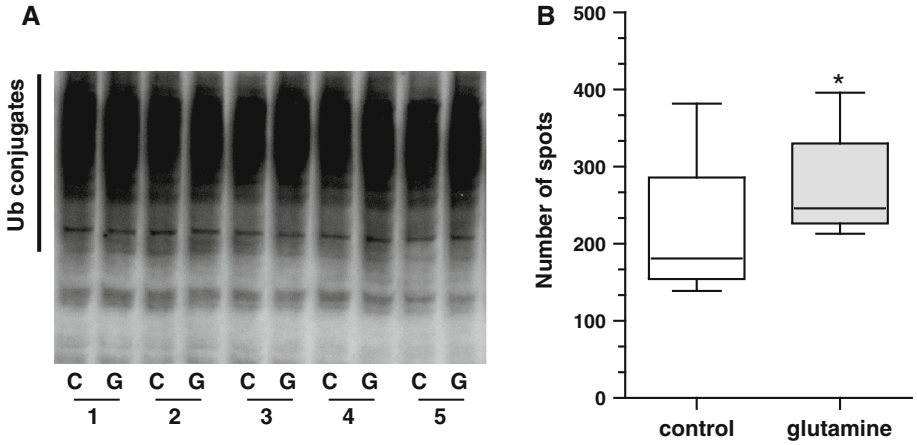

Fig. 1 Polyubiquitinated proteins and number of ubiquitinated proteins detected in nitrocellulose membrane. **a** 1D gel immunoblot using anti-polyubiquitin antibody. **b** 2D immunoblots using anti-ubiquitin antibody revealed an increased of ubiquitinated proteins after glutamine infusion. *C* control, *G* glutamine; $*P < 0.05$ vs control

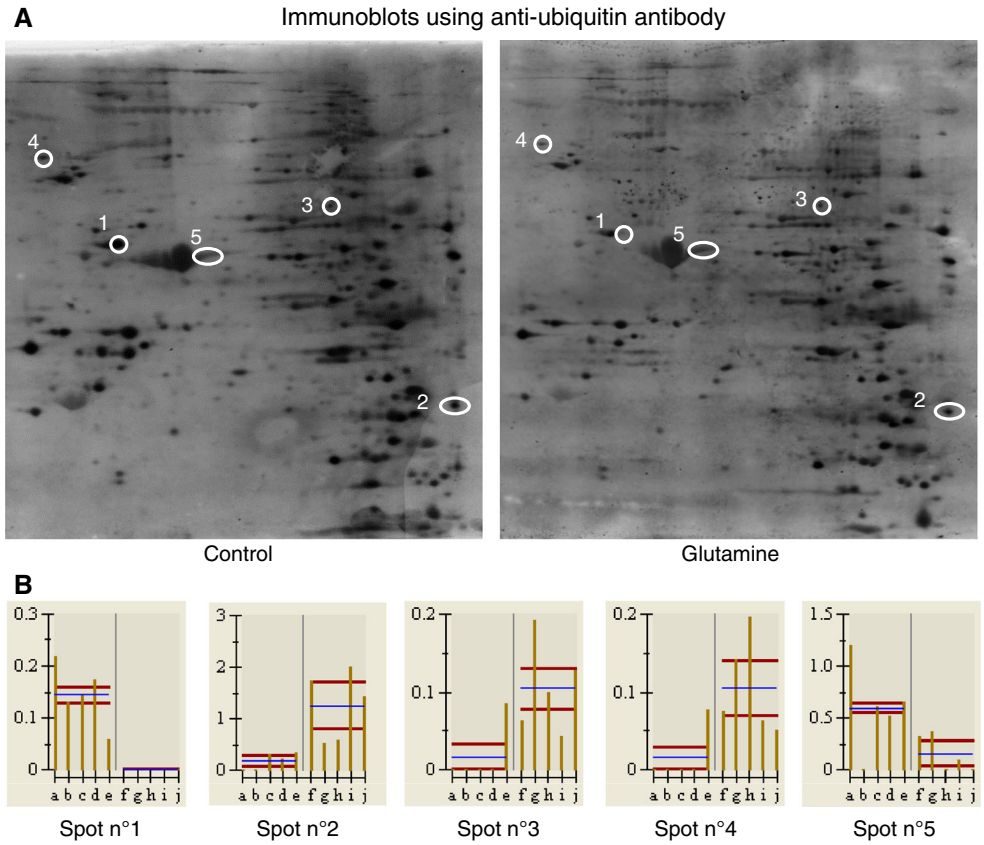

Fig. 2 Ubiquitinated protein pattern in duodenal mucosa after glutamine infusion. **a** Differentially ubiquitinated proteins (i.e., at least ±1.5-fold modulated) between control and glutamine conditions were determined on immunoblot by statistical analysis (Student's *t* test, $P < 0.05$). **b** Results of differential expression of 5 spots. *a–e* Represent controls and *f–j* represent glutamine conditions

Among upregulated ubiquitinated proteins, we identified the Heat Shock Protein 74 kDa or Heat shock 70 kDa protein 4 (Hsp74) encoded by the hspa4 gene (spot no 4, Fig. 2) which is a member of Hsp110 protein family, the Protein transport protein Sec23A (Sec23A, spot no 3, Fig. 2), part of the COPII coat, involved in protein transport between endoplasmic reticulum and Golgi apparatus and the Alcohol dehydrogenase 4 (Adh4, spot no 2, Fig. 2) involved in alcohol transformation in ketone or aldehyde.

We confirmed that Grp75 was less ubiquitinated after glutamine compared with control condition by immunoprecipitation using anti-Grp75 antibody followed by immunoblot using anti-ubiquitin antibody (Fig. 4). We also showed that free Grp75 expression was increased after

Table 2 Identification of duodenal ubiquitinated proteins differentially expressed after 5 h of glutamine infusion in healthy volunteers

Spot number	Swiss-Prot accession number	Protein name	Short name	pI[a]	MW[a] (kDa)	Ratio [b]	*P* value[$]	Score on mascot	Sequence coverage (%)	Identified peptides number
1	P38646	Stress-70 protein, mitochondrial	Grp75	5.87	73.6	−P/A	0.0055	555	51	28
2	P08319	Alcohol dehydrogenase 4	Adh4	8.25	40.2	+2.66	0.0230	413	48	20
3	Q15436	Protein transport protein Sec23A	Sec23A	6.64	86.1	+2.3	0.0048	333	30	25
4	P34932	Heat shock 70 kDa protein 4	Hsp74/ Apg2	5.18	94.2	+2.24	0.0284	594	24	26
5	P02768	Serum albumin	Albu	5.92	69.3	−1.8	0.033	605	44	44

[a] Theoretical isoelectric point and molecular mass

[b] + upregulated, − downregulated, *P/A* present/absent in control/glutamine conditions

[$] Paired Student's test

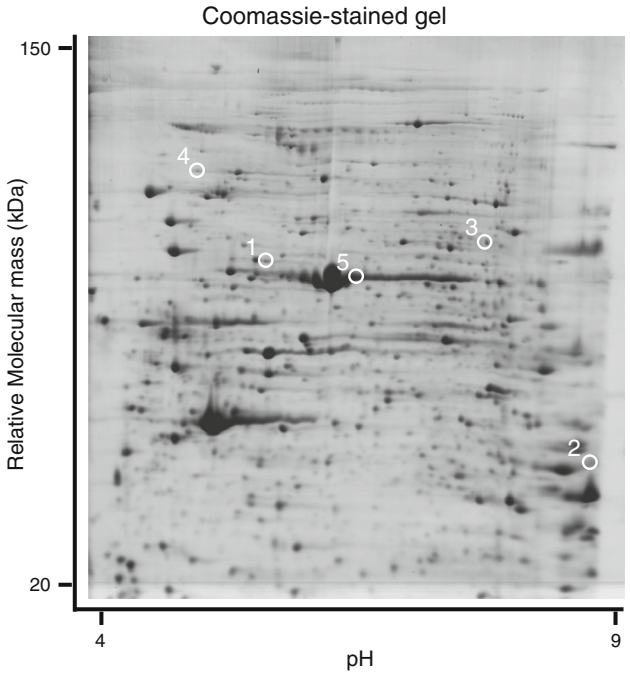

Fig. 3 Coomassie blue-stained 2-dimensional gel image representing total proteins extracted from duodenal mucosa. After determination of differentially ubiquitinated proteins on immunoblot, spots were checked and picked on classic Coomassie-stained gel and analyzed by liquid chromatography–tandem mass spectrometry. Protein identification results are depicted in Table 2

glutamine (Fig. 4). In contrast, free hsp74 expression was reduced after glutamine compared with control condition.

Discussion

In the present work, we studied ubiquitinated proteins modifications after glutamine infusion and we report that glutamine affected the ubiquitination level of specific proteins, i.e., heat shock proteins, in the duodenal mucosa in humans.

Glutamine is the preferential substrate of rapidly dividing cells such as epithelial cells (Rhoads et al. 1997) and regulates many cellular processes as immune and inflammatory response (Calder and Yaqoob 1999), redox status (Belmonte et al. 2007), nucleic acids synthesis (Labow and Souba 2000), for instance. Intestinal glutamine content is decreased during intestinal inflammation (Dechelotte et al. 2006; Sido et al. 2006) and glutamine supplementation may regulate gut barrier function (Beutheu et al. 2013a; Amasheh et al. 2009). In critically ill conditions, plasma glutamine is reduced (Dechelotte et al. 2006; Houdijk et al. 1998) and intravenous or enteral glutamine supplementation was associated with a reduction of complicated outcomes (Dechelotte et al. 2006; Houdijk et al. 1998). Pretreatment with glutamine has also been

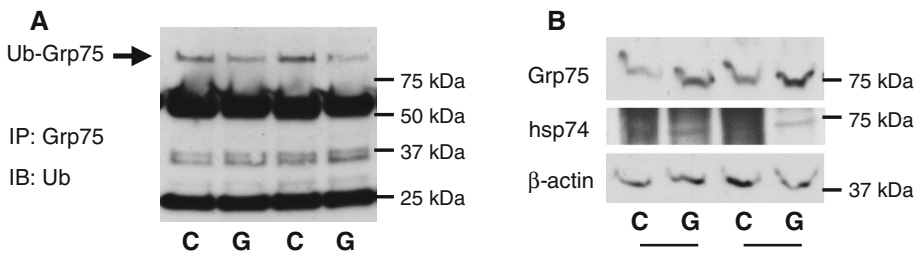

Fig. 4 Expression of Grp75 and hsp74 in human duodenal mucosa after glutamine infusion. Expression of ubiquitinated (**a**) and free (**b**) forms of Grp75 and hsp74 in duodenal mucosa of healthy subjects receiving either control (C) or glutamine-supplemented solution (G)

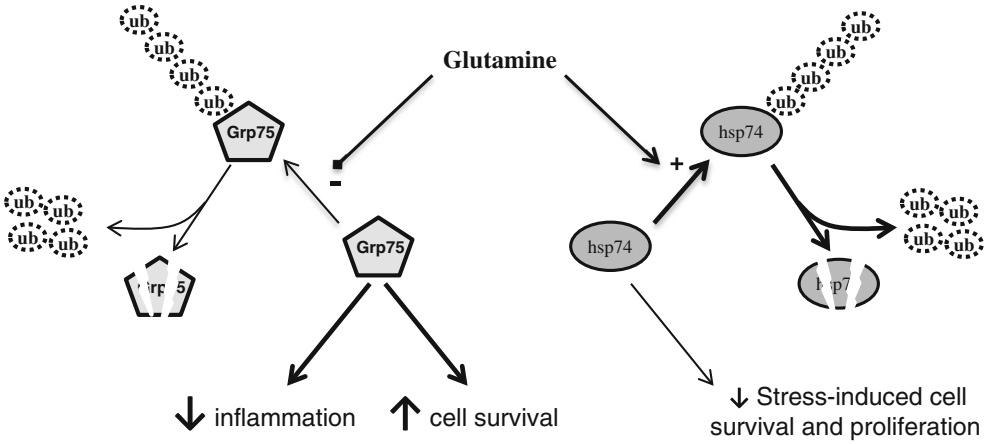

Fig. 5 Effects of glutamine on Grp75 and hsp74 ubiquitination processes and putative consequences on inflammation, cell proliferation and survival. Glutamine decreases Grp75 ubiquitination and thus increased free Grp75 expression which has been shown to reduce inflammatory response and enhance cell survival. Glutamine increased hsp74 ubiquitination and thus reduced free hsp74 expression that may regulate stress-induced cell survival and proliferation

reported to limit chemotherapy or surgery-induced intestinal injury (Yue et al. 2013; Owari et al. 2012; Zheng et al. 2006; Xue et al. 2011; Beutheu et al. 2013a, b). In addition, we previously showed that enteral glutamine infusion decreased ubiquitin mRNA compared with control isonitrogenous amino acids mixture in the duodenal mucosa of healthy volunteers (Coeffier et al. 2003). Similar results were observed in piglets (Adegoke et al. 2003). However, glutamine did not affect proteasome activities (Hubert-Buron et al. 2006; Coeffier et al. 2003, 2013). We thus focused on the pattern of ubiquitinated proteins to better understand the effects of glutamine on the ubiquitin proteasome system.

In our experiments, we did not use deubiquitinase inhibitors in sample lysis buffer. Deubiquitinases play a key role in the regulation of ubiquitination of proteins as reviewed (Clague and Urbe 2010). In addition, the type of ubiquitin linkage also contributes to the future of the protein, i.e., degradation by the 26S proteasome or cell localization or activation (Clague and Urbe 2010). In our tested conditions, glutamine modified the expression of five ubiquitinated proteins that are involved in different cellular processes. Among these five proteins, we chose to focus on two proteins that belong to heat shock protein family.

Grp-75, a member of Hsp 70 family (Wadhwa et al. 1993a), is involved in many processes including intracellular trafficking, proliferation, tumorigenesis and stress response (Sitja-Bobadilla et al. 2008). Grp-75 has a protective function especially in mitochondria homeostasis and interacts with newly synthesized mitochondrial proteins (Mizzen et al. 1991). Grp-75 was first described as mortalin for its immortalization properties on mouse and human embryonic fibroblasts (Wadhwa et al. 1993a, b). Grp-75 was also called mtHsp70 due to the predominant mitochondrial localization of this protein (Bhattacharyya et al. 1995). We recently reported that Grp-75 expression was decreased in intestinal inflammatory conditions due to an increase of its ubiquitination (Bertrand et al. 2013b). Interestingly, Grp-75 overexpression induced a reduction of inflammatory response in murine microglia cells through the regulation of NF-κB pathway (Volobouéva et al. 2013). In the present study, glutamine reduced ubiquitinated Grp-75 and increased free Grp-75 expression. Our results are in accordance with a previous study showing that glutamine enhanced PC12 cell viability by increasing Grp-75 expression (Liu et al. 2005). The upregulation of Grp-75 expression by glutamine may contribute to the protective effects of glutamine on intestinal cell viability or inflammatory response (Coeffier et al. 2010b). It was also suggested that beneficial effects of glutamine was mediated by an increase of hsp70 in humans (Ziegler et al. 2005). In accordance with available data, we thus speculate that the regulation of Grp-75 ubiquitination may occur in the beneficial effects of glutamine (Fig. 5). Further studies should be done to confirm this hypothesis in stressed models and to identify the ubiquitin ligase E3 specific for Grp-75 that remains unknown.

Interestingly, we also observed that Protein transport protein Sec23A (Sec23A), which covers ER-derived vesicles involved in transport from the endoplasmic reticulum to the Golgi apparatus, is more ubiquitinated after glutamine suggesting a downregulation. In a recent study, Szczyrba et al. (2011) showed that inhibition of Sec23A by siRNA stimulated cell proliferation. Given that glutamine is the preferential substrate of rapid dividing cells, upregulation of Sec23A could be a part of the cell proliferation enhancement by glutamine.

Hsp74, also called Apg-2 (Kaneko et al. 1997), belongs to the Hsp110 family and was first described as hsp70 RY from B cells (Fathallah et al. 1993) and is encoded by hspa4 gene. Apg-2 has been shown to regulate indirectly cell proliferation, differentiation or cell survival. Indeed Apg-2 compete with Zonula Occludens 1 (ZO-1) -associated nucleic acid binding protein (ZONAB) to interact with the Src homology 3 (SH3) domain of ZO-1 (Tsapara et al. 2006). ZONAB is a transcription factor regulating intracellular signaling pathways in epithelial cells (Balda and Matter 2000). In the present study, glutamine increased Apg-2 ubiquitination and reduced Apg-2 expression. We can thus speculate that ZONAB should be more sequestrated in the cytosol by linking to ZO-1 (Aijaz et al. 2007; Tsapara et al. 2006) (Fig. 5). Interestingly, hspa4 gene expression is increased during intestinal inflammation induced by TNBS in zebrafish (Crawford et al. 2011). The effect of glutamine on Apg-2 ubiquitination and expression should be further evaluated in inflammatory conditions as glutamine was previously described to have anti-inflammatory properties (Coeffier et al. 2010b).

In conclusion, we demonstrated that glutamine may regulate ubiquitination processes of proteins involved in stress response, Grp75 and Apg-2. Further studies should be done to confirm these results in pathophysiological conditions and to precisely elucidate the contribution of these mechanisms both in the regulation of Grp75 and Apg-2 function and in the beneficial effects of glutamine.

Acknowledgments This study was support by the French Agency for Research (Agence Nationale pour la Recherche, ANR-07-PNRA-020) and by the Nutricia Research Foundation. These funders did not interfere in the design, implementation, analysis and interpretation of the data.

Conflict of interest The authors declare that they have no conflict of interest.

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