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Effect of alanyl-glutamine on leucine and protein metabolism in irradiated rats

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Summary. The mechanism by which glutamine produces a favorable effect in the treatment of sepsis, injury, burns and abdominal irradiation is not completely understood. The main aim of this study was to evaluate the effect of alanyl-glutamine (AlaGln) administration on the metabolism of proteins in irradiated rats. The rats were exposed to whole-body irradiation (8 Gy) and then fed intragastrically with a mixture of glucose and amino acids either with AlaGln or without AlaGln. At 48 hours after irradiation, parameters of whole-body protein metabolism and DNA synthesis in intestinal mucosa were investigated using a primed, continuous infusion of [1-^{14}C]leucine and [^{3}H]thymidine. In addition, we evaluated the effect of irradiation and AlaGln on gut morphology, blood count and amino acid concentrations in blood plasma and skeletal muscle. Control rats were not irradiated but were given identical treatment. An increase in whole-body leucine oxidation, and insignificant changes in whole-body proteolysis and in protein synthesis were observed after irradiation. In irradiated rats we observed a decrease in muscle glutamine concentration, a decrease in protein synthesis in jejunum, colon and heart, and an increase in synthesis of proteins of blood plasma and spleen. Morphological examination and measurement of DNA synthesis failed to demonstrate any favorable effect of AlaGln supplementation on irradiated gut. However, administration of AlaGln resulted in a decrease in whole-body proteolysis and leucine oxidation which caused an increase in the fraction of leucine incorporated into the pool of body proteins. We conclude that the data obtained demonstrate that irradiation induces metabolic derangement associated with increased oxidation of essential branched-chain amino acids

(valine, leucine and isoleucine) and that these disturbances can be ameliorated by administration of AlaGln.

Keywords: Amino acids – Glutamine – Alanine – Ionising radiation – Leucine – Metabolism – Nutrition – Protein synthesis

Introduction

Gamma irradiation induces breakdown of skeletal muscle that results in the release of amino acids from irradiated muscle into the blood stream (Schwenen et al., 1989). This alteration in protein metabolism is probably the principal pathogenic factor of radiation myopathy, fatigue and muscle wasting in patients receiving radiation therapy (Kurohara et al., 1961). However, it should be pointed out that enhanced release of the amino acids from skeletal muscle can be of significant physiological importance. Especially in the case of maldigestion and malabsorption caused by radiation injury of the gastrointestinal tract the amino acids released by skeletal muscle to the blood stream begin to be an essential substrate for several tissues.

The amino acids which are released in the largest quantities from skeletal muscle are alanine and glutamine (Ruderman and Berger, 1974; Chang and Goldberg, 1978). Alanine is used mainly in the liver for gluconeogenesis which is activated in radiation disease (Borovikova et al., 1985). Glutamine is an important energy substrate and donates nitrogen for synthesis of nucleotides, ammonia and amino sugars. Important users of glutamine are the kidneys, small intestine, liver and cells of the immune system (Souba et al., 1985; Calder, 1994; Buchman, 1996). Some studies demonstrated increased utilization of glutamine, often associated with its decreased plasma and tissue levels, in sepsis, injury, burns, surgery and endurance exercise. Provision of exogenous glutamine in these conditions appears to improve nitrogen balance and the speed of patient recovery (Hammarqvist et al., 1989; Hickson et al., 1996). However, the mechanism of the favorable effect of glutamine on protein metabolism is obscure (Ziegler et al., 1996; Fürst, 1998). The healing effect of glutamine has been reported also after abdominal irradiation. Glutamine supplementation increased survival, diminished diarrhoea and the incidence of bowel perforation in irradiated rats. These metabolic improvements were associated with a marked increase in villous height, villous number, and number of mitoses per crypt (Klimberg et al., 1990). The beneficial effect of glutamine on the integrity of intestinal mucosa and bacterial translocation to mesenteric lymph nodes after abdominal radiation was demonstrated also by Karatzas et al. (1991).

The cause of the grater release of alanine and glutamine from skeletal muscle into the blood stream than of the other amino acids is the active synthesis of these amino acids in muscle tissue. The principal donor of nitrogen for *de novo* synthesis of alanine and glutamine in skeletal muscle is a group of branched-chain amino acids (BCAA), i.e. valine, leucine and isoleucine (Ruderman and Berger, 1974; Odessey et al., 1974; Harper et al., 1984). On the basis of the significance of BCAA in the synthesis of alanine and glutamine it can be hypothesized that administration of alanine and glutamine

may affect BCAA metabolism and that some metabolic effects of administration of glutamine and alanine are related to their effect on BCAA metabolism. The regulatory effect of BCAA on protein metabolism has been demonstrated by many studies (Harper et al., 1984).

The main task of this study was to evaluate the effect of alanine and glutamine on leucine turnover and on protein metabolism in irradiated rats. In addition, we estimated the effect of supplementation of alanine and glutamine on irradiated gut mucosa. Since glutamine is very unstable, the stable dipeptide alanyl-glutamine (AlaGln) was used in this study. The further advantage of supplementation of alanine and glutamine in the form of dipeptides is that more amino acids can be administered per unit volume in peptide form than in free form. Therefore, some problems with hypertonicity and fluid overload can be reduced by the use of AlaGln when parenteral administration is necessary.

Materials and methods

Animals

Male Wistar rats (Velaz, Prague, Czech Republic) were housed in standardized cages in quarters with controlled temperature and a 12-hour light-dark cycle and received Velaz-Altromin 1320 laboratory chow and drinking water *ad libitum*. All procedures involving animals were performed according to guidelines set by the Institutional Animal Use and Care Committee of Charles University. The experiments were started between 7 and 8 AM to minimize the influence of diurnal variations of food intake and hormone levels.

Materials

L-[1-^{14}C]leucine and [^{3}H]thymidine were purchased from Amersham International (Buckinghamshire, UK), [^{14}C]bicarbonate was obtained from Du Pont-NEN (Bad Homburg, Germany). Leucine, methylbenzethonium hydroxide, Folin-Ciocalteu phenol reagent and albumin were purchased from Sigma Chemical (St. Louis, MO). AlaGln was obtained from Ajinomoto Co. (Tokyo, Japan). The remaining chemicals were obtained from Lachema (Brno, CR). Amino acid concentrations in deproteinized samples of blood plasma or tissues were determined with high-performance liquid chromatography (Waters, Milford, MA) after precolumn derivatization with *o*-phthaldialdehyde. The radioactivity of the samples was measured with the liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, Fullerton, CA).

Experimental design

The experiments were performed separately as follows. The rats were exposed to irradiation of the whole body with 8 Gy generated by a ^{60}Co-source (Chirana, Prague, CR). Immediately and at 8, 24, 32 and 40 hours after irradiation, 3 ml of mixture of 60% glucose either with amino acids or amino acids plus AlaGln (see Table 1) was administered intragastrically. By intragastric feeding we provided 298 kJ/kg/day (approximately 25% of the daily needs of energy) and 0.4 g N/kg/day. The dose of AlaGln was 0.83 g/kg/day. Non-irradiated animals treated in the same manner served as controls. As we did not cover the remaining nutritional needs, the energy and nitrogen amount provided to all groups was identical. At 48 hours, each animal was placed in a glass

Table 1. Composition of the used solutions (3 ml)

	Control mixture	AlaGln mixture
Glucose	1.8 g	1.8 g
Isoleucine	13.2 mg	8.8 mg
Leucine	25.8 mg	17.2 mg
Lysine	17.3 mg	11.5 mg
Methionine	12.0 mg	8.0 mg
Phenylalanine	13.8 mg	9.2 mg
Threonine	12.0 mg	8.0 mg
Tryptophane	5.4 mg	3.6 mg
Valine	16.8 mg	11.2 mg
Arginine	32.4 mg	21.6 mg
Histidine	13.2 mg	8.8 mg
Alanine	23.4 mg	15.6 mg
Glycine	20.4 mg	13.6 mg
Aspartate	23.0 mg	15.3 mg
Proline	25.2 mg	16.8 mg
Serine	27.6 mg	18.4 mg
Tyrosine	24.6 mg	16.4 mg
Alanyl-glutamine	0.0 mg	100.0 mg
Total AA	300.6 mg	300.4 mg
Nitrogen content	45.9 mg	49.9 mg
Energy	35.7 kJ	35.7 kJ

metabolic cage to enable the collection of expired air, and was infused intravenously with L-[1-^{14}C]leucine (1.85 μCi/ml) and [^{3}H]thymidine (23.31 μCi/ml) in saline or in 20% AlaGln (for rats to which diet supplemented with AlaGln was administered). A priming dose of 0.7 ml (i.e. 1.30 μCi of [1-^{14}C]leucine and 16.32 μCi of [^{3}H]thymidine) was followed by a constant infusion at a rate 0.36 ml/h for 200 min. The rats were killed by exsanguination via the abdominal aorta exactly at the 201st min from the beginning of the infusion. Afterwards the liver, gastrocnemius muscle, spleen, kidney, small intestine, colon, lungs and heart were quickly removed and immediately frozen in liquid nitrogen.

Parameters of leucine metabolism

The parameters of whole body leucine metabolism were evaluated at steady-state conditions by the procedure as described in detail previously (Holeček et al., 1996, 1997). The expired CO_2 was trapped at 10-min intervals between 125th and 185th min of infusion by monoethanolamine. The average value of six measurements of $^{14}CO_2$ radioactivity in expired air at steady-state condition was used for calculations of leucine oxidation rate. The $^{14}CO_2$ recovery factor (FR) was about 90% in both control and experimental animals. The blood plasma leucine specific activity was measured in the blood collected at termination of infusion. Leucine specific activity, turnover, clearance and decarboxylation rates were calculated by the following formulas:

$$\text{Specific activity } \left(SA_{Leu}\right) \left(\text{dpm} \cdot \mu\text{mol}^{-1}\right) = \frac{\text{leucine radioactivity } \left(\text{dpm} \cdot \text{ml}^{-1}\right)}{\text{leucine concentration } \left(\mu\text{mol} \cdot \text{ml}^{-1}\right)}$$

$$\text{Turnover rate } \left(Q_{Leu}\right) \left(\mu mol \cdot h^{-1}\right) = \frac{\text{infusion rate } \left(dpm \cdot h^{-1}\right)}{SA_{Leu} \text{ in plasma } \left(dpm \cdot \mu mol^{-1}\right)}$$

$$\text{Clearance rate } \left(C_{Leu}\right) \left(ml \cdot h^{-1}\right) = \frac{Q_{Leu} \left(\mu mol \cdot h^{-1}\right)}{\text{plasma leucine } \left(\mu mol \cdot ml^{-1}\right)}$$

$$\text{Decarboxylation rate } \left(D_{Leu}\right) \left(\mu mol \cdot h^{-1}\right) = \frac{^{14}CO_2 \text{ production rate } \left(dpm \cdot h^{-1}\right)}{SA_{Leu} \text{ in plasma } \left(dpm \cdot \mu mole^{-1}\right) \cdot FR}$$

Whole body leucine metabolism was considered to take place within a common metabolic pool, represented by free plasma leucine. Leucine leaves the pool is either incorporated into protein (In) or oxidized (D). Due to the fact that exogenous leucine intake (E) was zero in our protocol, leucine turnover (Q) estimates the leucine released from protein, i.e. the protein breakdown (B) as described by the equation: Q = In + D = B + E. Using this formula, rates of leucine incorporation into protein, the oxidized fraction of leucine (OF = D·100/Q) and the fraction of leucine incorporated into protein (IF = In·100/Q) were calculated.

Protein synthesis and protein content

The samples for measurement of protein synthesis were processed as described elsewhere (Holeček et al., 1997). The fractional protein synthesis rates were calculated by using the equation derived by Garlick et al. (1973) and expressed as the fraction of protein mass renewed each day, in percent per day (Ks). Protein content was measured according Lowry et al. (1951).

DNA synthesis and DNA content

Samples of liver and jejunal mucosa to measure the DNA specific activity were established according to Bucher and Swaffield (1964). The DNA content was determined by the diphenylamine reaction (Burton, 1956).

Haematological examination

Blood was mixed in tube containing K_3EDTA (1.5 mg/ml blood). Blood count was evaluated using a blood particles analyzer Coulter Counter JT3 (Coulter Electronics, Luton, UK).

Histology

Samples of jejunum and colon were stained with haematoxylin and eosin and the degree of injury and repair was evaluated.

Statistical analysis

Results are expressed as the mean ± SEM. For statistical analysis the analysis of variance (two fixed factors) was used. Then a multiple post-hoc Bonferroni t-test followed. This test adjusts the significance level for the fact that multiple comparisons are made (6 comparisons with control). The overall significance level used was 0.05. Statistical software NCSS60 was used for analysis.

Results

Characteristics of the experimental animals (Table 2). A more significant decrease in body weight was observed in the irradiated rats than in the non-irradiated rats. Leukopenia developed within two days after irradiation. In addition, in irradiated animals a lower red blood cell count, hematocrit, haemoglobin concentration and platelet number were detected. These changes (related both to a shortened life-span of erythrocytes and to an inhibition of erythropoietic activity) were not affected by AlaGln. Lower weights of the small intestine and spleen were observed in the irradiated rats than in the non-irradiated rats, while the liver weight was higher in the irradiated animals. The lower weights of the small intestine and spleen were associated with a lower protein content. Because of a lower concentration of protein in the hepatic tissue of irradiated animals, there were no differences in the content of liver protein. The increase in liver weight in irradiated rats is caused mainly by an accumulation of glycogen and lipids (Streffer, 1966). We did not observe an obvious effect of AlaGln supplementation on tissue weights and protein contents.

DNA in intestinal mucosa and morphological examination of the gut. Significantly lower values of DNA concentration and higher values of labeled thymidine incorporation in DNA were observed in the jejunal mucosa of irradiated animals (Table 3). The changes in DNA synthesis that were not affected by AlaGln supplementation probably reflect the recovery of the damaged gut mucosa. The histological examination of jejunum and colon two days after irradiation (Fig. 1) revealed shorter villi, increased cellular infiltration of the lamina propria and thinner mucosa with decreased lymphatic infiltration of nodules. The differences between AlaGln treated and untreated animals were undetectable.

Parameters of leucine metabolism (Table 4). In irradiated rats we observed an increase in whole-body leucine oxidation which caused a marked increase in the leucine oxidized fraction and a decrease in the leucine fraction incorporated into the pool of body proteins. Infusion of AlaGln caused a marked decrease in leucine turnover, leucine oxidation, the leucine oxidized fraction and an increase in the leucine fraction incorporated in proteins.

Protein synthesis in specific tissues (Table 5). The fractional rate of protein synthesis at 48 h after irradiation was significantly lower in jejunum, colon and heart and higher in plasma and spleen than in the non-irradiated animals. In the jejunum of irradiated rats treated by AlaGln, a higher protein synthesis was observed than in rats treated by the mixture without AlaGln. Unfortunately, this increase was not significant based on our criteria.

Table 2. Characteristics of experimental animals

Variable	Non-irradiated animals		Irradiated animals		Significance
	−AlaGln (n = 7)	+AlaGln (n = 6)	−AlaGln (n = 6(5))	+AlaGln (n = 6)	
Body weight					
– initial (g)	248 ± 2	243 ± 3	243 ± 4	241 ± 4	
– final (g)	228 ± 3	219 ± 5	210 ± 3[c]	209 ± 2[c]	[a]
– difference (%)	−8.1 ± 1.0	−10.0 ± 1.6	−13.4 ± 0.5[c]	−13.0 ± 1.0[c]	[a]
Blood					
– WBC (10^9/l)	6.4 ± 0.9	4.7 ± 0.4	1.5 ± 0.1[c]	1.5 ± 0.2[c]	[a]
– RBC (10^{12}/l)	8.2 ± 0.1	8.5 ± 0.1	7.8 ± 0.1	7.5 ± 0.2[c]	[a]
– HCT (%)	46.4 ± 0.7	49.0 ± 0.6	44.4 ± 0.8	43.2 ± 1.2[c]	[a]
– HGB (g/l)	160 ± 3	165 ± 2	158 ± 1	156 ± 3	[a]
– PLT (10^9/l)	997 ± 48	705 ± 115	603 ± 100[c]	597 ± 114[c]	[a]
– plasma protein (mg/ml)	68 ± 4	69 ± 4	58 ± 2	60 ± 6	[a]
Liver					
– weight (g/kg b.w.)	28.5 ± 0.9	28.3 ± 1.5	34.3 ± 0.9[c]	34.6 ± 0.7[c]	[a]
– protein (mg/g)	198 ± 7	199 ± 9	169 ± 13	160 ± 9[c]	[a]
(mg/kg b.w.)	5,663 ± 331	5,640 ± 448	5,780 ± 350	5,540 ± 308	
Muscle					
– protein (mg/g)	155 ± 10	164 ± 7	160 ± 4	146 ± 5	
Kidney					
– weight (g/kg b.w.)	3.51 ± 0.16	3.52 ± 0.17	3.76 ± 0.12	3.79 ± 0.11	
– protein (mg/g)	156 ± 5	140 ± 7	134 ± 15	153 ± 9	
(mg/kg b.w.)	545 ± 22	496 ± 42	501 ± 49	581 ± 38	
Small intestine					
– weight (g/kg b.w.)	30.9 ± 2.5	30.8 ± 1.5	24.3 ± 0.6[c]	23.9 ± 0.3[c]	[a]
– protein (mg/g)	88 ± 6	84 ± 5	81 ± 5	79 ± 4	
(mg/kg b.w.)	2,648 ± 126	2,557 ± 69	1,953 ± 107[c]	1,905 ± 117[c]	[a]
Colon					
– weight (g/kg b.w.)	6.63 ± 0.52	6.12 ± 0.56	5.53 ± 0.35	5.92 ± 0.27	
– protein (mg/g)	90 ± 8	94 ± 1	104 ± 4	95 ± 8	
(mg/kg b.w.)	604 ± 71	578 ± 56	569 ± 26	553 ± 29	
Heart					
– weight (g/kg b.w.)	3.25 ± 0.15	3.10 ± 0.11	3.25 ± 0.11	3.27 ± 0.10	
– protein (mg/g)	131 ± 7	133 ± 4	123 ± 8	114 ± 6	[a]
(mg/kg b.w.)	429 ± 36	411 ± 15	400 ± 30	375 ± 27	
Spleen					
– weight (g/kg b.w.)	2.04 ± 0.09	1.82 ± 0.05	1.08 ± 0.03[c]	1.27 ± 0.03[c]	[a]
– protein (mg/g)	130 ± 9	140 ± 5	171 ± 7[c]	169 ± 9[c]	[a]
(mg/kg b.w.)	263 ± 13	254 ± 7	185 ± 9[c]	213 ± 11[c]	[a]

Values are means ± SEM. [a] $p < .05$ statistically significant effect of irradiation; [c] $p < .05$ vs. non-irradiated rats without AlaGln.

Table 3. DNA content and DNA synthesis in jejunum

Variable	Non-irradiated animals		Irradiated animals		Significance
	−AlaGln (n = 7)	+AlaGln (n = 6)	−AlaGln (n = 6)	+AlaGln (n = 6)	
DNA concentration (μg/g)	2,257 ± 264	2,337 ± 455	1,427 ± 107	1,245 ± 136	[a]
DNA synthesis (dpm/μg DNA)	125 ± 17	95 ± 7	224 ± 36[c]	216 ± 30[c]	[a]

Values are means ± SEM. [a] $p < .05$ statistically significant effect of irradiation; [c] $p < .05$ vs. non-irradiated rats without AlaGln.

Table 4. Parameters of whole body leucine metabolism

Variable	Non-irradiated animals		Irradiated animals		Significance
	−AlaGln (n = 7)	+AlaGln (n = 6)	−AlaGln (n = 6)	+AlaGln (n = 6)	
Turnover (μmol·kg^{-1}·h^{-1})	184 ± 13	174 ± 11	205 ± 9	151 ± 9[d]	[b]
Oxidation (μmol·kg^{-1}·h^{-1})	27.2 ± 3.7	12.7 ± 1.1	45.0 ± 7.2[c]	18.2 ± 3.9[d]	[a,b]
Incorporation (μmol·kg^{-1}·h^{-1})	157 ± 14	162 ± 11	160 ± 10	133 ± 7	
Oxidized fraction (%)	15.2 ± 2.1	7.5 ± 0.9	22.1 ± 3.4	11.7 ± 1.9[d]	[a,b]
Incorporated fraction (%)	84.8 ± 2.1	92.5 ± 0.9	77.9 ± 3.4	88.3 ± 1.9[d]	[a,b]
Clearance (ml·kg^{-1}·h^{-1})	995 ± 58	1,016 ± 24	934 ± 24	872 ± 41	[a]

Values are means ± SEM. [a] $p < .05$ statistically significant effect of irradiation; [b] $p < .05$ statistically significant effect of AlaGln; [c] $p < .05$ vs. non-irradiated rats without AlaGln; [d] $p < .05$ vs. irradiated rats without AlaGln.

Table 5. Fractional rate of protein synthesis in various tissues (%·day^{-1})

Variable	Non-irradiated animals		Irradiated animals		Significance
	−AlaGln (n = 7)	+AlaGln (n = 6)	−AlaGln (n = 5)	+AlaGln (n = 6)	
Blood plasma	409 ± 55	508 ± 176	913 ± 140[c]	623 ± 62	[a]
Liver	18.5 ± 3.0	22.1 ± 5.4	30.9 ± 4.8	27.0 ± 5.0	
Muscle	1.1 ± 0.2	1.8 ± 0.2	1.3 ± 0.4	1.0 ± 0.1	
Kidney	41.9 ± 6.0	28.9 ± 4.1	32.3 ± 8.3	30.0 ± 3.3	
Jejunum	112 ± 18	104 ± 7	60 ± 12[c]	97 ± 8	[a]
Colon	39.2 ± 3.9	34.6 ± 2.6	22.8 ± 3.4[c]	27.3 ± 3.6	[a]
Heart	9.8 ± 1.3	8.4 ± 1.5	3.3 ± 0.4[c]	4.3 ± 0.7[c]	[a]
Spleen	58 ± 16	109 ± 23	250 ± 57[c]	199 ± 29[c]	[a]

Values are means ± SEM. [a] $p < .05$ statistically significant effect of irradiation; [c] $p < .05$ vs. non-irradiated rats without AlaGln.

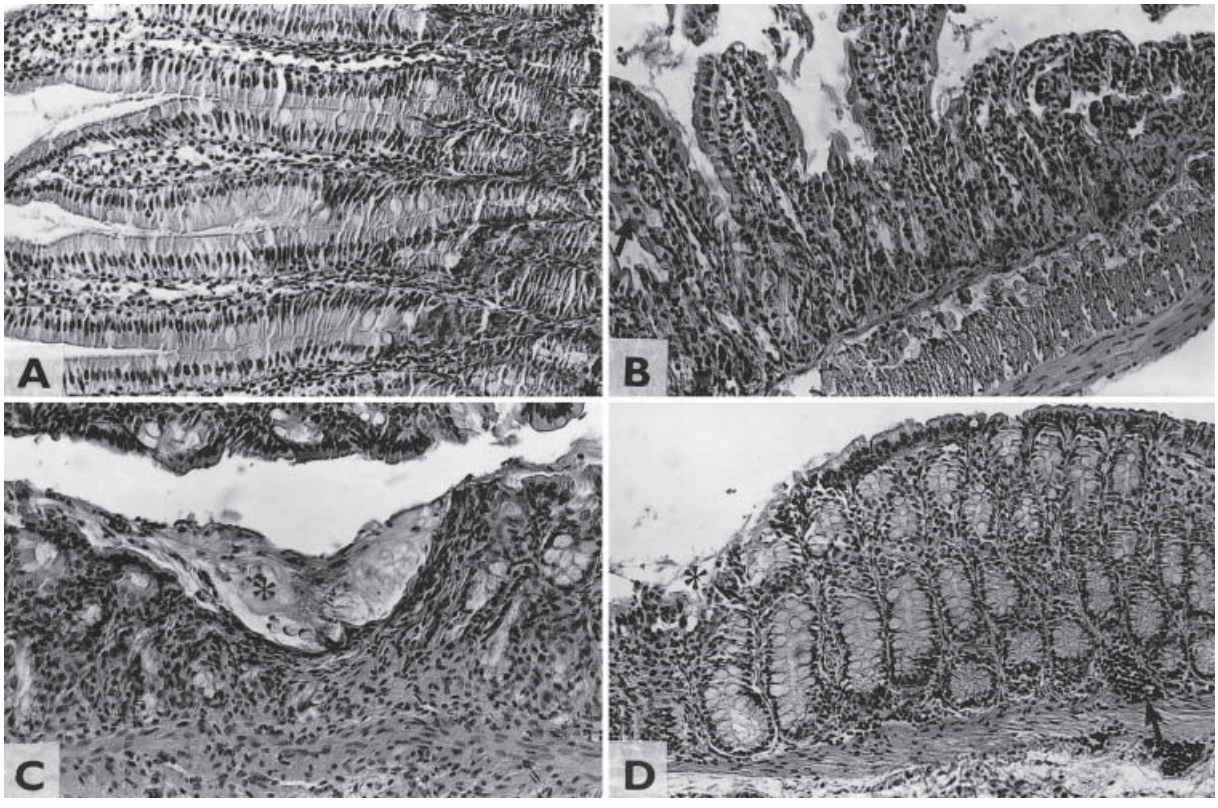

Fig. 1. Morphological examination of jejunum and colon. **A** Normal morphological picture of jejunum with regular villi shape, absorptive epitelium and crypts of Lieberkühn (right). H&E × 125. **B** Jejunum after irradiation, H&E × 120. The villi are shorter, degraded rarely (right) with increased cellular infiltration of lamina propria. Some enterocytes are dilated with pale cytoplasm (↑). The mucosa is thinner with decreased lymphatic infiltration of nodules. We did not observe any distinct effect of AlaGln administration on these alterations. **C** Colon after irradiation, H&E × 120. Small mucosal lesions (*) without covering epithelium and crypts with increased cellular infiltration of lamina propria. **D** Colon after irradiation of AlaGln treated animals, H&E × 100. The thinner mucosa region with absence of covering epithelium rarely (*). Dilatation of lymphatic vessels (↑) is probably not associated with AlaGln administration

Amino acid concentrations in blood plasma (Table 6). We did not observe an obvious effect of irradiation on plasma amino acid levels. However, administration of AlaGln caused a decrease in the total of BCAA and in the total of amino acids (after subtraction of alanine and glutamine levels) in irradiated animals. The significant decrease was observed in plasma levels of threonine, serine, methionine, leucine, tyrosine, phenylalanine, ornithine, lysine and histidine.

Amino acid concentrations in skeletal muscle (Table 7). There were small differences in amino acid concentrations in gastrocnemius muscle between irradiated and non-irradiated animals. The infusion of AlaGln caused an

Table 6. Amino acid concentrations in blood plasma (μmol/l)

Variable	Non-irradiated animals		Irradiated animals		Significance
	−AlaGln (n = 7)	+AlaGln (n = 6)	−AlaGln (n= 6)	+AlaGln (n = 6)	
Taurine	179 ± 18	210 ± 19	207 ± 38	161 ± 9	
Aspartate	5 ± 1	10 ± 2[c]	4 ± 1	8 ± 1	[b]
Threonine	267 ± 20	168 ± 15[c]	266 ± 12	176 ± 19[c,d]	[b]
Serine	288 ± 22	204 ± 15[c]	273 ± 14	185 ± 19[c,d]	[b]
Asparagine	54 ± 3	44 ± 3	54 ± 3	50 ± 8	
Glutamate	7 ± 1	13 ± 2	8 ± 1	18 ± 4[c,d]	[b]
Glutamine	493 ± 18	642 ± 66[c]	526 ± 12	603 ± 49	[b]
Glycine	283 ± 10	275 ± 34	219 ± 8	210 ± 32	[a]
Alanine	479 ± 25	688 ± 77[c]	498 ± 27	666 ± 65	[b]
Valine	177 ± 8	165 ± 15	217 ± 10	186 ± 13	[a]
Methionine	31 ± 2	24 ± 4	35 ± 2	25 ± 4	[b]
Isoleucine	98 ± 7	93 ± 17	108 ± 11	81 ± 8	
Leucine	185 ± 9	171 ± 7	220 ± 10[c]	174 ± 11[d]	[b]
Tyrosine	67 ± 4	52 ± 4	76 ± 4	54 ± 6[d]	[b]
Phenylalanine	73 ± 4	64 ± 8	80 ± 5	62 ± 4	[b]
Tryptophan	122 ± 13	112 ± 12	134 ± 8	116 ± 9	
Ornithine	20 ± 1	19 ± 2	17 ± 0	11 ± 1[c,d]	[a,b]
Lysine	175 ± 9	149 ± 13	184 ± 11	144 ± 15	[b]
Histidine	247 ± 11	223 ± 13	251 ± 9	214 ± 19	[b]
Arginine	100 ± 8	96 ± 6	96 ± 5	92 ± 9	
Derived values					
Ala + Gln	972 ± 26	1,329 ± 140[c]	1,024 ± 34	1,269 ± 112	[b]
BCAA	460 ± 16	429 ± 38	544 ± 20	441 ± 28[d]	[b]
Total AA	3,350 ± 62	3,418 ± 232	3,469 ± 116	3,234 ± 250	
Total − (Ala + Gln)	2,379 ± 73	2,089 ± 144	2,445 ± 98	1,965 ± 155[d]	[b]

Values are means ± SEM. [a]$p < .05$ statistically significant effect of irradiation; [b]$p < .05$ statistically significant effect of AlaGln; [c]$p < .05$ vs. non-irradiated rats without AlaGln; [d]$p < .05$ vs. irradiated rats without AlaGln.

increase in tissue levels of alanine and glutamine and a decrease in asparagine, glycine, valine and isoleucine in irradiated animals.

Discussion

As we provided only 25% of the daily energy needs to all groups, a significant decrease in body weight was observed in all animals within 48 hours. The higher loss of weight in the irradiated animals than in those not irradiated demonstrates that cachexia developing after irradiation is related to several factors, and not only to anorexia and decreased food intake. Other factors include maldigestion and malabsorption caused by the damage to the gastrointestinal tract and systemic disturbances in metabolism of all nutrients. The involvement of a specific metabolic derangement is clearly demonstrated by heterogeneous changes in the weights of individual tissues. In irradiated

Table 7. Amino acid concentrations in skeletal muscle (μmol/g)

Variable	Non-irradiated animals		Irradiated animals		Significance
	−AlaGln (n = 7)	+AlaGln (n = 6)	−AlaGln (n= 6)	+AlaGln (n = 6)	
Taurine	20.94 ± 1.05	22.39 ± 0.88	18.90 ± 2.23	17.39 ± 1.56	[a]
Aspartate	0.54 ± 0.07	0.64 ± 0.07	0.58 ± 0.14	0.54 ± 0.06	
Threonine	1.70 ± 0.11	1.32 ± 0.14	1.32 ± 0.21	1.00 ± 0.07[c]	[a,b]
Serine	1.07 ± 0.13	0.89 ± 0.10	1.15 ± 0.30	0.85 ± 0.10	
Asparagine	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.04	0.13 ± 0.01[d]	
Glutamate	2.63 ± 0.27	3.91 ± 0.55	3.09 ± 0.56	2.73 ± 0.44	
Glutamine	10.25 ± 0.47	23.49 ± 0.92[c]	8.45 ± 1.73	17.00 ± 1.31[c,d]	[a,b]
Glycine	4.60 ± 0.24	4.12 ± 0.31	4.10 ± 0.26	3.12 ± 0.17[d]	[a,b]
Alanine	5.09 ± 0.17	7.44 ± 0.46[c]	5.27 ± 0.52	6.56 ± 0.32[c]	[b]
Valine	0.49 ± 0.05	0.55 ± 0.06	0.70 ± 0.17	0.39 ± 0.04[d]	
Methionine	0.11 ± 0.02	0.11 ± 0.02	0.15 ± 0.03	0.08 ± 0.01	
Isoleucine	0.24 ± 0.02	0.29 ± 0.03	0.35 ± 0.07	0.21 ± 0.01[d]	
Leucine	0.53 ± 0.07	0.74 ± 0.10	0.82 ± 0.21	0.48 ± 0.04	
Tyrosine	0.16 ± 0.03	0.21 ± 0.04	0.29 ± 0.08	0.19 ± 0.02	
Phenylalanine	0.29 ± 0.02	0.34 ± 0.05	0.35 ± 0.11	0.21 ± 0.03	
Tryptophan	0.14 ± 0.02	0.14 ± 0.01	0.16 ± 0.04	0.12 ± 0.01	
Ornithine	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	
Lysine	0.27 ± 0.02	0.22 ± 0.03	0.21 ± 0.07	0.19 ± 0.02	
Histidine	0.42 ± 0.02	0.57 ± 0.04	0.46 ± 0.10	0.53 ± 0.06	
Arginine	1.25 ± 0.07	1.28 ± 0.09	1.13 ± 0.10	1.42 ± 0.12	
Derived values					
Ala + Gln	15.3 ± 0.6	30.9 ± 0.7[c]	13.7 ± 2.2	23.6 ± 1.5[d]	[a,b]
BCAA	1.3 ± 0.1	1.6 ± 0.2	1.9 ± 0.4	1.1 ± 0.1	
Total AA	50.8 ± 1.4	68.7 ± 1.3	47.5 ± 6.1	53.0 ± 2.0	[a,b]
Total − (Ala + Gln)	35.5 ± 1.2	37.8 ± 1.0	33.8 ± 4.1	29.5 ± 1.7	[a]

Values are means ± SEM. [a]$p < .05$ statistically significant effect of irradiation; [b]$p < .05$ statistically significant effect of AlaGln; [c]$p < .05$ vs. non-irradiated rats without AlaGln; [d]$p < .05$ vs. irradiated rats without AlaGln.

animals, the significant decrease in weight and protein content was seen in the intestine and spleen, while the weight of hepatic tissue increased.

One of the topics of this study was to evaluate the effect of supplementation with alanine and glutamine on the recovery of irradiated gut. Unfortunately, in AlaGln treated animals we did not observe any apparent improvement in gut morphology or favorable changes in protein content, DNA content and DNA synthesis. The only data which supports the results of the studies of Klimberg et al. (1990) and Karatzas et al. (1991) who reported the beneficial effect of glutamine on healing of the irradiated intestine, was an insignificant increase in protein synthesis in the jejunum in the group of irradiated rats supplemented with AlaGln. The possible explanation of our finding is a lower dose of glutamine administered in the form of AlaGln (0.56 g Gln/kg/day) in comparison with the dosages administered in the above mentioned studies (5.6 g Gln/kg/day in Klimberg's and 5.4 g Gln/kg/day in Karatzas's study).

Probably the most important findings obtained by the amino acid analysis was the decrease in glutamine concentration in skeletal muscle of irradiated animals and the decrease in blood plasma BCAA and some other amino acids after AlaGln treatment. The decreased glutamine level in skeletal muscle of irradiated animals was corrected by AlaGln. We assume that the main cause of the decrease in BCAA and in some other plasma amino acids after AlaGln administration was due to a lower rate of release of amino acids from protein breakdown. Another possibility is the influence of high extracellular levels of alanine and glutamine on amino acid transport across cell membranes.

The main effect of irradiation on leucine metabolism is undoubtedly the increase in whole-body leucine oxidation, indicating a wasting of essential BCAA (which is known as an important substrate and a regulator of protein metabolism). The increased leucine oxidized fraction, under the conditions of decreased food intake, impaired digestion and/or absorption, can cause a net loss of body proteins. The marked decrease in body weight and in protein content of the small intestine and spleen of irradiated animals, in spite of identical food intake, supports this explanation. We failed to prove the significant increase in proteolysis in irradiated rats that was demonstrated by others using isolated perfused muscle and/or on the basis of the measurement of creatinuria (Schwenen et al., 1989; Kurohara et al., 1961; Altman and Schwenen, 1987). In the present study, we evaluated the changes in whole-body proteolysis and it can be assumed that opposite changes in tissues other than skeletal muscle can significantly affect changes in whole-body proteolysis. To our knowledge, there is no other study evaluating the effect of irradiation on the whole-body protein breakdown using primed, continuous infusion of labeled leucine.

The principal new information is provided by the data that describes the effects of AlaGln on leucine turnover. Administration of AlaGln resulted in a significant decrease in whole-body leucine oxidation and the leucine oxidized fraction both in irradiated and non-irradiated animals. In irradiated animals it caused the decrease in leucine turnover indicating the decrease in whole-body proteolysis. These results imply a marked influence of AlaGln on leucine and protein metabolism and also the importance of BCAA, particularly of leucine, in synthesis of alanine and glutamine. We assume that the delivery of alanine or glutamine to the site of their synthesis should decrease the flux of nitrogen from BCAA and thus decrease the rate of BCAA catabolism. This decreased catabolism of BCAA can favorably affect the disturbances in protein metabolism, particularly in skeletal muscle. It should be noted that the changes in BCAA catabolism should not necessarily be associated with changes in their concentration. The reason could be alterations in transport across cell membrane and/or changes in protein synthesis and proteolysis. The significant decrease in proteolysis was demonstrated after AlaGln administration in irradiated rats in the present study. A similar effect of AlaGln on leucine oxidation and the leucine oxidized fraction was also observed in our other study in which we evaluated the effect of endotoxin and AlaGln infusion on leucine turnover (Holeček et al., 2000).

In conclusion, the obtained data demonstrated that irradiation induces metabolic derangements which are associated with increased oxidation of essential BCAA and that these disturbances can be ameliorated by administration of AlaGln.

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