Amino Acids (1999) 16:1–11

Amino
Acids

Plasma amino acid levels after carbon tetrachloride induced acute liver damage. A dose-response and time-response study in rats

M. Holeček[1], **H. Skalská**[2], and **J. Mráz**[3]

Departments of [1]Physiology and [3]Histology, Charles University, School of Medicine, and [2]Faculty of Management and Information Technology, Hradec Králové, Czech Republic

Accepted October 13, 1997

Summary. The aims of the present study were to assess the changes of individual plasma amino acid levels in relation (1) to the severity of liver damage and (2) to the process of liver recovery. Acute liver injury was induced by an intragastric administration of CCl_4 diluted in olive oil in doses of 2, 4 and /or 6 g of CCl_4 per kg b.w. The control rats received olive oil only. Animals were sacrificed at 16, 24, 48 and 96 hours after treatment. The severity of liver injury was assessed by histological examination, by changes in ALT and AST in the blood plasma and by changes in liver weight. Statistical analysis was carried by ANOVA, $p < 0.05$ was considered significant. The Spearman rank correlation coefficient was used as a measure of the degree of linear relationship between variable and dose. In the period of the development of acute liver damage, i.e. at 16 and 24 hours after treatment, an increase in blood plasma amino acid levels and positive correlations with the dose of CCl_4 were observed for most individual amino acids. The only exception was arginine which decreased in a dose dependent manner. At a phase of liver recovery, i.e. at 48 and 96 hours after CCl_4 treatment, the concentrations of some individual amino acids decreased below the control values. The negative correlation with the dose of CCl_4 occurred for taurine and isoleucine (at 48 hours) and taurine, threonine, valine, methionine, isoleucine and leucine (at 96 hours).

Keywords: Amino acids – Liver – Hepatic damage – Carbon tetrachloride

Introduction

Fulminant hepatic failure is associated with a marked increase in plasma amino acids, with resulting aminoaciduria (Iber et al., 1957; Record et al., 1976; Rosen et al., 1977). However, the pathogenesis of changes of individual plasma amino acid levels caused by acute liver damage is not understood very well. The main causes of the rise in plasma amino acids after severe liver damage are undoubtedly the leak of amino acids into the circulation from

injured hepatocytes and impaired utilization of plasma amino acids by reduced liver cell mass. Changes in concentration of some amino acids are significantly affected also by impaired-whole body metabolism, altered nutrition, and by hormonal effects. There is no data about the effect on aminoacidemia of recovery of damaged hepatic tissue.

We suppose that the familiar knowledge of the changes in plasma amino acid levels in relation to the severity of acute liver damage and liver recovery may be both of theoretical and practical importance. However, it should be noted, that for an unacceptable contaminating variable, it is very difficult to organize such a study on human subjects. The convenient tools to study this problem are animal models of acute liver damage. These models enable both induction of liver injury of variable degree and subsequent evaluation of changes reflecting the process of liver recovery. The aim of the present study was to assess the effect of the severity of acute rat liver damage induced by various doses of CCl_4 and subsequent recovery on changes in plasma amino acid levels.

Material and methods

Animals

Adult male Wistar rats weighing between 220 and 260 g were obtained from Velaz (Prague, CR). Rats were housed in standardized cages in quarters with controlled temperature under a 12-hour light-dark cycle and received Velaz-Altromin 1320 laboratory chow (Velaz, Prague, CR) and drinking water ad libitum. All procedures involving animals were performed according to the guidelines set by the Institutional Animal Use and Care Committee of Charles University.

Study protocol

Carbon tetrachloride was diluted in olive oil (1:1) and administered intragastrically in a dose of 2, 4 and/or 6 g of CCl_4/kg b.w. The doses of CCl_4 were chosen on the basis of previous studies (Holeček et al., 1995; Holeček et al., 1996) and our preliminary experiments. The dose of 6 g of CCl_4 corresponds to $LD_{50/48h}$ in our experimental conditions. The control animals received olive oil only in a dose of 4 g/kg b.w. The rats were killed by blood withdrawal under light ether narcosis at 16, 24, 48 and/or 96 hours after treatment. To exclude nutritional effects on aminoacidemia, all rats fasted 24 hours before sacrifice. In addition, the control rats killed at 48 and 96 hours after treatment were pair-fed with rats given 4 g of CCl_4 per kg b.w. Immediately after sacrifice the liver was removed, weighed, and a sample of hepatic tissue was taken from the medium lobule for histological examination.

Analytical methods

Free amino acid concentrations were measured in samples of blood plasma deproteinated by sulphosalicylic acid using ion-exchange chromatography (amino acid analyzer T339, Mikrotechna, Prague, CR). Because tryptophan is bound to plasma proteins and it is partially lost during deproteinization with sulphosalicylic acid (Milson et al., 1979), this amino acid was not determined. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using commercial kits (Lachema, Brno, CR). The liver tissue samples were stained with haematoxylin-eosin for histology.

Statistical analysis

Statistical package SPSS was used for statistical analysis of differences of group means. For each time interval group means for different doses were compared by a one-way analysis of variance. If null hypothesis (that all population means are equal) was rejected we used Duncan test for multiple comparison between means. Separately for each time interval, Spearman rank correlation coefficient was used as a measure of the degree of linear relationship between variable and dose. Coefficient and

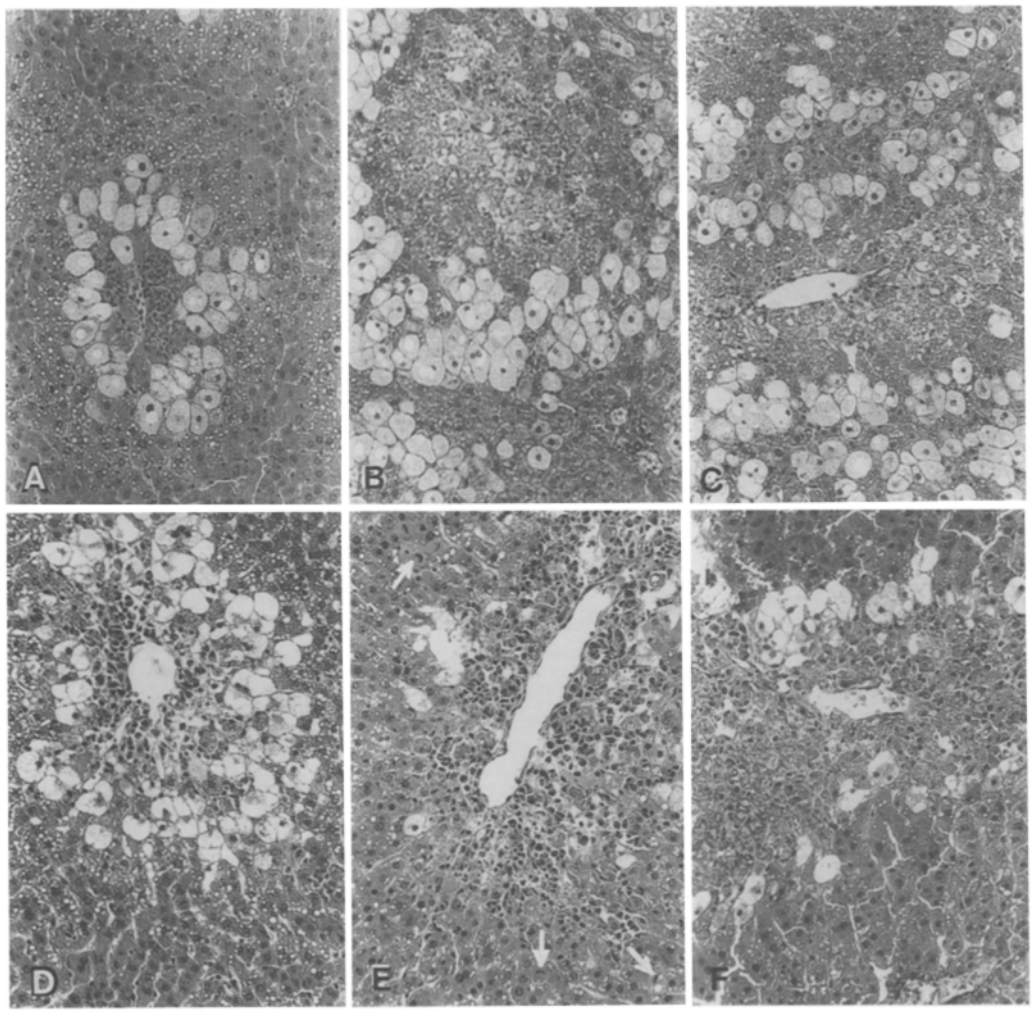

Fig. 1. Histological examination, H&E × 180 (**ABC** / **DEF**). Twenty four hours after CCl_4 treatment (**A**: 2 g of CCl_4; **B**: 4 g of CCl_4; **C**: 6 g of CCl_4 per kg b.w.). The peripheral microvesicular steatosis (**A**), centrizonal necroses (the extent correlated with the dose of CCl_4 administered) and the hydropic degeneration of hepatocytes in midzone (**A**) and in periphery (**C**). Forty eight hours after CCl_4 treatment (**D**: 2 g of CCl_4; **E**: 4 g of CCl_4; **F**: 6 g of CCl_4 per kg of b.w.). The centrizonal necroses with macrophages presence, hyperemia, and peripheral microvesicular steatosis. The extent of hydropic degeneration of hepatocytes in midzone (**D**) and in periphery (**E**, **F**) was less pronounced than 24 hours after CCl_4 treatment (above). The hepatocyte mitotic activity was markedly increased at this period (arrows)

significance levels were computed with procedure NONPAR CORR (Norusis, 1993). Statistically significant value at level $p < 0.05$ (signed by *) means that there is linear association between the variable and the dose of carbon tetrachloride. Negative value of coefficient means decreasing value of variable with increasing level of dose and vice versa. Positive value of coefficient indicates changes in the same direction. Values greater than /0.8/ means strong or very strong linear association between variable and dose.

Results

Liver damage patterns

Liver damage patterns evaluated on the basis of liver histology (Fig. 1), ALT and AST activities in blood plasma (Figs. 2 and 3) and changes in liver weight (Fig. 4) revealed a rapid development of liver damage within 24 hours after treatment. The highest correlation between the dose of CCl_4 and ALT and AST activities and/or liver weight was observed at 24 hours (Table 5). Morphological examination of liver samples showed intensive process of liver recovery at 48 hours and almost complete restoration at 96 hours after CCl_4 treatment.

Plasma amino acid patterns

Plasma concentrations of individual amino acids after administration of various doses of CCl_4 are given in Tables 1–4. Great positive correlations between the dose of CCl_4 and blood plasma amino acid concentration were observed in most of the individual amino acids at 16 and 24 hours after treatment. The

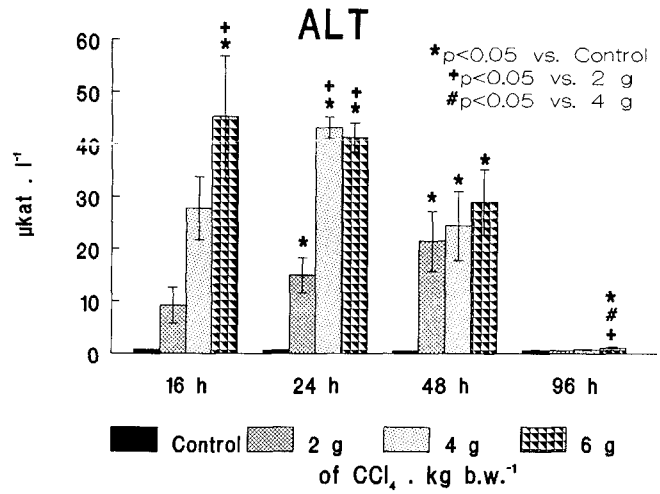

Fig. 2. Plasma ALT levels of rats at intervals after administration of various doses of CCl_4. Mean ± SE. The number of animals in particular groups is the same as given on Tables 1–4

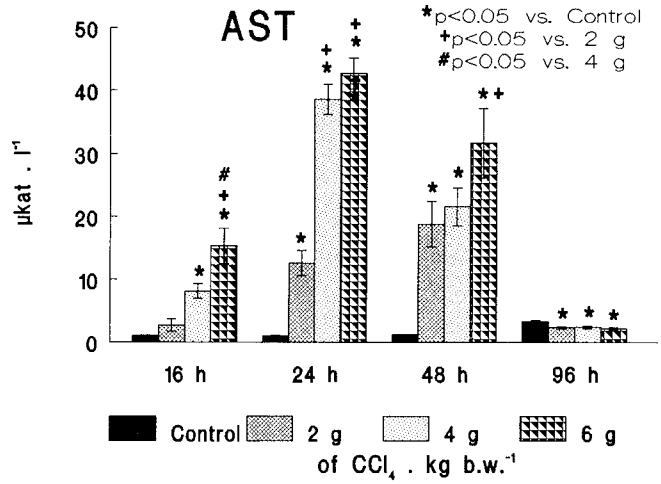

Fig. 3. Plasma AST levels of rats at intervals after administration of various doses of CCl_4. Mean ± SE. The number of animals in particular groups is the same as given on Tables 1–4

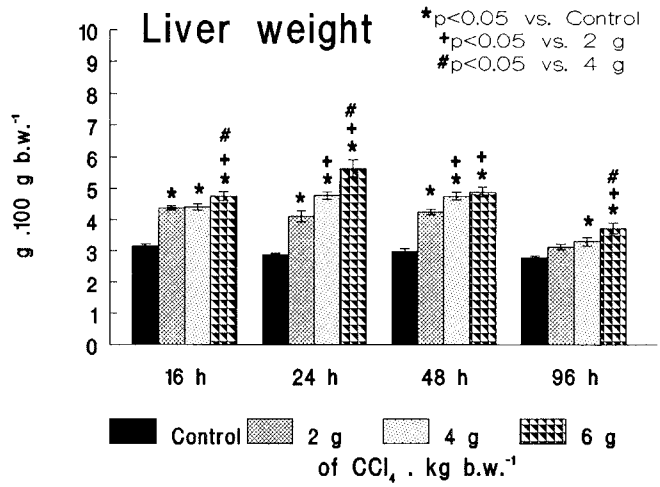

Fig. 4. Liver weight (g. 100g b.w.$^{-1}$) of rats at intervals after administration of various doses of CCl_4. Mean ± SE. The number of animals in particular groups is the same as given on Tables 1–4

strongest relationships were with aspartate, glutamine, glycine, alanine, valine, ornithine, lysine and histidine. The relationship between the dose and amino acid concentration was not significant for taurine (at 24h), cystine and citrulline. Only plasma levels of arginine showed a negative relationship.

At 48 hours the positive relationship between the CCl_4 dose and plasma concentration was observed only in eight amino acids (aspartate, serine,

Table 1. Plasma amino acid concentrations 16 hours after administration of various doses of CCl_4

Amino acid	Control (n = 5)	2 g CCl_4 (n = 5)	4 g CCl_4 (n = 5)	6 g CCl_4 (n = 8)
Taurine	437 ± 21	566 ± 35	721 ± 69*+	635 ± 38*
Aspartate	52 ± 3	78 ± 10	151 ± 32*+	167 ± 14*+
Threonine	218 ± 20	511 ± 122	824 ± 282*	1272 ± 126*+
Serine	213 ± 15	428 ± 91	608 ± 164*	791 ± 86*+
Asparagine	93 ± 8	121 ± 26	170 ± 49	282 ± 22*+#
Glutamate	198 ± 22	253 ± 33	565 ± 192*+	456 ± 43
Glutamine	613 ± 46	846 ± 117	1929 ± 911	3891 ± 352*+#
Proline	125 ± 6	194 ± 16*	204 ± 15*	233 ± 15*
Glycine	220 ± 13	279 ± 37	761 ± 308	1298 ± 183*+#
Alanine	354 ± 21	1229 ± 254*	2395 ± 214*+	831 ± 255*+
Citrulline	52 ± 7	58 ± 18	66 ± 15	69 ± 14
Valine	179 ± 16	222 ± 19	327 ± 28*+	357 ± 9*+
Cystine	45 ± 1	31 ± 11	32 ± 10	43 ± 8
Methionine	52 ± 5	57 ± 5	76 ± 14	91 ± 14*
Isoleucine	98 ± 7	107 ± 12	159 ± 15*+	165 ± 5*+
Leucine	187 ± 12	169 ± 32	285 ± 25*+	302 ± 11*+
Tyrosine	56 ± 9	47 ± 4	37 ± 7	69 ± 10#
Phenylalanine	46 ± 11	37 ± 2	63 ± 11	65 ± 8+
Ornithine	25 ± 4	174 ± 49*	287 ± 27*+	318 ± 13*+
Lysine	411 ± 20	426 ± 41	964 ± 203*+	1683 ± 130*+
Histidine	42 ± 7	64 ± 18	149 ± 32*	257 ± 36*+#
Arginine	159 ± 11	15 ± 12*	2 ± 1*	0 ± 0*
Total AA	3875 ± 88	5915 ± 614	10775 ± 2440*+	15276 ± 1078*+#
BCAA	464 ± 18	498 ± 62	771 ± 68*+	824 ± 22*+
AAA	102 ± 18	84 ± 3	100 ± 14	134 ± 17+
BCAA/AAA	5.20 ± 0.95	6.06 ± 0.94	8.03 ± 0.70	6.81 ± 0.85

Results are expressed as $\mu mol \cdot l^{-1}$. Number of animals in parentheses. Mean ± SE. *$p < 0.05$ vs. control; +$p < 0.05$ vs. rats treated by 2 g of $CCl_4 \cdot kg\ b.w.^{-1}$; #$p < 0.05$ vs. rats treated by 4 g of $CCl_4 \cdot kg\ b.w.^{-1}$. *Total AA*, the sum of all amino acids; *BCAA*, branched-chain amino acids (isoleucine + valine + leucine); *AAA*, aromatic amino acids (tyrosine + phenylalanine).

glycine, alanine, methionine, phenylalanine, ornithine, and histidine). The negative value of arginine's correlation coefficient was lower than at previous intervals. The most exciting observations at 48 hours were shifts of correlation coefficients of taurine and isoleucine from positive values at 16 and/or 24 hours (only isoleucine) to negative ones.

At 96 hours after the CCl_4 treatment, the concentrations of some amino acids decreased below the control values, particularly in rats administered with a highest dose of CCl_4. These amino acids (taurine, threonine, valine, methionine, isoleucine, and leucine) showed also a significant negative correlation with the dose of CCl_4 administered. The negative correlation between the dose of CCl_4 and arginine levels observed in previous intervals was absent in this period. Positive correlations with the dose of CCl_4 were shown only for glycine, ornithine and histidine.

Table 2. Plasma amino acid concentrations 24 hours after administration of various doses of CCl_4

Amino acid	Control (n = 5)	2 g CCl_4 (n = 5)	4 g CCl_4 (n = 6)	6 g CCl_4 (n = 4)
Taurine	419 ± 44	652 ± 124	496 ± 37	395 ± 19
Aspartate	46 ± 8	74 ± 8	147 ± 22*+	157 ± 26*+
Threonine	233 ± 25	428 ± 83	758 ± 234	1410 ± 387*+#
Serine	222 ± 16	379 ± 74	672 ± 207	1242 ± 244*+#
Asparagine	95 ± 12	136 ± 9	192 ± 38*	308 ± 48*+#
Glutamate	205 ± 19	242 ± 13	459 ± 66	710 ± 251*+
Glutamine	665 ± 41	944 ± 14	1759 ± 468	4093 ± 1119*+#
Proline	129 ± 6	170 ± 20	196 ± 16*	196 ± 11*
Glycine	249 ± 14	252 ± 7	1001 ± 241*+	1694 ± 229*+#
Alanine	430 ± 37	1020 ± 218	2340 ± 176*+	3249 ± 408*+#
Citrulline	45 ± 4	71 ± 3*	61 ± 11	75 ± 5*
Valine	204 ± 25	301 ± 15*	396 ± 39*+	487 ± 30*+
Cystine	40 ± 7	47 ± 3	34 ± 10	50 ± 13
Methionine	52 ± 5	62 ± 4	92 ± 15	167 ± 62*+
Isoleucine	110 ± 13	127 ± 7	172 ± 14*+	176 ± 9*+
Leucine	185 ± 20	229 ± 15	318 ± 28*+	347 ± 55*+
Tyrosine	66 ± 9	70 ± 6	101 ± 15	135 ± 37*+
Phenylalanine	39 ± 4	53 ± 8	91 ± 11*+	110 ± 13*+
Ornithine	33 ± 4	144 ± 24*	340 ± 25*+	376 ± 51*+
Lysine	391 ± 34	443 ± 16	1046 ± 179*+	1665 ± 304*+#
Histidine	45 ± 10	86 ± 3	190 ± 36*+	356 ± 67*+#
Arginine	117 ± 27	76 ± 17	0 ± 0*+	0 ± 0*+
Total AA	4021 ± 284	6006 ± 516	10859 ± 1722*+	17394 ± 3285*+#
BCAA	99 ± 57	657 ± 33	887 ± 72*+	1010 ± 95*+
AAA	105 ± 11	123 ± 10	192 ± 24*	244 ± 47*+
BCAA/AAA	4.82 ± 0.47	5.54 ± 0.63	4.83 ± 0.41	4.50 ± 0.69

Results are expressed as $\mu mol \cdot l^{-1}$. Number of animals in parentheses. Mean ± SE. $^*p < 0.05$ vs. control; $^+p < 0.05$ vs. rats treated by 2 g of $CCl_4 \cdot kg\ b.w.^{-1}$; $^\#p < 0.05$ vs. rats treated by 4 g of $CCl_4 \cdot kg\ b.w.^{-1}$. *Total AA*, the sum of all amino acids; *BCAA*, branched-chain amino acids (isoleucine + valine + leucine); *AAA*, aromatic amino acids (tyrosine + phenylalanine).

Discussion

The results of the present study demonstrate characteristic changes of individual amino acid levels in blood plasma within the course of development of acute liver damage and subsequent recovery.

Phase of liver damage development

The first 24 h after CCl_4 resembles the period of acute liver damage development. During this period the concentration of most amino acids substantially increased, mostly in good correlation with the dose of administered CCl_4. The highest increase was observed in amino acids that are in a high concentrations in cytosol of rat liver cells (glutamine, glutamic acid, alanine, glycine, lysine, threonine, histidine, valine, leucine and isoleucine) (Fürst, 1985), and/or me-

Table 3. Plasma amino acid concentrations 48 hours after administration of various doses of CCl_4

Amino acid	Control (n = 5)	2 g CCl_4 (n = 7)	4 g CCl_4 (n = 4)	6 g CCl_4 (n = 4)
Taurine	408 ± 12	316 ± 24*	297 ± 29*	237 ± 26*+
Aspartate	43 ± 2	65 ± 3*	68 ± 10*	72 ± 14*
Threonine	203 ± 4	235 ± 23	233 ± 26	268 ± 36
Serine	195 ± 4	226 ± 19	229 ± 22	269 ± 21*
Asparagine	83 ± 6	85 ± 19	77 ± 11	117 ± 20
Glutamate	174 ± 16	179 ± 13	176 ± 29	181 ± 13
Glutamine	594 ± 36	825 ± 55*	612 ± 49+	808 ± 83*#
Proline	134 ± 7	129 ± 4	132 ± 10	137 ± 16
Glycine	219 ± 10	273 ± 15*	328 ± 23*	299 ± 30*
Alanine	368 ± 14	592 ± 74	610 ± 112	704 ± 88*
Citrulline	55 ± 6	63 ± 4	68 ± 6	31 ± 11*+#
Valine	166 ± 4	174 ± 10	193 ± 15	177 ± 17
Cystine	34 ± 7	38 ± 5	46 ± 6	48 ± 9
Methionine	53 ± 1	57 ± 7	63 ± 9	70 ± 5
Isoleucine	115 ± 8	97 ± 6	99 ± 12	74 ± 15*
Leucine	164 ± 10	159 ± 10	157 ± 19	136 ± 19
Tyrosine	67 ± 5	85 ± 15	79 ± 20	111 ± 15
Phenylalanine	34 ± 3	73 ± 13*	53 ± 11	75 ± 13*
Ornithine	25 ± 5	186 ± 18*	198 ± 36*	215 ± 34*
Lysine	390 ± 26	389 ± 25	414 ± 28	396 ± 38
Histidine	29 ± 8	42 ± 10	63 ± 15	59 ± 4
Arginine	144 ± 22	21 ± 17*	15 ± 9*	5 ± 4*
Total AA	3695 ± 104	4309 ± 232	4210 ± 247	4487 ± 431
BCAA	445 ± 9	430 ± 26	449 ± 45	387 ± 49
AAA	100 ± 4	159 ± 28	132 ± 24	186 ± 19*
BCAA/AAA	4.46 ± 0.13	3.05 ± 0.37*	3.74 ± 0.68	2.07 ± 0.14*#

Results are expressed as $\mu mol \cdot l^{-1}$. Number of animals in parentheses. Mean ± SE. *$p < 0.05$ vs. control; +$p < 0.05$ vs. rats treated by 2 g of $CCl_4 \cdot$ kg b.w.$^{-1}$; #$p < 0.05$ vs. rats treated by 4 g of $CCl_4 \cdot$ kg b.w.$^{-1}$. *Total AA*, the sum of all amino acids; *BCAA*, branched-chain amino acids (isoleucine + valine + leucine); *AAA*, aromatic amino acids (tyrosine + phenylalanine).

tabolized predominantly in the liver (tyrosine, phenylalanine, methionine, cystine). Considering that only a moderate increase of plasma amino acid levels may be observed after two-thirds hepatectomy (Holeček et al., 1996), it is undoubted that the principle cause of a marked increase of aminoacidemia in acute liver failure is the leak of amino acids from dying hepatocytes into the circulation.

Remarkable changes were observed in amino acids of the ornithine cycle. Ornithine concentrations were extremely high while citrulline was almost unaffected and arginine levels decreased almost to zero. We suppose that arginase, whose content in hepatocytes is very high (Ratner, 1973), is released from injured hepatocytes into the blood stream where it splits intravascular arginine into ornithine and urea and thus decreases arginine and increases ornithine plasma levels. Slight, mostly insignificant

Table 4. Plasma amino acid concentrations 96 hours after administration of various doses of CCl_4

Amino acid	Control (n = 7)	2 g CCl_4 (n = 8)	4 g CCl_4 (n = 8)	6 g CCl_4 (n = 7)
Taurine	356 ± 12	306 ± 10*	302 ± 14*	212 ± 10*+#
Aspartate	52 ± 5	50 ± 3	62 ± 7	54 ± 6
Threonine	235 ± 13	252 ± 19	215 ± 9	172 ± 10*+#
Serine	225 ± 6	207 ± 6	225 ± 11	251 ± 30
Asparagine	90 ± 1	92 ± 7	94 ± 6	89 ± 7
Glutamate	200 ± 8	180 ± 9	193 ± 10	196 ± 29
Glutamine	603 ± 21	541 ± 47	517 ± 43	580 ± 52
Proline	144 ± 5	133 ± 8	133 ± 6	149 ± 15
Glycine	221 ± 15	229 ± 12	271 ± 15*	312 ± 22*+
Alanine	340 ± 24	304 ± 14	300 ± 16	309 ± 31
Citrulline	73 ± 5	72 ± 4	73 ± 66	77 ± 4
Valine	165 ± 13	144 ± 9	135 ± 8	120 ± 9*
Cystine	37 ± 4	43 ± 3	34 ± 3	49 ± 4*#
Methionine	49 ± 2	45 ± 2	43 ± 2*	40 ± 2*
Isoleucine	94 ± 6	86 ± 5	84 ± 3	76 ± 2*
Leucine	143 ± 9	122 ± 8	125 ± 7	110 ± 8*
Tyrosine	65 ± 4	68 ± 5	65 ± 4	53 ± 4+
Phenylalanine	45 ± 3	41 ± 2	39 ± 2	38 ± 2
Ornithine	28 ± 1	33 ± 2	33 ± 2	41 ± 3*+#
Lysine	421 ± 7	393 ± 21	416 ± 34	377 ± 29
Histidine	48 ± 2	49 ± 2	57 ± 5	60 ± 4*+
Arginine	191 ± 9	187 ± 6	188 ± 9	168 ± 10
Total AA	3826 ± 124	3576 ± 137	3607 ± 160	3534 ± 233
BCAA	403 ± 27	351 ± 21	345 ± 17	306 ± 18*
AAA	110 ± 5	108 ± 18	105 ± 5	91 ± 6*+
BCAA/AAA	3.66 ± 0.11	3.26 ± 0.13*	3.29 ± 0.11*	3.36 ± 0.10

Results are expressed as $\mu mol \cdot l^{-1}$. Number of animals in parentheses. Mean ± SE. *$p < 0.05$ vs. control; +$p < 0.05$ vs. rats treated by 2 g of $CCl_4 \cdot$ kg b.w.$^{-1}$; #$p < 0.05$ vs. rats treated by 4 g of $CCl_4 \cdot$ kg b.w.$^{-1}$. *Total AA*, the sum of all amino acids; *BCAA*, branched-chain amino acids (isoleucine + valine + leucine); *AAA*, aromatic amino acids (tyrosine + phenylalanine).

changes in citrulline concentration demonstrate unaffected function of the kidney.

Phase of liver recovery

As demonstrated by histological examination, the interval between 48 and 96 h after CCl_4 administration is a period when the process of liver injury development is completed and an intensive process of liver recovery develops. The most striking feature of this period was a decrease of some plasma amino acids below the values of control animals associated with a shift from positive correlation between the dose of CCl_4 and plasma amino acid levels at 16 and 24 hours to a negative one. The earliest and most pronounced shift to negative values of Spearman correlation coefficient were observed for isoleucine and taurine.

Table 5. Spearman correlation coefficients between the variable and the dose of CCl_4 administered at four intervals after administration of CCl_4

	16 hours (n = 23)	24 hours (n = 20)	48 hours (n = 20)	96 hours (n = 30)
Liver weight	0.669*	0.913*	0.858*	0.683*
ALT	0.811*	0.869*	0.563*	0.615*
AST	0.869*	0.904*	0.740*	−0.496*
Taurine	0.576*	−0.50	−0.712*	−0.799*
Aspartate	0.815*	0.868*	0.579*	0.153
Threonine	0.809*	0.707*	0.330	−0.612*
Serine	0.667*	0.669*	0.568*	0.019
Asparagine	0.762*	0.754*	0.173	0.043
Glutamate	0.781*	0.858*	−0.040	−0.104
Glutamine	0.840*	0.868*	0.207	−0.084
Proline	0.727*	0.643*	−0.131	−0.136
Glycine	0.781*	0.832*	0.642*	0.654*
Alanine	0.854*	0.912*	0.567*	−0.081
Citrulline	0.402	0.478*	−0.143	0.182
Valine	0.861*	0.880*	0.277	−0.525*
Cystine	−0.001	0.016	0.319	0.249
Methionine	0.470*	0.666*	0.456*	−0.546*
Isoleucine	0.769*	0.740*	−0.509*	−0.488*
Leucine	0.770*	0.774*	−0.294	−0.407*
Tyrosine	0.207	0.564*	0.376	−0.280
Phenylalanine	0.533*	0.804*	0.551*	−0.347
Ornithine	0.834*	0.916*	0.698*	0.626*
Lysine	0.848*	0.856*	0.080	−0.131
Histidine	0.861*	0.948*	0.532*	0.497*
Arginine	−0.796*	−0.894*	−0.610	−0.283
Total AA	0.902*	0.912*	0.379	−0.149
BCAA	0.787*	0.840*	−0.274	−0.506*
AAA	0.402	0.713*	0.596	−0.389*
BCAA/AAA	0.278	−0.102	−0.617*	−0.276

* = statistically significant linear association between the variable and the dose of CCl_4 at level $p < 0.05$. *Total AA*, the sum of all amino acids; *BCAA*, branched-chain amino acids (isoleucine + valine + leucine); *AAA*, aromatic amino acids (tyrosine + phenylalanine).

A remarkable result obtained at 96 hours after CCl_4 administration is a decrease of plasma branched-chain amino acids (BCAA: valine, leucine, and isoleucine) concentrations associated with the negative relationship between the dose of CCl_4 and plasma levels of these amino acids. Considering that BCAA are predominantly utilised in protein synthesis, we suppose that the main mechanism decreasing plasma BCAA levels in this period is caused by their intensive uptake and utilization in protein synthesis within the process of liver recovery. The decrease of ratio of BCAA over aromatic amino acids (AAA: phenylalanine and tyrosine) is often considered as an unfavourable

manifestation of liver disease development (McCullough, 1981) and it could be a numerical indicator of the grade of encephalopathy (Fischer et al., 1976). However, a number of papers demonstrated certain shortcomings of this index (Fischer, 1982; Shiels et al., 1985). We demonstrated the more pronounced decrease of BCAA than of aromatic amino acids during the recovery period of liver damage induced by CCl_4 in this study. Thus we assume that a decrease of BCAA and/or BCAA/AAA ratio may, in the case of acute liver damage, be a favourable indicator of liver recovery and not a negative one.

Acknowledgements

This study was supported by a grant from the Charles University No. 152/95.

References

Fischer JE (1982) Amino acids in hepatic coma. Digest Dis Sci 27: 97–102

Fischer JE, Rosen HM, Ebeid AM, James JH, Keane JM, Soeters PB (1976) The effect of normalization of plasma amino acids on hepatic encephalopathy in man. Surgery 80: 77–91

Fürst P (1985) Regulation of intracellular metabolism of amino acids. In: Bozzetti F, Dionigi R (eds) Nutrition in cancer and trauma sepsis. Karger, Basel, pp 21–53

Holeček M, Skopec F, Šprongl L (1995) Protein metabolism in cirrhotic rats: effect of dietary restriction. Ann Nutr Metab 39: 346–354

Holeček M, Mráz J, Tilšer I (1996) Plasma amino acids in four models of experimental liver injury in rats. Amino Acids 10: 229–241

Iber FL, Rosen H, Levenson SM, Chalmers TC (1957) The plasma amino acids in patients with liver failure. J Lab Clin Med 50: 417–425

McCullough AJ, Czaja AJ, Jones JD, Go VLW (1981) The nature and prognostic significance of serial amino acid determinations in severe chronic active liver disease. Gastroenterology 81: 645–652

Milson JP, Morgan MY, Sherlock S (1979) Factors affecting plasma amino acid concentrations in control subjects. Metabolism 28: 313–319

Norusis MJ (1993) SPSS for Windows. Release 6.0. SPSS Inc., Chicago

Ratner S (1973) Enzymes of arginine and urea synthesis. Adv Enzymol 39: 1–90

Record CO, Buxton B, Chase RA, Curzon G, Murray-Lyon IM, Williams R (1976) Plasma and brain amino acids in fulminant hepatic failure and their relationship to hepatic encephalopathy. Europ J Clin Invest 6: 387–394

Rosen HM, Yoshimura N, Hodgman JM, Fischer JE (1977) Plasma amino acid patterns in hepatic encephalopathy of differing etiology. Gastroenterology 72: 483–487

Shiels MT, Czaja AJ, Ludwig J, McCullough AJ, Jones JD, Go VLW (1985) Diagnostic and prognostic implications of plasma amino acid determinations in chronic active hepatitis. Digest Dis Sci 30: 819–823

Authors' address: Dr. Milan Holeček, Department of Physiology, Charles University School of Medicine, Šimkova 870, 500 01 Hradec Králové, Czech Republic, Fax ++42-49-5210190

Received June 4, 1997