Nutritional Effects of a d-Methionine-containing Solution on AH109A Hepatoma-bearing Rats

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The effects of a d-methionine-containing solution (DMCS) on the nutritional status of AH109A hepatoma-bearing rats receiving total parenteral nutrition were studied. The DMCS solution inhibited the decrease of transferrin in the plasma of tumor-bearing rats when compared with the effect of an l-methionine-containing solution. The survival time was also significantly prolonged in the DMCS-treated rats. These results indicate that DMCS had a beneficial effect on the malnutrition induced in tumor-bearing rats and would be a useful amino acid solution for the nutritional support of cancer patients.

Key words: d-methionine; total parenteral nutrition; survival period; nutritional support; hepatoma-bearing rat

Several studies have reported the beneficial effects of an amino acid solution for total parenteral nutrition (TPN) in cancer patients, and such solutions have also been studied in our laboratory. In a previous paper, we reported that increases in the protein and DNA contents of the liver of tumor-bearing rats were observed when they were treated with a d-methionine-containing solution, which suggested nutritional improvement by the d-methionine-containing solution. Methionine is the chief methyl donor for the methylation of DNA, RNA and protein, and in tumor cells, the requirement for methionine was found to be higher. In general, animals cannot utilize d-amino acids directly, conversion of the d-amino acids to l-isomers needing two steps. They are first oxidatively deaminated to the corresponding α-keto acids by d-amino acid oxidase, the α-keto acids then being converted by stereospecific transamination to form their l-amino acids. In humans and rats, d-methionine can be used as a nutrient in place of l-methionine, among the several d-amino acids, has the highest substrate specificity for d-amino acid oxidase. Naylor et al. have reported that d-amino acid oxidase was not retained in many tumor cells as a result of growth of the cells. In addition, it was reported that d-amino acids showed more preferential incorporation into tumor cells than did l-amino acids. In the present study, we prepared a new amino acid solution containing d-methionine and investigated the effect on the host of this d-methionine-containing solution by comparing it with that of the conventional amino acid solution.

Male Domyru rats, purchased at 4 weeks of age (70 to 100 g) from Nihon Rat Co. (Saitama, Japan), were used. Animal feed (CRF-1, Oriental Yeast Co., Tokyo, Japan) and water were provided to the rats ad libitum until the designated time during the study. The rats were maintained in an environment with a 12-h light/dark cycle at 23±2°C for a minimum of 7 days before the study. The AH109A hepatoma tumor cell line was used to establish a model of tumor-bearing rats. On day 7 of the acclimatization, the rats were subcutaneously inoculated with 1.0×10⁶ viable AH109A hepatoma tumor cells per rat in the right flank. On day 7 after the inoculation, the tumor-bearing rats were assigned according to their body weight and tumor volume to two similar AH109A hepatoma-bearing groups; one received the conventional solution (control group, n=8), and the other group was treated with the d-methionine-containing solution prepared in our laboratory (DMCS group, n=8). A reference group of animals, which were non-tumor-bearing rats, received the conventional solution (NTB group, n=8). The tumor volume was evaluated by the following formula for a prolate spheroid:

V = L · W · D · π / 6 (mm³)

where L is the length, W is the width, and D is the depth of the solid tumor in mm. All animals underwent surgical placement of a silastic catheter in the superior vena cava according to the method of Steiger et al., the rats being fasted overnight before this operation. After the venous cannulation, the animals in each group were intravenously infused (i.v.) with the respective solutions at 230 ml/kg/day for 7 days.

The body weight of each rat was measured on days 0 and 7 (final day) of the TPN infusion. Blood samples were collected in heparinized tubes from the abdominal aorta after the infusion, and plasma samples from heparinized blood centrifuged at 3000 rpm at 4°C for 10 min were stored at -25°C until needed for use. The albumin (Alb), total protein (TP) and transferrin (TF) in

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Abbreviations: DMCS, d-methionine-containing solution; NTB, non-tumor-bearing rat; TPN, total parenteral nutrition; Alb, albumin; TP, total protein; TF, transferrin
the plasma were measured by an autoanalyzer (model 7150, Hitachi, Tokyo, Japan). Each tumor was surgically removed and the wet weight was recorded. The survival time was also examined for the control group (n = 12) and DMCS group (n = 10), the survival period being defined as the number of days of survival after the start of TPN. The amino acid compositions used for the TPN infusions are shown in Table 1. Although there was a difference in total amino acid contents between the two amino acid solutions, the TPN solutions were adjusted to have the same total nitrogen content before being infused to the rats. Each TPN solution was prepared with a glucose-electrolyte solution supplemented with the appropriate amino acid solution and vitamins (Fuso Pharmaceutical Ind., Osaka, Japan). The composition of each TPN solution was glucose (208.3 g/l), amino acids (control group, 38.1 g/l; DMCS group, 35.6 g/l), total nitrogen (5.79 g/l) and total energy (control group, 985.6 kcal; DMCS group, 975.6 kcal).

Each result is expressed as the mean ± S.E. The data for the experimental groups (tumor weight and tumor/carcass weight ratio) were analyzed by using Student’s t-test, while the body weight and biochemical data (Alb, TP and Tf) were analyzed by using Dunnett’s test. The survival data were analyzed by using the Kaplan-Meier method, and the significance of differences between the survival curves was analyzed by using the log-rank test. Differences are considered significant when p was < 0.05.

The Alb and Tf concentrations are shown in Fig. 1. The Alb and Tf values, indicators of nutritional status, were significantly lower in the control group due to the development of cancer. The Tf value was significantly higher in the DMCS group than in the control group, the plasma Alb level also tending to be higher (p<0.1) in the DMCS group than in the control group. There was no significant difference in TP values among the three groups. The survival time was also significantly longer in the DMCS group than in the control group (Fig. 2). These results suggest that the d-methionine-containing solution could be more valuable for improving cancer-induced protein malnutrition than the l-methionine-containing solution. The body weights after TPN for 7 days were almost the same among the control (157.3±3.7 g), DMCS (156.1±2.8 g) and NTB (161.1±3.6 g) groups. The DMCS group did not show any significant inhibition of the tumor weight (control, 24.6±2.3 g; DMCS, 21.5±2.4 g) or of the tumor/carcass ratio (control, 18.2±1.7; DMCS, 16.2±2.0).

It has been reported that cancer-induced malnutrition is associated with various physiologically active substances produced by tumor cells and/or some host cells that subsequently change the metabolism of the host.19,20 Although there was no significant difference in
the tumor weight, the present results suggest that the d-methionine-containing solution might inhibit the production of such physiologically active substances. This suggestion is supported by the improved nutritional status of the rats receiving the d-methionine-containing solution. A possibility that the inhibition of tumor metastasis by the d-methionine-containing solution might have led to the improved nutritional status of the tumor-bearing rats cannot be ruled out. The use of TPN for cancer patients is intended for nutritional support. In recent years, while amino acid-TPN preparations deprived of methionine\textsuperscript{21–23} induced the inhibition of tumor growth, they also had a negative influence on the host.\textsuperscript{24} The present results suggest that a d-methionine-containing solution could be adopted as an amino acid solution suitable for cancer patients that would be beneficial for their nutritional support.

References


