Human Myeloma Cell Line (KHM-4) Established from A Patient with Multiple Myeloma Associated with Hyperammonemia

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A cell line of plasma cells with high ammonia (NH₃) production (KHM-4) was established from a patient with multiple myeloma complicated by hyperammonemia and abnormal serum concentrations of amino acids. Surface marker studies of KHM-4 cells showed that the cells were positive for cytoplasmic immunoglobulins (IgA kappa), HLA-DR, and T 10. Secretion of ammonia by the KHM-4 cells was detected by the addition of L-glutamine and L-arginine into the culture medium of amino acid-free RPMI 1640. In the presence of L-glutamine, KHM-4 cells secreted a greater amount of ammonia than the T cell line, CEM. However, production of ammonia by L-arginine was not observed in other cell lines. These observations provide evidence for the existence of a peculiar amino acid metabolism in the myeloma cells causing hyperammonemia and serum amino acid disturbance.

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Key words: amino acid, ammonia, arginine, glutamine

Introduction

Hyperammonemia occurs in a diverse range of disorders such as liver failure (1, 2), Reye's syndrome (3), inborn errors of urea synthesis (4), homocitrullinuria syndrome (5), lysinuric protein intolerance (6), and transient hyperammonemia of the newborn (7). It may also occur as a complication of urinary tract infection (8), asparaginase therapy (9), valproic acid therapy (10), and systemic carnitine deficiency (11). Recently, we encountered and reported two patients with multiple myeloma who had hyperammonemia, serum amino acid disturbances, and disturbance of consciousness (12). Malignant plasma cells have proved to be very difficult to establish; however, we established a myeloma cell line (KHM-4) from one patient with hyperammonemia. In this study, we analyzed the mechanism of hyperammonemia using KHM-4 cells.

Materials and Methods

Case report. A 51-year-old female with IgA kappa type multiple myeloma was readmitted to our hospital with a huge plasmacytoma of the right chest wall in September 1988. Despite intensive chemotherapy, her condition did not improve and she developed hyperammonemia (100–160 µg/dl) and somnolence. The serum levels of valine, leucine, isoleucine, threonine, cystine, glutamine, arginine, histidine, tryptophan, citrulline, and ornithine were significantly low. However, the concentrations of glycine and aspartic acid were high. Her somnolence transiently improved after intensive chemotherapy, but she died of complications related to pleural effusion secondary to myeloma cells in May 1989.

Autopsy revealed almost normal liver histology (12).

Cell culture. Heparinized pleural effusion fluid from the patient was layered onto the Ficoll-Conray (specific gravity, 1.078), and centrifuged at 400 g for 30 minutes. The interphase cells, which consisted almost entirely of myeloma cells, were collected and seeded into culture plates at approximately 10⁶ cells/ml after washing with complete medium. In primary culture and the early passages, RPMI 1640 medium containing 20% fetal calf serum (FCS) was used, and when the cells began to grow steadily, the medium was changed to RPMI 1640 containing 10% FCS. Throughout the study, the
cells were maintained under conditions of 5% CO₂ in humidified air at 37°C.

**Immunologic Marker Studies.** Measurements of the following cell surface markers were performed: HLA-DR, CD 2 (T 11), CD 10 (CALLA), CD 19 (B 4), CD 20 (B 1), CD 38 (T 10), and PCA-1 (Becton Dickinson, Mountain View, CA). Cytoplasmic immunoglobulins were analyzed by immunoperoxidase stain.

**Assay for ammonia in the culture media.** KHM-4 cells were inoculated onto culture plates at 2 x 10⁶ cells/ml in RPMI 1640 with or without amino acid. After 4 or 8 hours of culture, ammonia levels in the culture media were measured by the enzymatic glutamate dehydrogenase method using Kyowa-Medix reagents and an autoanalyzer (RA-XT, Kyowa-Medix, Tokyo). T cell line (CEM), B cell line [KHM-2B (13)], and two myeloma cell lines [KHM-1A (14) and KMS-12-PE (15)] were used as the controls.

**Results**

*Morphology of cultured cells.** Myeloma cells were collected and cultured from the patient's pleural effusion. KHM-4 cells floated in the culture medium as single cells or soft clusters. In Wright-Giemsa-stained smears, KHM-4 cells were morphologically similar to typical plasma blasts, with nuclei containing nucleoli and basophilic cytoplasm (Fig. 1A). Immunoperoxidase studies showed that KHM-4 cells reacted with antibodies against alpha (Fig. 1B) and kappa chains (Fig. 1C) of immunoglobulin.

*Surface markers.** KHM-4 cells were positive for HLA-DR and CD 38 (T 10) but negative for CD 2 (T 11), CD 10 (CALLA), CD 19 (B 4), CD 20 (B 1), and PCA-1 (data not shown).

*Secretion of ammonia.** Fresh myeloma cells from the pleural effusion secreted ammonia into the culture medium of RPMI 1640 without L-glutamine, and its concentration increased almost linearly from the time of cell seeding (Fig. 2). To determine the effect of amino acids on the accumulation of ammonia, each amino acid of the same concentration as RPMI 1640 medium was added to the amino acid-free culture medium. After an 8 hour culture of KHM-4 cell without amino acid, the ammonia level in the culture medium was 145 ± 35 μg/dl,
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Similar to the level prior to culture. A slight increase in ammonia by L-cystine (262 ± 69 µg/dl), a significant increase by L-arginine (338 ± 55 µg/dl), and a marked increase by L-glutamine (1,393 ± 51 µg/dl) were detected; however, other amino acids such as asparagine, aspartic acid, glutamic acid glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine did not induce the secretion of ammonia (Fig. 3).

A smaller amount of ammonia secretion was also detected in the culture medium of CEM cells with the addition of L-glutamine (Fig. 4). In the presence of L-arginine, no increase of ammonia was detected in the culture medium of CEM cells (Fig. 4) and cell lines, KHM-1A, KHM-2B, and KMS-12-PE (data not shown). These data indicate that the metabolism of arginine by KHM-4 cells is qualitatively different from that of other cell lines. Figure 5 shows the dose effect of L-glutamine and L-arginine on the accumulation of ammonia. Accumulation of ammonia by KHM-4 cells was detected during similar to the level prior to culture. A slight increase in ammonia by L-cystine (262 ± 69 µg/dl), a significant increase by L-arginine (338 ± 55 µg/dl), and a marked increase by L-glutamine (1,393 ± 51 µg/dl) were detected; however, other amino acids such as asparagine, aspartic acid, glutamic acid glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine did not induce the secretion of ammonia (Fig. 3).

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![Figure 2](image_url)  
Fig. 2. Accumulation curve of ammonia per 2 × 10⁶ cells/ml in RPMI 1640 without L-glutamine. Ammonia in the supernatant of cultures of fresh myeloma cells from the patient increased linearly from the time of cell seeding.

![Figure 3](image_url)  
Fig. 3. Accumulation curve of ammonia from KHM-4 cells in RPMI 1640 with amino acids. KHM-4 cells secreted a smaller amount of ammonia in the presence of L-glutamine but not in the presence of L-arginine.

![Figure 4](image_url)  
Fig. 4. Accumulation curve of ammonia in RPMI 1640 with L-glutamine (300 µg/ml) and L-arginine (200 µg/ml). CEM cells secreted a smaller amount of ammonia in the presence of L-glutamine but not in the presence of L-arginine.
Discussion

Hyperammonaemia is mainly seen in hepatic failure (1,2) and congenital metabolic disorders (4-6), however, there was no evidence of liver dysfunction or an occult congenital metabolic disorder in this patient, and the liver was found to be almost normal at autopsy (12). Tsunoda et al. have reported four cases of multiple myeloma with hyperammonaemia and disturbance of consciousness in which liver function was within the normal range (16). The mechanism of hyperammonaemia was not known, but they suggested that the plasma of some patients with multiple myeloma contained unidentified factors which increased the plasma ammonia concentration. We demonstrated that KHM-4 cells established from the patient with multiple myeloma and hyperammonaemia secreted ammonia in the presence of L-glutamine and L-arginine.

The cause of hyperammonemia in this patient seems to be directly related to the myeloma cells, due to the production of ammonia by KHM-4 cells in the presence of normal serum concentrations of L-glutamine and L-arginine.

It is suggested that serum amino acid alterations are a more important factor than hyperammonemia in the induction of disturbance of consciousness in hepatic failure. In particular, the decrease in branched-chain amino acids (leucine, isoleucine, and valine) and the increase in aromatic amino acids (phenylalanine and tyrosine) which results in a low Fischer ratio, is regarded as the most important factor. Serum amino acid alterations and a low Fischer ratio (1.29; normal range 2.6-4.3) were detected in our patient. The branched-chain amino acids had a very low concentration, but the aromatic amino acids were not increased. However, the concentration of tyrosine was low. We demonstrated that the arginine metabolism of KHM-4 cells was completely different from that of other cell lines. KHM-4 may become a useful tool for the biochemical analysis of hyperammonemia and serum amino acid alterations in multiple myeloma.

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