Journal of Pediatric Surgery (2007) 42, 608–612

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Original articles

Hypoxia upregulates amino acid transport in a human neuroblastoma cell line

Hideki Soh, Masafumi Wasa*, Masahiro Fukuzawa

Department of Pediatric Surgery, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan

Index words:
Hypoxia;
Amino acid transport;
Human neuroblastoma cell line

Abstract

Background/Purpose: Some tumor cells survive and even grow in hypoxic conditions. We examined the effect of hypoxia on amino acid transport in a human neuroblastoma cell line SK-N-SH.

Methods: Cells were incubated under hypoxic conditions (1% O_2–5% CO_2–94% N_2). After 0, 8, 16, and 24 hours, the transport of ^{3}H-glutamine, ^{3}H-glutamate, and ^{3}H-leucine was assayed. ^{3}H-Thymidine and ^{3}H-leucine incorporation was measured for the determination of DNA and protein synthesis, respectively.

Results: Hypoxia increased Na^+-dependent glutamine and Na^+-independent leucine transport significantly at 16 and 24 hours compared with control ($P < .01$) by a mechanism that increased V_{max} without affecting K_m. These increases were completely blocked by actinomycin D and cycloheximide. There was no significant difference in Na^+-dependent glutamate transport between control and hypoxic groups. DNA and protein synthesis significantly decreased in the hypoxic condition compared with control ($P < .01$).

Conclusions: This study demonstrated that hypoxia upregulates amino acid transport in a human neuroblastoma cell line. This mechanism may allow cells to survive and even grow under hypoxic conditions.

Human neuroblastomas are biologically remarkable in that some regress spontaneously without chemotherapy and other show invasive and progressive growth behavior [1]. They can often outgrow their blood supply with tumor growth, leading to the tumor hypoxia and necrosis [2]. Some tumor cells survive and even grow in these conditions where oxygen and nutrient resources are limited [3]. However, a precise mechanism for this has not yet been elucidated.

Amino acid transport across the plasma membrane is essential for supplying cells with amino acids for cellular metabolism. Malignant cells display uncontrolled rates of cellular proliferation and require an increased supply of precursor amino acids to support key biosynthetic pathways [4]. As a result, these cells have very efficient transport systems and can transport amino acids across the plasma membrane faster than normal cells [5]. We have previously shown that adaptive increase in amino acid transport is observed and amino acid transport is regulated by type I insulin–like growth factor receptor under glutamine-deprived conditions in a human neuroblastoma cell line [6,7]. However, it remains unclear how neuroblastoma cells regulate amino acid transport when the availability of oxygen is limited.

The purpose of this study was to examine the effect of hypoxia on amino acid transport in an SK-N-SH human neuroblastoma cell line, which provides a well-characterized in vitro model system to study the growth mechanism.

* Corresponding author. Tel.: +81 6 6879 3753; fax: +81 6 6879 3759.
E-mail address: wasa@pedsurg.med.osaka-u.ac.jp (M. Wasa).

0022-3468/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2006.12.010

1. Materials and methods

1.1. Chemicals

Radiolabeled amino acids (^{3}H-L-glutamine, ^{3}H-L-glutamate, ^{3}H-L-leucine) and ^{3}H-thymidine were obtained from Amersham (Arlington Heights, Ill). Cell culture media was from GIBCO/BRL (Gaithersburg, Md). Amino acids and all biochemicals were purchased from Sigma Chemical Inc (St Louis, Mo) and fetal bovine serum was from JRH Biosciences (Lenexa, Ks). Tissue culture plates were obtained from Corning (New York, NY). Neuroblastoma cell line, SK-N-SH, was provided by Dr Tadao Ohno (RIKEN Cell Bank, Tsukuba, Japan).

1.2. Cell culture

Neuroblastoma cells were cultured at 37°C under a humidified atmosphere of 5% CO_2/95% air. The cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 1000 U/mL penicillin, and 1000 U/mL streptomycin. The culture medium was changed every 3 days until cells were confluent, at which point the cells were used for experiments.

Hypoxic conditions were obtained by transferring the culture dishes to a modular incubator (Personal CO_2 Incubator/Multi Gas Incubator, Astec, Fukuoka, Japan) which was flushed with 1% O_2–5% CO_2–94% N_2. Hypoxia was verified by blood gas analysis (IL 1400 BGElectrolyte Analyzer, Instrumentative Laboratory, Milan, Italy) of culture media. The PO_2 of the culture media was found to be 30% ± 4% of normal levels (150 ± 6 mm Hg) at 30 minutes after changing to the hypoxic conditions, and these oxygen levels were maintained until the end of the experiment.

1.3. Measurement of amino acid transport

Cells were seeded into 24-well tissue culture plates (0.5 mL per well). After getting 100% cell confluence, the culture medium was changed and incubated in the hypoxic condition. Amino acid transport was measured at 0, 8, 16, and 24 hours, and transport values at 0 hour were chosen as the control.

Amino acid transport was measured by the cluster tray method of Gazzola et al [8]. Before the transport assays, the cells were rinsed twice with warm sodium-free Krebs-Ringer phosphate buffer (CholKRP, which was made by replacing the corresponding sodium salts with choline chloride and choline phosphate) to remove extracellular sodium and amino acids. The transport of radiolabeled amino acid (5 μCi ^{3}H-amino acid per milliliter) was performed for 1 minute at 37°C at 10 μmol/L unlabeled amino acid in both sodium Krebs-Ringer phosphate and CholKRP buffers. The transport reaction was terminated by discarding the uptake buffer and rinsing the cells 3 times with ice-cold buffer. The wells containing the cells were solubilized in 200 μL of 0.2N NaOH/0.2% sodium dodecyl sulfate solution. One hundred microliters of the cell extract was neutralized with 10 μL 2N HCL and subjected to scintillation spectrophotometry. The remaining 100 μL in each well was used for the protein assay by the bicinchoninic acid protein method [9].

The sodium-dependent transport values were obtained by subtracting the transport values in CholKRP from those in sodium Krebs-Ringer phosphate buffer. Saturable sodium-independent transport values were determined in CholKRP by subtracting the values in the presence of excess (10 mmol/L) unlabeled amino acid from those in its absence.

1.4. Measurement of DNA and protein synthesis

For the determination of DNA and protein synthesis, we measured the incorporation of ^{3}H-thymidine and ^{3}H-leucine, respectively. After obtaining 100% cell confluence, the cells were incubated in the hypoxic condition. After 8 and 16 hours, ^{3}H-thymidine and ^{3}H-leucine (1 μCi/mL) were added to the culture medium. The assay was terminated after 2.5 hours, when the cells were washed twice with phosphate-buffered saline and fixed by washing 3 times with ice-cold 10% trichloroacetic acid. Thereafter, cells were rinsed twice with 70% and 95% ethanol, respectively.

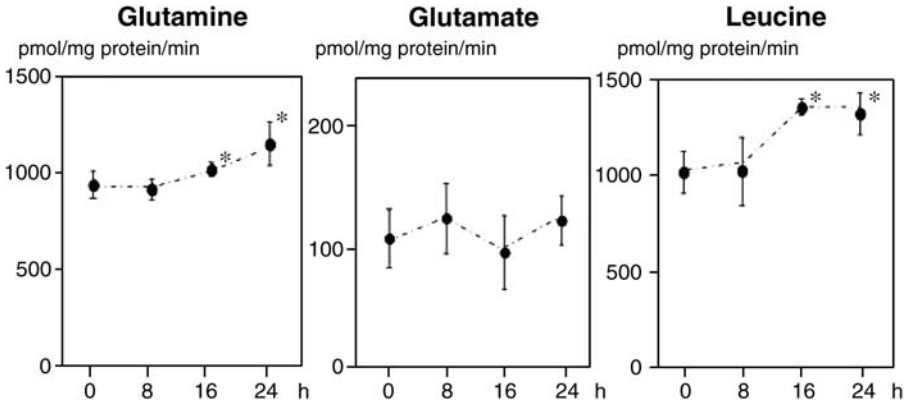

Fig. 1 Time-dependent effect of hypoxia on Na^+-dependent glutamine, glutamate, and Na^+-independent leucine transport in SK-N-SH. Amino acid transport was assayed at 0, 8, 16, and 24 hours of hypoxia. Data are presented as mean ± SD of quadruplicate determinations. *$P < .01$ vs 0 hour.