

Glutamine addiction: A fatal ignorance in prescription of glutamine supplementation

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INTRODUCTION

Glutamine is a functional amino acid ^[1] that constituted 20% of free amino acid in circulating biological fluids and more than 50% of the amino acids in skeletal muscles.^[2] It is essential for the growth and the survival of physiologically and rapidly dividing cells particularly the enterocyte.^[3] It maintains the gut barrier function and prevents the permeability of toxins and pathogens in the gut.^[4] As glutamine is required for the protein synthesis, it involves in many functional processes in organs and tissues of the gastrointestinal, cardiovascular and immune systems.^[5] Its effect on the protein building blocks is mediated by stimulation the phosphorylation of mammalian target of rapamycin-1 (mTOR1).^[6] In addition, it increases the gene expression of antioxidants and inhibits the expression of certain cytokines in organs and tissues such as gut and adipose tissue.^[7]

Glutamine is converted to glutamate by glutaminase or other amidases and the glutamate is undergone deamination process to produce α -ketoglutarate.^[8] Therefore, glutamine behaves as anaplerotic substrate in the tricarboxylic acid cycle (TCAC). By anaplerotic TCAC means that α -ketoglutarate is sufficient to overcome the proliferative arrest which induced by glutamine withdrawal or starvation.^[9]

Glutamine is a nutritional supplement that approved by Food and Drug Administration (FDA) for patients who are critically ill, those admitted in intensive care units, malignancies and hematological diseases.^[10-12]

Glutamine Addiction

Cancer cells differed from normal cells by having the ability to take up more glucose and producing lactic acid, such effect is called Warburg effect.^[13-15] Warburg found that cancer cells exhibited a high glucose uptake and glycolytic flux leading to the reduction of the pyruvate which generated by glycolysis to lactate (aerobic glycolysis) rather than undergoing mitochondrial oxidation via the TCAC.^[16]

The activation of glucose uptake is induced by oncogenes.^[17] As a result of excess glycolytic

metabolites, the metabolism of glutamine tended to be inefficient.^[18,9] Therefore, the survival of cancer cells will be impossible without exogenous glutamine supplement and therefore, these cells exhibited “glutamine addiction” ^[19], that is, the cancer cell utilized glutamine in a high proportion compared with normal cell and is considered a potential input into the TCAC.^[20] Therefore, the excess utilization of glutamine by cancer cell and the acceleration of cancer cell metabolism that producing TCAC metabolites (i.e. cataplerotic output) are shared in the growth of tumor cells.^[21] This means that the cancer cells depended on the excess glutamine for their growth, a phenomenon called *glutamine addiction*.^[22]

Glutamine Transporters

Glutamine transporters play a major role in the developing the glutamine addiction phenomenon. The influx and efflux of the glutamine is under the control of the plasma membrane transporters which are characterized at the molecular level.^[23] The assignment of these transporters is according to the classically-identified transport systems ASC (neutral amino acid transporters from the solute carrier 1 (SLC1) family, and systems A and N (SLC38 members) ^[24,25] in addition, there is another system named L system (light chain amino acid transport). In respect to the organ, there are several glutamine transporters (Box-1). The influx of glutamine into cancer cells achieved by SLC1A5 while the efflux of glutamine is carried directly through the SLC7A5–SLC3A2 (LAT1–4f2hc) complex.

Glutamine influx as an exchange substrate for essential amino acid is mediated by the two transporter SLC1A5 and 4F2hc/LAT1 which are act as a tandem fashion in cancer cells.^[26] Also glutamine is required in the several biosynthetic pathways and it is necessary to maintain redox balance through glutathione synthesis ^[9]. On the other side, the oncogenic transformation involved in (a) up-regulates the expression of ASCT2 transporter, (b) up-regulates the enzymes that able to capture the glutamine for use in the TCAC ^[26, 27] and (c) up-regulates the glutamine transporters SNAT1 (SLC38A1) and SNAT2 (SLC38A2) in the activated

immune cells. [28] Moreover, expression of ATB^{0,+} (SLC6A14); ATB^{0,+} transporter by estrogen hormone plays a role in the oncogenesis process via increasing the amino acids transport including glutamine. [29]

Box-1: Glutamine transporters system

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| <p>i. System A: These transporters are sodium-coupled and act as <i>influx transporters</i> under physiological conditions.</p> <ol style="list-style-type: none">1. ATA1/SNAT1 (amino acid transporter A1 or sodium-coupled neutral amino acid transporter 1; SLC38A1),2. ATA2/SNAT2 (amino acid transporter A2 or sodium coupled neutral amino acid transporter 2; SLC38A2), SN1/ <p>ii. System N: these transporters are coupled to sodium-or-hydrogen gradient. They mediate the transport of glutamine in one direction whereas the hydrogen into opposite direction i.e. they are glutamine <i>efflux or influx transporters</i></p> <ol style="list-style-type: none">3. SNAT3 (system N1 or sodium-coupled neutral amino acid transporter 3; SLC38A3),4. SN2/SNAT5 (System N2 or sodium coupled neutral amino acid transporter 5; SLC38A5). <p>iii. System L: Na-independent transport systems for glutamine</p> <ol style="list-style-type: none">5. LAT1/4F2hc6. LAT2/4F2hc |
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Pharmacological intervention against glutamine addiction

The development of the pharmacological agents that are used in management of glutamine addiction are still the infancy period. Therefore, the possible pharmacological strategy to combat the glutamine addiction is included:

1. Glutamine starvation. The effect of glutamine starvation on the cancer cells seems to be dual effect. As a results of glutamine starvation, the reactive oxygen species levels are increased leading to cell death and, on the contrary, leads to generation of reactive oxygen species and thereby cell death and on the opposite direction, the reactive oxygen species that resulted the loss of

the tumor suppressor may cause glutamine addiction as a defense mechanism against oxidative stress. [30] Experimental depletion of glutamine by using the Crisantaspase (anti-leukemic agent) or methionine-L-sulfoximine (glutamine synthase enzyme inhibitor) halted the growth of hepatocellular cancer cell line. [31]

2. Using SLC1A5 inhibitors. The Glutamine influx is essentially mediated by the cooperation of the SLC1A5 and SLC7A5/3A2 transporters. [32] N-γ-aryl-glutamine is a potent competitive inhibitor o SLC1A5 transporter [33] and it may be applicable against glutamine addiction in cancer cell.
3. Suppressing the glutamine anaplerosis. Pyruvate and glutamate are the major sources of anaplerosis. [34] Cancer cells showed high rate of proliferation and they required pyruvate carboxylase and glutamine synthetase-mediated pathways for anaplerosis. Therefore, suppression of these enzymes activities can prevent glutamine addiction and inhibition the growth of cancer cells. Methionine sulfoximine inhibits the activity of glutamine synthetase enzyme and thereby reduced the glutamine synthesis. Methionine sulfoximine (MSO) depleted the glutamine pool and thereby alleviate the glutamine addiction that shared in cancer cell proliferation. [35]
4. Inhibiting the glutamine-dependent reprogramming of cancer cells in mitochondria by using metformin. In prostate cancer cell line, metformin inhibits the growth by 50% via activation the activated mitogen phosphokinase pathway. [36] Metformin-doxorubicin combination killed cancer cells, reduced tumor mass and prevented the relapse of the tumor in experimental animal model. [37]
5. Blocking the activity of the transporters via inhibition of the glutamine-dependent mammalian target of rapamycin (mTOR) pathway. [38] Everolimus is a specific (mTOR) inhibitor and it is used in management of advanced renal cell carcinoma, pancreatic neuroendocrine cancer and breast cancer. [39-41]
6. Reducing the blood glutamine level by using l-asparaginase enzyme [42] or deplete the plasma glutamine by using phenylbuterate, a compound that approved by FDA for the treatment of hyperammonia in acute liver disease. [43]

On the other side, Some researchers believed that glutamine improves the effectiveness and reduces the adverse reactions of cancer chemotherapy e.g. doxorubicin, methotrexate, and 5-fluorouracil and paclitaxel. According to the University of Maryland Medical Center report, glutamine may stimulate the growth of tumors, therefore, there is no need to use glutamine supplementation as the body can naturally synthesis this amino acid. The use of glutamine supplementation in the sport medicine for building the muscle mass or in management of wasting syndromes as with cachexia that associated with cancer or used as a nutrient may cause glutamine addiction. The daily recommended glutamine in healthy subjects is 0.1 g/kg body weight and up to 0.8 g/kg in patients with diseases associated with glutamine deficiency. Therefore, in healthy adults (weighing 70 kg body weight) who used glutamine supplementation more than 7g/day or 7 glutamine capsules (the strength of l-glutamine is 1000 mg/capsule) are potentially at risk of developing glutamine addiction and may flare up the occult cancer.

Conclusion

It concludes that prescription of glutamine supplementation is not free from the risk of glutamine addiction in patients with occult cancer or in the early stages of the malignancies.

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