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Amino acid depletion as a means of cancer treatment: a rebirth of an old treatment paradigm

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Titolo
La deplezione di aminoacidi come mezzo per il trattamento del cancro: la rinascita di un vecchio paradigma di trattamento

Key words
Arginine, arginase, hepatocellular carcinoma, melanoma, pegylated

Parole chiave
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Summary
Nutritional deprivation/depletion studies in laboratory/veterinary animals have led to great scientific discoveries and better understanding of the biosyntheses and metabolisms of the studied nutrients, such as fatty acids, vitamins, amino acids and hormones. In cancer cell cultures, depletion of a number of amino acids such as arginine, 5-hydroxytryptophan, leads to cell death in a broad range of cancer cells. This provides the scientific basis of amino acid depletion in cancer treatment.

Riassunto
Studi di deplezione/deprivazione nutrizionale in animali di laboratorio e animali sottoposti a cure veterinarie hanno portato a grandi scoperte scientifiche e a una migliore comprensione della biosintesi e del metabolismo dei nutrienti studiati, come gli acidi grassi, le vitamine, gli aminoacidi e gli ormoni. In colture di cellule cancerose, l'esaurimento di un certo numero di aminoacidi come l'arginina, 5-idrossitriptofano, porta alla morte cellulare in una vasta gamma di cellule tumorali. Ciò fornisce la base scientifica della deplezione di aminoacidi nel trattamento del cancro.

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Nutritional deprivation/depletion studies in laboratory/veterinary animals have led to great scientific discoveries and better understanding of the biosyntheses and metabolisms of the studied nutrients, such as fatty acids, vitamins, amino acids and hormones. *In vitro*, amino acid depletion results in normal somatic cells entering into quiescence (G_0). Upon repletion of the amino acid, they immerge from quiescence into G_1 and normal cell cycle resumes. Cancer cells behave rather differently in the presence of amino acid restriction. Even in amino acid depletion, they continue to go into cycle resulting in 'arrests' at various cycling phases such as G2/M; some die of apoptosis (1). This suggests defects in different nutritional checkpoints at different phases of the cell cycle in cancer cells. In cancer cell cultures, depletion of a number of amino acids such as ar-

ginine, 5-hydroxytryptophan, leads to cell death in a broad range of cancer cells. This provides the scientific basis of amino acid depletion in cancer treatment.

In the seventies and eighties, L-asparaginase, an L-asparagine depleting enzyme, came into clinical prominence in the treatment of acute lymphoid leukemias (ALL) and lymphomas (2, 3). The drug restricts L-asparagine by enzymatically converting it to L-aspartate. Since leukemic cells lack the enzyme asparagine synthase (AS) to regenerate L-asparagine, they die rapidly with dramatic clinical benefits. Normal somatic cells on the other hand, with their intact AS, survive L-asparagine restriction. The drug is highly effective against acute ALL and certain non-Hodgkin's lymphomas, even as a single agent. However, resistance to the drug also quickly develops thought to be due to rapid up-regulation of the AS gene (4, 5).

Current research indicates that the amino acid depletion of which causes the greatest perturbation in cancer cell proliferation and least "detrimental effect" to the host is arginine, which, arguably, is one of the most versatile and indispensible amino acid in the body (6). The amino acid plays a key role in virtually all biochemical pathways and is present in almost all peptides and enzymes. It is intimately involved in a myriad of biochemical reactions including the urea cycle in the liver, polyamine and creatine biosyntheses and production of nitric oxide in the vascular tone control, etc. (7). It has been well documented that dietary restriction of arginine resulted in retardation of tumor growth in laboratory animals; the converse is also in arginine-enriched diet (8).

Conceptually, arginine is such an important amino acid that the body cannot afford to be deficient of, let alone tolerating its complete absence. An important enzymatic system exists in the mitochondria of somatic cells in which a tightly bound dual enzyme system with Argininosuccinate Synthetase/ Argininosuccinate Lyase (ASS/ ASL) endogenously converts citrulline to arginine, thus providing an alternative source of the amino acid (9). Citrulline is found in abundance in the blood, tissue fluid and intracellular space. In time of deficiency or period of rapid growth, this system goes into overdrive ensuring adequate supply of arginine. This dual source of arginine is the reason why, despite its great prominence in the body, arginine is still classified as a non-essential amino acid since the body can synthesize its own arginine (10).

With immunohistochemistry and RT-PCR technique a number of tumors, including hepatocellular carcinoma (HCC) and melanoma, were found to lack or under-express ASS/ASL, and they are particularly prone to arginine restriction both *in vitro* and *in vivo* (1-12). This is a rather simplistic way to rationalize the mechanism of cell death in these tumors under the condition of arginine depletion. We have ample scientific data to indicate that arginine depletion causes a multitude of intracellular perturbations, which in turn lead to the demise of these tumors, irrespective to their ASS status.

In vivo, arginine depletion can be achieved with an arginine degrading enzyme such as arginase or arginine deiminase (ADI); the former is a mammalian hepatic urea cycle enzyme now produced in vast quantities with recombinant DNA technique (12) while the latter is a protein derivative of a *Mycoplasma*, either *argini* or *homoni (*13). Unlike in *in vitro* conditions, arginine depletion in real life animals poses a number of problems since these native enzymes have very short *in vivo* half-lives. This problem can be resolved with the process of pegylation, which lengthens the enzymes circulatory half lives substantially (14). Another problem is the development of auto-antibodies which occur readily even after short exposures, such as weeks, rendering the enzymes ineffectual. This appears to be a major pro-

blem for ADI and not arginase, being a humanized hepatic enzyme. Arginine depletion with pegylated arginase and ADI has now been shown to be an effective way of tumor control: pegylated ADI has completed Phase II clinical trials vs. HCC and melanoma in Italy, Taiwan and the US (15). Pegylated recombinant hepatic arginase has completed phase I clinical in HK in a group of HCC patients.

Pegylated recombinant hepatic arginase (Coded-named BCT-100) was co-developed by the Hong Kong Polytechnic University and Bio-Cancer Treatment International Ltd., a Hong Kong-based biotech company. The drug is now being produced in strict GMP conditions in Northern China and is ready for clinical trials in both the US and the PRC. Phase I study of the drug at Queen Mary Hospital, University of Hong Kong, started in May 2008 and has recently closed. Clinical data confirmed that the drug is effective and safe in depleting circulatory arginine in human subjects in a dose-dependent manner. Of the 8 patients who completed all 12 weeks of depletion, 4 had stabilized disease with time to progression over 2.8 months. The drug is extremely well tolerated with excellent QoL profile. Phase II is due to start in late 2009.

References

1. Scott L, et al. Single amino acid (arginine) deprivation: rapid and selective death of cultured transformed and malignant cells. Br J Cancer 2000; 836: 800-10.
2. Capizzi RL, et al. L-asparaginase: clinical, biochemical, pharmacological and immunological status. Ann Int Medicine 1971; 74: 893-901.
3. Sallan SE, et al. Influence of intensive asparaginase in the treatment of childhood non-T-cell acute lymphoblastic leukemia. Cancer Res 1983; 43: 5601-7.
4. Chakrabarti R, Schuster SM. L-asparaginase: perspectives on the mechanisms of action and resistance. Int J Ped Hem/Oncol 1997; 4: 597-611.
5. Jousse C, et al. Amino acids as regulators of gene expression: molecular mechanisms. Biochem Biophys Res Commun 2004; 313: 447-52.
6. Wheatley DN, et al. Arginine catabolism, liver extracts and cancer. Pathol Oncol Res 2002; 8 (1): 18-25.
7. Morris SM Jr. Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr 2002; 22: 87-105.
8. Gonzalez GG, Byus CV. Effect of dietary arginine restriction upon ornithine and polyamine metabolism during two-stage epidermal carcinogenesis in the mouse. Cancer Res 1991; 51: 2932-9.
9. Schimke RT. Enzymes of arginine metabolism in mammalian cell culture. I. Repression of argininosuccinate synthetase and argininosuccinase. J Biol Chem 1964; 239: 136-45.
10. Hoogenraad N, et al. Inhibition of intestinal citrulline synthesis causes severe growth retardation in rats, Am J Physiol 1985; 249: G792–G799.
11. Dillon BJ, et al. Incidence and distribution of argininosuccinate synthetase deficiency in human cancers: a method for identifying cancers sensitive to arginine deprivation. Cancer 2004; 100; 826-33.
12. Cheng PN, et al. Pegylated recombinant human arginase (rhArg-peg5,000mw) inhibits the in vitro and in vivo proliferation of human hepatocellular carcinoma through arginine depletion. Cancer Res 2007; 67: 309-17.
13. Takaku H, et al. Anti-tumor activity of arginine deiminase from *Mycoplasma argini* and its growth-inhibitory mechanism. Jpn J Cancer Res 1995; 86: 840-6.
14. Holtsberg FW, et al. Poly(ethylene glycol) (PEG) conjugated arginine deiminase: effects of PEG formulations on its pharmacological properties. J Control Release 2002; 80: 259-71.
15. Izzo F, et al. Pegylated arginine deiminase treatment of patients with unresectable hepatocellular carcinoma: results from phase I/II studies. J Clin Oncol 2004; 22: 1815-22.