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Review Article

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L-Tryptophan Depletion Using a New Bioreactor: A Possible New Cancer Therapy

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Abstract

Not all cancer therapeutic strategies known to date are adequate for all cancer patients. Most of them are followed by a high rate of severe side effects and complications. L-Tryptophan metabolism plays a key role in organism development, as well as in the occurrence and development of tumors. By degrading certain amino acids, tumor growth can be limited while maintaining the body's normal nutritional requirements. The L-tryptophan depletion bioreactor is described as a possible new method of cancer therapy. L-tryptophan is an essential amino acid that has been recognized as an important cancer nutrient and its removal can lead to destruction of the tumor cells. Tumor cells or normal human cells cannot synthesize L-tryptophan and therefore tumor resistance is unlikely to develop. L-tryptophan is also a constituent for different biomolecules such as Sero-tonin, Melatonin, and is needed for other synthesis processes in the cell growth. L-tryptophan degrading enzymes with three iso-enzymes called tryptophan side chain oxydase (TSO) I, II, III have different molecular weights and different effectiveness. All the TSO enzymes have heme that can catalyze essentially similar reactions involving L-tryptophan as a substrate. The most effective TSO is the type TSO III. A column, which contained TSO, immobilized on silica beats as a bioreactor, was integrated in a plasmapheresis unit and tested it in different animals. In sheep and rabbits, L-tryptophan depletion in plasma was shown at 95% and 100% rates respectively by a single pass through the bioreactor. In 20 different tumor cell lines, there were different efficacies. Brest cancer and medulloblastoma showed the greatest efficacy of L-tryptophan degrading. The gene technology of TSO production from Pseudomonas is associated with formation of endotoxins. These endotoxins must be eliminated. Bioreactors with TSO III are developed to treat cancer diseases successfully and has low side effects. A combination of L-tryptophan depletion with all available cancer thera-pies is possible.

Keywords: L-tryptophan, Tryptophan metabolism, L-tryptophan side chain oxidase (TSO), TSO bioreactor, antitumor enzyme

1. Introduction

Since several years is known, that L-Tryptophan (Trp) metabolism is involved in cancer development and immune suppression [1]. L-Tryptophan is an essential amino acid which is required for different protein biosynthesis and its metabolites to activate their growth and evade defenses [2, 3]. L-Tryptophan is also a biochemical precursor of metabolites including gastrointestinal functions, immunity, metabolism, and the nervous system [2]. Cancers utilize Trp and its metabolites to promote their growth and evade host defenses [3]. They get Trp through upregulation of TRP transporters, and up-regulate key enzymes of Trp degradation, and down-regulate others [3]. Enzymes and metabolites of Trp metabolism promote many effects and are influenced by different body systems. They have large effects of the immune system, which can directly or indirectly influence cancer therapy strategy. Most of the free Trp is degraded into several biological active compounds through the kynurenine pathway (KP) or serotonin pathway [4]. Through KP, nicotinamide adenine dinucleotide (NAD*) increases, a main and product with antioxidant activity under the action of indoleamine-2,3-dioxygenase (IDO) or tryptophan-2,3 dioxygenase (TDO). In the serotonin pathway, serotonin can be further converted into N-acetyl-serotonin and melatonin [4]. The intestinal flora is important Trp absorption.

In preclinical models and clinical cases, IDO, TDO and kynurenine have been shown to accelerate tumorigenesis, proliferation, invasion, and metastasis [4]. The Trp metabolism leads to the production of several essential substances for host physiology, which intervenes in many diseases such as neurologic, psychiatric, metabolic, infections, intestinal diseases, and cancer cells [2]. Therefore, Trp metabolism in cancer cells and/or cancer related cancer-associated stromal cells contributes to the suppression of antitumor immune response [5]. L-Tryptophan has been recognized as an important cancer nutrient and its removal from blood can lead to destruction of the tumor [6]. Normal or tumor cells cannot synthesize L-Tryptophan and tumor resistance is unlikely to develop [7]. Since several years, different authors try to develop therapy strategies for degrading

Trp and the metabolite to influence various cancers in animal experiments and humans [8-10].

Cancer treatment has been always considered one of the most critical and vital themes of clinical science. Many approaches have been developed, depending on the type and the stage of tumor. However, approximately10 percentage of all malignant diseases in a progressive stage can be cured. A great problem of the most administered chemotherapy regimens is often the development of resistance against different cancers [7, 11, 12]. In many cases, the resistance exists primarily before the chemotherapy is administered, or the oncogenes of cancer cells can be mutated during the chemotherapy. The result is a resistance against the administered chemotherapy [11]. Resistance to chemotherapy can to specific mechanisms intrinsic cancer biology or general mechanisms common to different tumor types or drug pharmacokinetics [13, 14]. The acquisition of chemo-resistance is a complex and multifactorial phenomenon related to the tumor micro-environment, and the mechanism has not been fully clarified. However, to date there have been few reports about the establishment of cancer cell lines resistant to chemotherapeutic drugs [15-17]. A comparable mechanism is observed for the new kinase inhibitors or for the monoclonal antibodies. The cancer cells can change their oncogenes by mutations resulting in resistance against the kinase inhibitors. In these cases, new drugs and therapeutic concepts must be developed continuously [7].

The pathology of various cancer diseases has shown that the primary oncogenetic defect shall be acquired resulting in genetic aberration which, independent of the cancer, leads to qualitative and quantitative changes in the production of special proteins [7]. These special proteins have a key function in the regulation system of cell growth and differentiation. Different proteins such as growth factors, receptors, cytoplasmatic proteins belong to these substances, which by dysregulation can induce a malignant disease [7].

Various new sophisticated therapeutic strategies were developed in recent years ago, of which some are summarized in Table 1. However, the new therapeutic strategies are only indicated for some different cancer diseases, therefore various therapeutic strategies or combination of these are necessary. No previous cancer therapeutic strategies are effective in all patients, and they are often associated with a high rate of severe side effects [7]. A large problem is the primary or acquired resistance to different chemotherapeutic drugs [11, 13-17]. The high rate of side effects and low effectiveness need the development of new drugs and new therapeutic methods constantly. Possibilities of treatment of different cancers with so-called anti-tumor enzymes, bioreactors, as an extra-corporeal tumor treatment are reported [7, 18-30]. The influence on the protein synthesis by depletion of essential amino acids such as L-Tryptophan is a new strategy [6, 31-33].

Besides Trp certain amino acids such as L-asparagine, and L-glutamine have been recognized as important cancer nutrients, and the removal of these amino acids can lead to decrease and destruction of the tumor [34-37]. Since these so-called anti-tumor enzymes are derived from bacterial or fungal sources, immunological responses are observed after parenteral administration [31].

The isolation of the L-tryptophan degrading enzyme, indolyl-3-alkane-αhydroxylase was described by Roberts et al. [31, 38]. They found later two isoenzymes and they were called tryptophan side chain oxydase (TSO). Blood tryptophan depletion by TSO resulted in a significant anti-neoplastic activity against mouse tumors in vivo. A new iso-enzyme was isolated from blood by Schmer et al, in 1978, and was called TSO III, and they developed a bioreactor to us in an extracorporeal system for degrading L-Tryptophan [39, 40].

• New endocrine and cytotoxic therapy like antiestrogen, aromatase inhibitors and cytotoxic drugs like Taxane, campothecin analog, etc. (18).
• High dose chemotherapy and stem cell transplantation in leukemia and solid tumors (19)
• Cancer vaccines and specific immunotherapy (20)
• Antibodies as specific cancer therapy with monoclonal antibodies (21)
• Immunotoxins (22)
• Human gene therapy (23)
• Tyrosine kinase inhibitors (24)
• Detection of tumor cell dissemination by immune cytology (25)
• IA with polyclonal ab against sTNFR (26)
• Neoangiogenesis and tumor growth (27)
• Transforming the TA into immunologic therapy (28)
• Cancer nanotechnology (29)

IA. Immunoadsorption, TA: therapeutic apheresis, sTNFR: tumor necrosis factor rec

Table 1: Modern Cancer Strategies (mod. after 7)

2. Methods

Treatment of certain tumors by deprivation of the essential amino acid L-tryptophan has the advantage over non-essential amino acid deprivation, because tumor cells cannot synthesize L-tryptophan [39]. This is an advantage over non-essential amino acids deprivation because host and tumor cells cannot synthesize L-tryptophan, and tumor resistance is therefore unlikely to develop. L-tryptophan cannot be produced in the organism itself

[7, 39]. L-tryptophan as an essential amino acid is an important amino acid for the cellular integrity, and is needed for different metabolic processes, etc.

L-tryptophan is essential for the protein synthesis and reduction, the genome replication and the growth of cell organelles etc. [7]. It is a constituent for different biomolecules such as serotonin, melatonin and is needed for other synthesis processes in the cell growth [4]. A lack of L-tryptophan is associated with different side effects and is followed by a destruction of cells, especially of cells with a high division rate.

Extracorporeal L-Tryptophan depletion can interrupt all Trp metabolism and effects on the immune system, and the following growth of cancer cells, therefore, the growth of the cancer can be stopped [3]. Schmeer et al. developed an extracorporeal bioreactor system, which contains TSO III, to study the toxic side effects and immunologic reactions in animal experiments [40, 41]. The bioreactor for removing the potential cancer nutrient L-tryptophan from blood was used in tumor bearing animals.

The isolated L-tryptophan degrading enzymes (indolyl3-alkane-α-hydroxylase, INDH) has three iso-enzymes and called tryptophan side chain oxydase (TSO) I, II, III. The first iso-enzyme TSO I has a molecular weight of about 60,000 Daltons, the second iso-enzyme TSO II has a molecular weight of about 44,000 Daltons, and the third iso-enzyme has a molecular weight of about 42,000 Daltons as determined by sodium dodecyl sulphat-polyacrylamid gel electrophoresis [7, 40, 42]. These isoenzymes can be differentiated by tryptic digestion.

The 3 TSO enzymes have been characterized as multi-enzyme complexes containing heme that catalyze essentially similar reactions involving L-tryptophan as a substrate. The TSO I and II are distinguishable by their subunit structure, antigenicity and by their reactivity and specificity for various substrates, indicating that TSO II and I are distinct enzymes [7, 39]. Schmer isolated, at the Sloan Kettering Institute for Cancer Research, New York, another TSO enzyme, which he named TSO III, which is more effective in degrading L-tryptophan than TSO I or II, in 1978 [33]. He tested the isolated TSO type III, the most effective of the 3 types, in animals (sheep, rabbits, and rats), naked immune supprimized rats and in 20 different human cell lines [40].

Enzymatic removal of L-tryptophan from blood of a patient by plasmapheresis and extracorporeal treatment by enzymatic degradation of L-tryptophan in the pheresed blood has long been perceived to have therapeutic benefits [35, 38]. Blood levels of L-tryptophan modulate synthesis and synaptic release of the neurotransmitter serotonin [4, 7]. Varying L-tryptophan blood levels provides a means to affect brain serotonin levels. The human kidneys will eliminate the metabolites, which are producing by the L-tryptophan degrading enzymes [7].

The extracorporeal bioreactor system containing TSO type III was developed by Schmer et al. [39]. The bioreactor is based on silica. The amino groups containing silica beads were activated with 25 % glutaraldehyde. The activated aminosilane beads were washed with distilled water and finally equilibrated with 0.2 M sodium acetate pH 5.5 [7]. The activated silica beads can be stored in this buffer at 4°C and remain fully active for more than 6 weeks. A solution of TSO in 0.2 M sodium acetate pH 5.5 was passed over the reactor column until the red colored enzyme solution appeared at the outlet. After different washing procedures the pre-activated micro-reactors, consisting of a polyacrylic-cellulose copolymer were equilibrated with 0.2 M sodium acetate with a pH 5.5 and filled with 1 % TSO solution in the same buffer. The reaction conditions, wash procedures and sterilization were identical to the procedure described for silica beads derived bioreactor. The enzyme then was eliminated from endotoxin by different washing procedures and/or using an endotoxin removing kit. The silica-based enzyme reactor was filled in columns, washed and sterilized [7].

The amount of TSO bound to the matrix was determined by pumping sodium phosphate through the bioreactor. The increase in absorbance at 333nm was then expressed in enzyme unit bounds per ml reactor bed. In vitro leakage was determined by pumping sodium phosphate solution through the bioreactor for 2 hours in a circuit. One ml of the solution was then mixed and the increase in absorbance at 333nm within one hour was observed as a sign of leakage [7].

3. Results

In a rigorous experiment, one could show that the enzyme reactor can degrade L-tryptophan. One liter of human plasma was perfused at 10 ml/min through the column. The concentration of L-tryptophan was significantly lower after the bioreactor column than the concentration of L-tryptophan before the bioreactor column [7, 43].

The TSO-bioreactor was tested in different animals [7, 40-43]. Schmer et al. tested in sheep and rabbits the TSO-bioreactor with a closed-circuit mini plasmapheresis unit. They observed that the Trp depletion in plasma was 100 % in sheep and 95 % in rabbits by a single pass through the bioreactor. L-tryptophan was effectively eliminated, excellent results [40].

In 9/10 immune supprimized rats a strong regression of the tumor was found in comparison to the control animals. The destruction of the tumor cells was not only in the center of the tumor but in the periphery of the tumor too, which was observed in histopathological investigations [40]. The treatment with TSO-bioreactor can be combined with vascular inhibiting substances [39]. Some different results were seen in 20 different tumor cell lines. Brest cancer and medulloblastoma showed the greatest efficacy of L-tryptophan degrading.

With interferon-γ, all cell lines showed a higher L-tryptophan use and therefore a rapid destruction of all cells [44]. In the culture medium of murine leukemia cells Trp limitation caused a decrease in DNA and histone synthesis followed by complete growth arrest [7]. The efficacy can be improved with the vascular inhibitors and/or interferon-γ. The anti-neoplastic effect of gamma-interferon-γ is most recently thought to be caused by intracellular L-tryptophan depletion via activation of indoleamine 2.3-dioxygenase [7, 40]. The precise mechanism of Interferon-γ in tryptophan degradation is not clarified.

When used as an agent for reducing blood L-tryptophan levels

in a human patient displaying the symptoms of a malignancy, a TSO enzyme composition is administered in an amount sufficient to achieve a dosage of 0.1 to 200 IU/kg body weight/day, and preferable 70 to 120 IU/kg body weight/day, and more preferable 75 to 95 IU/kg body weight/day when given either as a single dose per course or in incremental doses [39, 40].

The new bioreactor for removing the potential cancer nutrient Trp from blood was used in a 58 years old female patient with metastatic uterus cancer. Despite surgery, radiation and a previous chemotherapy, the cancer showed a rapid progression with lymphedema of the left leg and liver metastases [45]. Over 3 weeks, 15 treatments with the bioreactor were performed. The columns with the TSO enzymes were turned on the filtrate line of a therapeutic plasma exchange unit. In total 5-8 L plasma were treated with the bioreactor per session. Measurements of Trp pre and post the bioreactor showed an elimination rate of 21 to 43 % of Trp per session. The lymphedema disappeared after 10 treatments; the tumor marker decreased significantly. The patient was in a better condition after the treatment period. The treatments were tolerated well. Only in three treatments side effects like shivering and fever were observed. The side effects could be stopped by reducing the filtrate rate of the blood separation and the application of steroids. This first treatment of a cancer patient showed the blood Trp depletion by TSO resulted in an antineoplastic activity against cancer.

On the data of Bambauer, Yefu et al. extracted and purified TSO from Pseudomonas [46]. The results of flow cytometry confirmed the TSO apoptotic activity. In animal experiments, they found the tumor suppressive effect was better in the oncotherapy group than in the intraperitoneal control group. TSO enzyme could inhibit tumor proliferation and promote tumor apoptosis, which was found in immune-histochemistry results. The new TSO enzyme which have a molecular weight of 219, can degrade Trp. The extraction/purification and amino sequencing obtained its basic information; then a preliminary of its anticancer effects was performed. The exact sequence of TSO was clarified [46]. TSO has a degrative effect on Trp and effected proliferation and migration of tumor cells in vitro and in vivo. Yefu et al. could show that TSO suppresses hepatocellular carcinoma through degradation of Trp. They develop now tryptophan-free foods for diet in cancer patients, especially for some weeks after cancer diagnosis and/or during the bioreactor treatment [47].

4. Discussion

In recent years, many authors published the results of their investigation such as small molecules originated from Trp as potential biomarkers or new treatment strategies of immune resistance in various cancers [48-51]. L-Tryptophan metabolism plays an important role in cancer, which can promote tumor progression by inhibiting anti-tumor immune responses and increasing the malignant properties of cancer cells. With the new bioreactor (TSO III) for degrading L-tryptophan created by Schmeer et al., a high effectiveness in anti-neoplastic effect with no resistance possibilities is found. With the degradation of Trp all followed metabolism steps could be stopped. In animal experiments with a closed-circuit bioreactor system, more than 95% of Trp in a single pass was reached [40]. Whole blood L-tryptophan levels changed little throughout the experiment indicating a vast extravascular tryptophan pool. The procedures were tolerated well by the animals without any change in vital signs [43].

L-tryptophan is an essential amino acid. L-tryptophan cannot be produced by human or animal cells [7]. Removal of this nutrient from blood cannot be overcome by a higher production in the cells, therefore making it possible to treat cancer cells repeatedly without the disadvantage of the cancer being able to overcome the “bottle neck” situation of nutrient deprivation.

The production of the TSO III enzyme by gene technology, production of the columns and sterilization is the first step, and the new cancer therapy could be started in a clinical trial with an apheresis unit after revised Declaration of Helsinki. The treatment with the TSO-bioreactor will be daily 4 to 5 hours and 5 days per week over 4 to maximum of 5 weeks. This is one treatment cycle (20-25 treatments). The duration of a minimum of 4 to 5 hours per day is necessary to keep the Trp blood concentration as low as possible to release Trp from the vulnerable cells of the tumor. In this phase, Trp could leave the vulnerable cells and invade intravascular, and could split by the TSO-bioreactor in metabolites which results in a very low L-tryptophan blood level. Between the treatments, the extravascular Trp invade to the intravascular space, the blood level of Trp increased. L-tryptophan is probably removed from cells to increase the blood levels [7, 45]. One treatment circle is 4 to 5 weeks because a longer treatment time could influence the Trp metabolisms in the healthy organs. A further treatment circle could be started again after 2 to 3 months, if no remission is reached. A combination with other cancer therapies is possible. However, one treatment circle could be sufficient to destroy or reduce the tumor and the metastases and a remission could be reached [7]. Side effects are very low such as a serotonin deficiency like anxiety, fatigue, cognitive impairment, agitation, chronic pain, feeling worse, etc., and side effects due to the extracorporeal circulation. The possibility of toxicity of endotoxin in the TSO III enzyme must be solved by different washing procedure or using an endotoxin removing kit. Endotoxins are only available by the production of TSO enzyme from Pseudomonas not from fungal source sources.

Yefu et al. investigate the degration effect on tryptophan, TSO protein was isolated and purified from Pseudomonas, and the reaction products were identified by high performance liquid chromatography (HPLC)) and high-performance liquid chromatography tandem mass spectrometry (HPLC-MS) (46). De novo sequencing provided them the complete amino acid sequence of TSO protein. The results of CCD-8, colony formation, transwell and angiogenesis confirmed that TSO inhibitory effects on the proliferation, migration of HCCLM3 cells. TSO significantly inhibited the invasion and migration of HCCLM3 cells and had a significant inhibitory effect on angiogenesis. The results of flow cytometry confirmed its apoptotic activity. In animal experiments, Yefu et al. found that the tumor suppressive effect was better in the oncotherapy group than in the intraperitoneal injection control group. The results of immunohistochemistry also suggested that TSO enzyme could inhibit tumor proliferation and promote tumor apoptosis. The novel enzyme can degrade L-tryptophan, and its basic information was obtained by extraction/purification and amino acid sequencing [46].

For example, alone in Germany 450,000 to 500,000 women, men, and children afflict by different cancers per year. 20 to 30% of these patients die in the first year after diagnosing of cancer [52]. The therapeutic measures to date have very different results in view of healing or quality life, etc. The treatment costs for one therapeutic cycle of Trp depletion of 4 to 5 weeks depend on the production costs of the columns, and the costs of 20 to 25 primary separation of the blood and the perfusion of plasma through the bioreactor column. The costs could be reduced by setting of one column for 4 to 5 weeks per patient and treatment cycle. The columns could be sterilized after every treatment and hold his activity for a minimum of 6 weeks. If only 1 to 10% of the new patients, who afflict the disease every year, will be treated, this would be a great benefit for the patients. The treatment cycles could be repeated after 2 to 3 months or more, if no remission is reached by the first treatment cycle. Between the cycles, a staging of the cancer is necessary. A further step could be the development of a direct blood perfusion through the bioreactor. During the treatment of depletion of Trp by the bioreactor, a diet with Trp-free food is useful

In conclusion, TSO extracted and purified from Pseudomonas had a degradative effect on L-tryptophan and affected proliferation and migration of tumor cells in vitro and in vivo. These findings may contribute to the development of anti-tumor therapies.

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