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Tryptophan Side-Chain Oxidase (TSO) Degrades L-Tryptophan, a Possible New Cancer Therapy

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ABSTRACT

Not all cancer therapeutic strategies known to date are adequate for all cancer types and patients. Many of them are followed by a high rate of 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. L-tryptophan depletion bioreactor is described as a possible new therapeutic strategy of cancer therapy. L-tryptophan is an essential amino acid, which has been recognized as an important cancer nutrient and its removal can lead to destruction of the tumor. Normal human cells or cancer cells cannot synthesize L-tryptophan and therefor tumor resistance is unlikely to develop. L-tryptophan is also a constituent for different bio-molecules such as Serotonin, Melatonin, and is needed for other synthesis processes in the cell growth. A column, which contained tryptophan side-chain oxidase (TSO) as a bioreactor, was integrated in a therapeutic plasma exchange unit, and tested it in different animals and cancer cell lines. Breast cancer, medulloblastoma, and hepatocellular carcinoma showed greatest efficacy of L-tryptophan degrading. TSO is developed to treat cancer diseases successfully, and has low side effects. A combination of L-tryptophan depletion with all available cancer therapies is possible.

Keywords

L-Tryptophan, Tryptophan side-chain oxidase, TSO bioreactor, Antitumor enzyme, Hepatocarcinoma cell line, Tryptophan-free food.

Introduction

A disadvantage is that approximately 10% of all cancer diseases in a progressive stage can be cured. A great problem of the most administered chemotherapy regimens is often a development of resistance against different cancers, which was published at the begin of the 90th [1,2]. Resistance to chemotherapy can attributed to specific mechanisms intrinsic cancer biology or general mechanisms common to different tumor types or drug pharmacokinetics [3,4]. The acquisition of chemo-resistance is a complex and multifactorial phenomenon related to the tumor microenvironment, 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 [5-7]. 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. In the last years, more and more cancer chemotherapy resistances were reported [8-13].

New knowledge in the pathology of various cancer diseases have 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. 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. Recent years ago, various new sophisticated therapeutic strategies were developed of which some are summarized in Table 1.

Table 1: Cancer therapy strategies of the last years (modified after [14].

- New endocrine and cytotoxic therapy like antioestrogene, aromatase inhibitors and cytotoxic drugs like Taxane, camptothecin analog, etc.
- High dose chemotherapy and stem cell transplantation in leukemia and solid tumors [15]
- Cancer vaccines and specific immunotherapy [16]
- Antibodies as specific cancer therapy with monoclonal antibodies [17]
- Immunotoxins [18]
- Human gene therapy [19]
- Thyrosine kinase inhibitors [20]
- Detection of tumor cell dissemination by immunocytology [21]
- Neoangiogenesis and tumor growth [22]
- IA with polyclonal antibodies against sTNFR [23]
- Transforming the TA into immunologic therapy [24]
- Cancer nanotechnology [25]

IA: Immunoadsorption; TA: Therapeutic apheresis; sTNFR: Tumor necrosis receptor.

Not all previous cancer therapeutic strategies are effective in all patients and they are often associated with a high rate of side effects. The high rate of side effects and low effectiveness need the development of new drugs and new therapeutic methods constantly. Various authors reported possibilities of treatment different cancers with so called anti-tumor enzymes, bioreactors as an extracorporeal tumor treatment [26,27]. One possibility is the influence of the protein synthesis by depletion of essential amino acids such as L-Tryptophan [28-31].

Kidd first published the use of serum amino depletion as an effective anticancer agent [32,33]. He reported that serum of normal guinea pig could induce regression in certain types of animal lymphomas. Broome showed that the enzyme L-asparaginase was the anti-neoplastic substance in normal guinea pig serum that depleted the serum of the nonessential amino acid L-asparaginase [34]. The principle of removing amino acids from blood as a form of cancer therapy has proven to be beneficial in cases of acute lymphoblastic leukemia using L-asparaginase to degrade the nonessential amino acid L-asparaginase, constituting an important tumor nutrient. However, L-asparaginase sensitive tumors can eventually become L-asparaginase resistant. This is usually due to the increased denovo synthesis of L-asparaginase by the tumor cells. L-asparaginase is a nonessential amino acid and can be synthesized by the human organism.

Roberts et al. described the isolation of the L-tryptophan degrading enzyme, indolyl-3-alkane-α-hydroxylase, later shown to consist of 2 isoenzymes and called tryptophan side chain oxydase 1, 2. Blood tryptophan depletion by TSO resulted in a significant anti-neoplastic activity against mouse tumors in vivo [27,35].

Methods

The deprivation of the essential amino acid L-tryptophan, as the treatment of certain tumors, has the advantage over non-essential amino acid deprivation, because tumor cells cannot synthesize L-tryptophan [14,36]. This offers the potential advantage over non-essential amino acid deprivation because host and tumor cells cannot synthesize L-tryptophan and tumor resistance is therefore unlikely to develop. L-tryptophan cannot be produced by human or animal cells itself. L-tryptophan is an important amino acid for the cellular integrity and is needed for many different metabolic processes.

The exposure of tumor cells to decreased levels of the essential L-tryptophan offers potential advantage in tumor treatment, since de novo, synthesis cannot occur, therefore preventing tumor resistance. Tumor cells sensitive to L-tryptophan depletion would repeatedly respond to this form of therapy in contrast to all chemotherapeutic approaches where resistance develops leading to the death of the patients. A removal of this nutrient from blood cannot overcome by a higher production in the cells, therefore making it possible to treat sensitive tumor cells over and again without the disadvantage of the tumor being able to overcome the "bottle neck" situation of nutrient deprivation [14].

To design a so called bioreactor for removing the potential cancer nutrient L-tryptophan from blood, the new L-tryptophan degrading enzyme TSO 3 was isolated by Schmer et al., and chemically bound to Glutaraldehyd activated gamma amino silane silica and to Zeta affinity micro columns consisting of a Glutaraldehyd activated polyacrylic cellulose copolymer. In animal experiments, a closed circuit bioreactor in a single pass was used. Zeta affinity bioreactors degraded L-tryptophan levels changed little throughout the experiment indicating a vast extra vascular tryptophan pool. Schmer et al. tested the TSO in sheep and rabbits with a closed circuit mini plasmapheresis unit [37]. The procedures were tolerated well by the animals without any change in vital signs Schmer et al. could show that the L-tryptophan depletion in plasma was 100 % in sheep and 95 % in rabbits by a single pass through the bioreactor. The investigations in immune supprimized rats with tumor like medulloblastoma were in 9/10 animals' strong regression of the tumor in comparison to the control animals. In 20 different tumor cell lines, there were some different results. Breast cancer and medulloblastoma showed the greatest efficacy of L-tryptophan degrading. With gamma interferon, all cell lines showed a higher L-tryptophan use and therefore a rapid destruction of all cells [37,38].

Enzymatic removal of L-tryptophan from blood of a patient by extracorporeal treatment by enzymatic degradation of L-tryptophan in the pheresed blood has long been perceived to have therapeutic benefits. For example, blood levels of L-tryptophan modulate synthesis and synaptic release of the neurotransmitter serotonin. 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 enzyme.

The new bioreactor for removing the potential cancer nutrient L-tryptophan from blood was used in a 57 years old female patient with a 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 [39]. 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. The bioreactor was perfused with plasma (20-30 ml/min.) won by a membrane plasma separator. In total 5 – 8 plasmas were treated with the bioreactor per 1 session. Measurements of L-tryptophan pre and post the bioreactor showed that 21 to 43 % of serum L-tryptophan were eliminated by one treatment. The lymphedema disappeared after 10 treatments, the tumor markers decreased significantly, and 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 was observed. These side effects could be stopped by reducing the filtrate rate and the application of steroids. This first treatment of a cancer patient showed the blood tryptophan depletion by TSO resulted in an antineoplastic activity against cancer.

On the data of Bambauer [14], Yefu et al. extracted and purified TSO from Pseudomonas [40]. The results of flow cytometry confirmed its apoptotic activity. In animal experiments, Yefu et al. found the tumor suppressive effect was better in the oncotherapy group than in the intraperitoneal injection group. The results of immunohistochemistry also suggested that TSO enzyme could inhibit tumor proliferation and promote tumor apoptosis. The novel enzyme that can degrade L-tryptophan was found, and extraction/purification and amino acid sequencing obtained its basic information; then a preliminary of its anticancer effects was performed [40]. TSO has a degradative effect on tryptophan and affected proliferation and migration of tumor cells in vitro and in vivo, especially TSO suppresses hepatocellular carcinoma through degration of L-tryptophan. Yefu et al. develop now tryptophan free foods for diet in cancer patients, especially during the bioreactor treatments, or for some weeks after cancer diagnosis.

Discussion

To treat the cancer successful, a new cancer therapy consisting of the L-tryptophan degrading enzyme TSO 3, has been developed, which will be won by gene technology from bacterial or fungal sources. The bioreactor based on Silica. The amino groups containing silica beats were activated with Glutaraldehyd. The activated aminosilane containing silica beats were then washed after different procedure. The activated silica beads can be stored in buffer at 4°C and remain fully active for more than 6 weeks. 20 to 30 ml of the activated beads filled in a column, sterilized and inserted in the filtrate line of an apheresis unit. Advantages of the L-tryptophan degrading enzymes. TSO 1-3 have an excellent stability, no development of a resistance to tumor cells and the combination on this new therapy with all other therapy measures especially with gamma interferon [14].

The production of the TSO 3 enzyme by gene technology, production of the columns and sterilization is the first step, then the new cancer therapy could be started in a clinical trial with an apheresis unit after revised Declaration of Helsinki. The treatment of one circle includes 5-6 treatments per week, daily treatment 4 to 5 hours and 3 to 4 weeks. The duration of a minimum of 4 hours per day is necessary to keep the L-tryptophan concentration in the blood as low as possible to release L-tryptophan from the vulnerable cells of the tumor. The duration of one circle is 4 weeks because a longer treatment can be influenced the L-tryptophan of the healthy organs. Another circle can be started again after 2 to 3 months, if no remission is reached. A combination with other cancer therapies is possible. However, one circle must be sufficient to reduce the tumor and the metastases and reaches a remission. Side effects are very low and are a serotonin deficiency like anxiety, fatigue, cognitive impairment, agitation, chronic pain, feeling worse etc., and side effects due to the extracorporeal circulation [14]. A further point is the toxicity of TSO. This point must be clarified with different washing procedures before introduction of this therapy in humans. Endotoxins are available only won by Pseudomonas sources. They can be eliminated by different washing procedures or by the gene technology from fungal sources.

For example, alone in Germany 450.000 to 500.000 women and men afflict by different cancers per year. Of these patients, 20 to 30 % die in the first year after diagnosing of the cancer. The therapeutic measures to date have very different results in view point of healing or quality of life, etc. The treatment costs for one therapeutic cycle of L-tryptophan depletion of 3 to 4 weeks depend on the production costs of the columns. The costs for 15 to 20 primary separation of the blood and the perfusion of plasma through the bioreactor column are comparable with the costs of the immunoadsorption. The costs could be reduced by producing TSO from fungal sources and a treatment set of one column for 3 to 4 weeks per patient and treatment cycle. If only 0.1 to 1 % of the new patients who afflict the disease every year will be treated, this would be a great benefit for the patients. The treatment 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 direct blood perfusion through the biore-actor.

The first step is done. The TSO enzyme was isolated and purified by Yefu et al. [40] and tested in human hepatocarcinoma cell line (HCCLM3), and HepG2 and animals. They found, TSO could inhibit proliferation and promote apoptosis. TSO degrade tryptophan and inhibit the growth of cancer cells. In combination with tryptophan free foods, we have a new and effective anti tumor therapy with no resistance possibilities is available.

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