

Review Article

L-Tryptophan depletion bioreactor: a possible cancer therapy

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Abstract. The cancer therapeutic strategies known to date are not adequate for all cancer patients. Most of them are followed by a high rate of side effects and complications. The L-tryptophan depletion bioreactor is described as a possible new method 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 tumour. Normal human cells or tumor cells cannot synthesize L-tryptophan and therefore 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. L-tryptophan degrading enzymes with 3 iso-enzymes called tryptophan side chain oxydase (TSO) I, II, III were isolated. The 3 iso-enzymes can be differentiated by tryptic digestion. They have different molecular weights with different effectivenesses. 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 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. The results in immune suppressed rats with tumors were impressive, too. In 20 different tumor cell lines there were different efficacies. Breast 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. This disadvantage can be prevented by different washing procedures or by using fungal sources for the TSO production. TSO III 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, L-tryptophan side chain oxidase (TSO), TSO bioreactor

Introduction

Approximately 10 % of all malignant diseases in a progressive stage can be cured. A great problem of the most administered chemotherapy regimens is often development of resistance against different cancers [1, 2]. 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 end result is a resistance against the administered chemotherapy [1]. A comparable mechanism is observed for the new kinase inhibitors. 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 various new sophisticated therapeutic strategies were developed of which some are summarized in Table 1.

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.

All the previous cancer therapeutic strategies are not effective in all patients and they are often associated with a high rate of side effects [2]. A further problem is the primary or acquired resistance to different chemotherapeutic drugs [1]. The high rate of side effects and low effectiveness need the development of new drugs and new therapeutic methods constantly.

Some new possibilities for cancer therapy for example with regard to modulation of the dysregulation of the cell growth are shown in Table 1. Various authors reported possibilities of treatment of different cancers with so-called anti-tumor enzymes, bioreactors, as an extracorporeal tumor treatment [13-15]. One possibility is the influence of the protein synthesis by depletion of essential amino acids such as L-Tryptophan [15-18].

Certain amino acids such as L-asparagine, L-glutamine and L-tryptophan have been recognized as important cancer nutrients, and the removal of these amino acids can lead to decrease and destruction of the tumor. Since these

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TABLE 1
CANCER THERAPY STRATEGIES

New endocrine and cytotoxic therapy like antioestrogene, aromatase inhibitors and cytotoxic drugs like Taxane, camptothecin analogs, etc.
High dose chemotherapy and stem cell transplantation in leukemia and solide tumours (3)
Cancer vaccines and specific immunotherapy (4)
Antibodies as specific cancer therapy with monoclonal antibodies (5)
Immunotoxins (6)
Human gene therapy (7)
Tyrosine kinase inhibitors (8)
Detection of tumor cell dissemination by immunocytology (9)
Neoangiogenesis and tumour growth (10)
IA* with polyclonal ab against sTNFR (11)
Transforming the TA** into immunologic therapy (12)

*IA; immunoadsorption, **TA; therapeutic apheresis, sTNFR; tumor necrosis factor receptor.

so called anti-tumor enzymes are derived from bacterial or fungal sources immunological responses are observed after parenteral administration [19].

The use of serum amino depletion as an effective anti-cancer agent was first published by Kidd in 1953 [20, 21]. He reported that serum of normal guinea pig could induce regression in certain types of animal lymphomas. Subsequently, in 1961 Broome showed that the enzyme L-asparaginase was the anti-neoplastic substance in normal guinea pig serum which depleted the serum of the non-essential amino acid L-asparaginase [22].

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 non-essential 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 [14, 23], later shown to consist of 2 iso-enzymes and called tryptophan side chain oxidase (TSO). Blood tryptophan depletion by TSO resulted in a significant anti-neoplastic activity against mouse tumors in vivo.

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 [24].

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 in the organism itself [19]. L-tryptophan is an essential amino acid. L-tryptophan is an important amino acid for the cellular integrity. L-tryptophan is needed for a lot of different metabolic processes.

The availability of tryptophan is essential for the protein synthesis and reduction, the genome replication

and the growth of cell organelles etc. L-tryptophan is a constituent for different bio-molecules such as serotonin, melatonin and is needed for other synthesis processes in the cell growth. 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.

With the extracorporeal L-Tryptophan depletion the growth of cancer cells can be interrupted and the growth of the cancer can be stopped. After the study of toxic side effects and immunologic reactions in animal experiments using TSO, an extracorporeal bioreactor system which contains TSO was developed by Schmer [25]. The bioreactor for removing the potential cancer nutrient L-tryptophan from blood was used in tumor bearing animals.

The isolated L-tryptophan degrading enzymes (indolyl-3-alkane- α -hydroxylase, INDH) has 3 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 sulphate-polyacrylamide gel electrophoresis [26]. These iso-enzymes can be differentiated by tryptic digestion.

All TSO enzymes have been characterized as multi-enzyme complexes containing heme that catalyze essentially similar reactions involving L-tryptophan as a substrate. However TSO I and TSO II are distinguishable by their subunit structure, antigenicity and by their reactivity and specificity for various substrates, indicating that TSO I and TSO II are distinct enzymes. In 1978, Schmer, at the Sloan Kettering Institute for Cancer Research, New York, isolated another TSO enzyme which he named TSO III, which is more effective in degrading L-tryptophan than TSO I or II [17]. Schmer tested the isolated TSO type III, the most effective of the 3 types, in animals (sheep, rabbits, and rats), knocked immunosuppressed rats and in 20 different human cell lines.

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 [26]. 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 metabolites which are producing by the L-tryptophan degrading enzymes will be eliminated by the human kidneys.

The extracorporeal bioreactor system containing TSO type I was developed by Schmer et al. [24]. 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. 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. The silica based enzyme reactor was filled in columns, washed and sterilized.

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.

TABLE 2
BLOOD LEVEL OF L-TRYPTOPHAN (TRP)

Treatment days	Pre-TRP ($\mu\text{g/ml}$)	Post-TRP* ($\mu\text{g/ml}$)	Percent decrease*
1	8	0.7	91.3
2	5.3	0	100
3	5	1.4	72
4	2.8	0	100
5	6	0.7	88.3
6	1.1	ND	ND
7	1.3	ND	ND
8	3.3	0	100
9	1.1	1	9.1

*ND indicates no data.

Results

In a rigorous experiment one could show that L-tryptophan can be degraded by the enzyme reactor. 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.

The TSO-bioreactor was tested in different animals [16, 24, 25, 27]. In sheep and rabbits the TSO-bioreactor was tested by Schmer with a closed circuit mini plasmapheresis unit. He could show that the L-tryptophan depletion in plasma was 100 % in sheep and 95 % in rabbits by a single pass through the bioreactor. These results are very excellent, because the L-tryptophan was effectively eliminated [25].

The investigations in immune suppressed rats with tumors like medulloblastoma were impressive, too. In 9/10 animals a strong regression of the tumor was observed in comparison to the control animals. In histopathological investigations it could be observed that the destruction of the tumor cells was not only in the center of the tumor but in the periphery of the tumor. This suggests that the treatment with TSO-bioreactor can be combined with vascular inhibiting substances [28].

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. Limitation of L-tryptophan in the culture medium of murine leukemia cells caused a decrease in DNA and histone synthesis followed by complete growth arrest. The efficacy can be improved with the vascular inhibitors and/or gamma interferon. The anti-neoplastic effect of gamma-interferon is most recently thought to be caused by intracellular L-tryptophan depletion via activation of indoleamine 2,3-dioxygenase [16, 29].

For instance, 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 [26].

L-tryptophan cannot be produced by human or animal cells [30]. L-tryptophan is an essential amino acid. Removal of this nutrient from blood cannot be overcome by a higher production in the cells, therefore making it possible to treat cancer cells over and over again without the disadvantage of the cancer being able to overcome the "bottle neck" situation of nutrient deprivation.

To design a so-called bioreactor for removing the potential cancer nutrient L-tryptophan from blood, the L-tryptophan degrading enzyme tryptophan side chain oxydase (TSO III) was chemically bound to glutaraldehyde activated gamma amino silane silica and to Zetafinity micro-columns consisting of a glutaraldehyde activated polyacrylic-cellulose copolymer [31].

The silica beads activated polyacrylic-cellulose copolymers (25-30 ml) are packed in a column. After the sterilization and elimination of endotoxins, one column is integrated in the plasma line of a plasmapheresis unit. The patient blood is separated by a hollow fiber membrane separator in blood cells and plasma, the plasma is then perfused through the TSO-bioreactor in which L-tryptophan is splitted in metabolites. The blood cells and the plasma with metabolites of L-tryptophan are then pumped back to the patient. The metabolites will be eliminated by the human kidney.

The treatment with the TSO-bioreactor will be daily 4 to 5 hours and 5 days per week over 3 to maximum of 4 weeks. This is one treatment cycle. The cycle can be repeated every 2 to 3 months until a remission is reached or the cancer can be effectively treated with surgery or radiation or both. Important is that L-tryptophan blood level will be kept on a very low level over some hours during the treatment. In this situation L-tryptophan could leave the cells and could invade into the blood, and could split by the TSO-bioreactor in metabolites which results in a very low L-tryptophan blood level.

In the next treatment the blood level of L-tryptophan increased. L-tryptophan is probably removed from cells to

increase the blood levels. This can be found in an example from the United States Patent for TSO I and II in 1993 as shown in Table 2 [26].

Discussion

A new bioreactor for degrading L-tryptophan (TSO III) created by Schmer and his group showed a high effectiveness in anti-neoplastic effect with no resistance possibilities. In animal experiments a closed circuit bioreactor in a single pass was used. Zetafinity bioreactors degraded L-tryptophan in animals to more than 95 % in a single pass [25]. 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 [32].

A new cancer therapy method consisting of the L-tryptophan degrading enzyme, which will be produced by gene technology from bacterial or fungal sources, L-tryptophan side chain oxydase III (TSO III), is developed to treat cancer disease successfully. The bioreactor based on silica. The amino groups containing silica beads were activated with glutaraldehyde. The activated aminosilane beads were then washed after different procedures. The activated silica beads can be stored in buffer solution at 4° C and remain fully active for more than 6 weeks. The activated beads (20 to 30 ml) are filled in a column, sterilized and inserted in the filtrate line of an apheresis unit. Advantages of the L-tryptophan degrading enzyme TSO are the excellent stability, no development of a resistance by tumor cells and the combination of this therapy with all other therapeutic measures, especially with vascular inhibiting substances and/or gamma interferon [24]. One column with TSO beads will be sufficient for the treatment for 3 to 4 weeks in one patient (5 treatments per week for a maximum of 4 weeks).

A serious disadvantage of the TSO is the formation of endotoxins during the TSO production from *Pseudomonas* by gene technology. The toxicity of endotoxin is high for patients and depends of on its level. Therefore intensive washing procedures and treatments to eliminate endotoxin are necessary before administration in patients [33]. L-tryptophan degrading enzyme TSO can be produced by gene technology from bacterial or fungal sources. The advantage of the fungal sources is to receive a TSO without endotoxin.

An important benefit of TSO enzyme having the indicated minimum specific activity is that the patient is exposed to less enzyme mass per unit of TSO enzyme activity and thereby is exposed to less endotoxin in a given treatment. Reduced exposure to endotoxin results in because of the disclosed TSO enzyme activity. Thus the patient can be exposed to greater amounts of TSO enzyme activity without risking endotoxin-induced histamine-response type side effects such as allergy, fever, sweating, bronchospasm, hypotension, sickness, severe shaking and anaphylaxis.

The side effects of a potential treatment with TSO-bioreactor can be the same, such as by serotonin deficiency such as anxiety, fatigue, cognitive impairment, negative

thoughts, agitation, chronic pain, feeling worse etc. In summary the side effects and the complications during an L-tryptophan degrading treatment could be by:

1) endotoxin, which can be minimized or stopped by a less amount of enzymes or by production of TSO from fungal sources ,

2) degrading L-tryptophan in the blood to a very low level which have the influence of the serotonin metabolism. This can be prevented by treatments of a maximum of 3 to 4 weeks with daily sessions of 4 hours (5 treatments per week). This is one treatment cycle, and can be repeated every 2 to 3 months later.

3) treatment cycles over a longer time may lead to an antibody development. These antibodies can be eliminated by plasmapheresis.

In a first step, the production of TSO III by gene technology from bacterial or fungal sources must be established then the sterilization of the bioreactor material and the sterile production of the columns with all necessary tests like sterilization or stability tests. All description of these measures is available. At the end of this first step is the industrial production of the TSO III-bioreactor.

A second step will be the clinical studies after the revised Declaration of Helsinki in different countries. After production of the TSO III enzyme by gene technology, sterilization and production of the sterile columns (first step), in the second step the new cancer therapy could be started in a clinical trial. Vascular access could be achieved by peripheral veins or by implementing a large bore catheter in the vena cava superior. After evaluation of the laboratory, clinical and other data of 30 to 50 cancer patients with 2 or more different cancers the bioreactor can be distributed.

The third step can be the distribution of the bioreactor TSO III, the implementation of the TSO III bioreactor after the study protocol and to summarize and evaluate all clinical and laboratory data to develop a more effective therapy concept.

Future aspects are the production of TSO III by gene technology from fungal sources. The advantage is that by fungal sources there is no endotoxin production, after which the chemical sterilization and animal tests if necessary may be performed to prove the lack of toxicity. Another point will be the investigation if the application of TSO III intravenously is possible, and if possible to find the amount of the TSO dose, the preparation, toxicity, and a dosage protocol. The next point will be the investigation to produce TSO enzyme as an oral drug.

The advantages of the TSO bioreactor are that no resistance of the TSO III-bioreactor is possible because human or animal cells cannot synthesize L-tryptophan. The possible cancer nutrient TSO III has a high anti neoplastic effect in breast cancer, medulloblastoma and other metastatic cancers.

TSO III enzyme has low side effects, which are not comparable with those of chemo- or radiation therapy. Side effects could be aggression, tiredness, or somnolence, etc. Therefore a limitation of the treatments of 15-20 daily treatments over 3 to 4 weeks is necessary. An L-tryptophan free diet is not necessary. This new therapy concept can be

combined with all other cancer therapies such as surgery, radiation, chemotherapy etc.

A further point is the toxicity of TSO. This point must be clarified with different washing procedures before the introduction of this therapy in humans. Endotoxins are available only won by *Pseudomonas* sources. They can be eliminated by different washing procedures. An antibody production against TSO enzyme is possible but not clarified and is only possible in longer therapy procedure. The antibodies can be eliminated by plasmapheresis.

Last but not least is the commercial aspect; for example in Germany alone 350,000 to 400,000 women and men afflict by different cancers per year. Of these patients, 20% to 30% die in the first year after diagnosing 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 (5-6 treatments per week, duration 3-4 weeks, daily treatment 4-5 hours) depend on the production costs of the column. The costs for 15-20 primary separation of the blood and the perfusion of plasma through the bioreactor column are lower than the plasmapheresis costs. The costs can be reduced by producing TSO from fungal sources and a treatment set of one column for 3-4 weeks per patient and one treatment cycle. If only 0.1%-1% of the new patients who afflict the disease every year will be treated, this would be a tremendous achievement. 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. The gene technology for production of the TSO enzyme (TSO III) and the producing of TSO-bioreactor columns are also patentable, provided that first works be performed.

Conflict of Interest

The author declares no conflicts of interest.

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