American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

A New Bioreactor For L-Tryptophan Depletion as A New Cancer Therapy

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To Cite This Article: *Rolf Bambauer*, Ralf Schiel, Karin Voit Bak and Richard Straube. A New Bioreactor For L-Tryptophan Depletion as A New Cancer Therapy. Am J Biomed Sci & Res. 2024 25(2) AJBSR.MS.ID.003296,* DOI: 10.34297/AJBSR.2024.25.003296

Received: December 09, 2024; **Published:** December 16, 2024

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 severe side effects and severe 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. 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 bio-molecules such as Serotonin, Melatonin, and is needed for other synthesis processes in the cell growth. L-tryptophan degrading enzymes with three iso-enzymes called tryptophan side chain oxidase I, II, III were isolated. The three iso-enzymes can be differentiated by tryptic digestion. They have different molecular weights with different effective nesses. All the tryptophan side chain oxidase enzymes have heme that can catalyze essentially similar reactions involving L-tryptophan as a substrate. The most effective tryptophan side chain oxidase is the type III. A column, which contained tryptophan side chain oxidase, 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. The results in immune supprimized rats with tumors were impressive, too. 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 tryptophan side chain oxidase 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 tryptophan side chain oxidase production, and type 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, Tryptophan metabolism, L-tryptophan Side Chain Oxidase (TSO), TSO Bioreactor, Antitumor enzyme

Introduction

Cancer treatment has been always considered one of the most critical and vital themes of clinical issues. Many approaches have been developed, depending on the type and the stage of tumor. However, approximately 10 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 [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 result is a resistance against the administered chemotherapy [1]. 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 chemoresistance 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 and Human Monoclonal Antibodies (HMA). In these cases, new drugs and therapeutic concepts must be developed continuously.

New knowledge in the pathology of various cancer diseases have shown that the primary onco-genetic 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.

No previous cancer therapeutic strategies are effective in all patients and they are often associated with a high rate of severe side effects [2]. A further problem is the primary or acquired resistance to different chemotherapeutic drugs [1,3-7]. The high rate of side effects and low effectiveness need the development of new drugs and new therapeutic methods constantly.

In the last years, various new sophisticated therapeutic strategies were developed 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. Some new possibilities for cancer therapy for example with regard to modulation of the dysregulation of the cell growth are shown (Table 1).

Table 1: Modern cancer strategies.

-	New endocrine and cytotoxic therapy like antioestrogene, aromatase inhibitors and cyto-toxic drugs like Taxane, campothecin analog, etc.
-	High dose chemotherapy and stem cell transplantation in leukemia and solid tumors [8]
-	Cancer vaccines and specific immunotherapy [9]
-	Antibodies as specific cancer therapy with monoclonal antibodies [10]
-	Immunotoxins [11]
-	Human gene therapy [12]
-	Tyrosine kinase inhibitors [13]
-	Detection of tumor cell dissemination by immune cytology [14]
-	Neo angiogenesis and tumor growth [15]
-	IA with polyclonal ab against sTNFR [16]
-	Transforming the TA into immunologic therapy [17]
-	Cancer nanotechnology [18]

Note*: IA: Immunoadsorption, TA: Therapeutic Apheresis, sTNFR: Tumor Necrosis Factor Receptor

Various authors reported possibilities of treatment of different cancers with so-called anti-tumor enzymes, bioreactors, as an extracorporeal tumor treatment [19-21]. One possibility is the influence of the protein synthesis by depletion of essential amino acids such as L-Tryptophan [22-24]. 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 so-called anti-tumor enzymes are derived from bacterial or fungal sources, immunological responses are observed after parenteral administration [25].

The use of serum amino depletion as an effective anticancer agent was first published by Kidd in 1953 [26,27]. 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 antineoplastic substance in normal guinea pig serum, which depleted the serum of the nonessential amino acid L-asparaginase [28]. The 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 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-αhydroxylase [20,29], later shown to consist of two isoenzymes and 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 [30].

Methods

The Indoleamine 2,3-Dioxygenase 1 (IDO1) is the key enzyme in the catabolism of L-tryptophan through Kynurenine Pathway

(KP), and this enzyme is found in advanced-stage cancers and is associated with poor disease prognosis and immune suppression [31,32]. It has an important role in tumor progression. The IDO1 activity during malignant tumor diseases seems to be a part of the tumoricidal immune defense strategy, when L-tryptophan deprivation and production of pro-apoptotic tryptophan catabolites counteract T cell responsiveness [33]. *Schmer, et al*. isolated the L-tryptophan-depleting enzyme indolyl-3-alkane-α-hydrolase from blood [30]. He found the 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.

This offers the potential advantage over nonessential 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 [30]. L-tryptophan is an essential amino acid, and is an important amino acid for the cellular integrity. L-tryptophan is needed for many different metabolic processes, etc. L-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.

The growth of cancer cells can be interrupted by extracorporeal L-Tryptophan depletion and the growth of the cancer can be stopped. *Schmer, et al*. developed an extracorporeal bioreactor system to study of toxic side effects and immunologic reactions in animal experiments using columns, which contains TSO [34]. 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-A-Hydroxylase, INDH) has three iso-enzymes and called tryptophan side chain oxidase 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 [35]. These isoenzymes 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 II and I 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. Schmer isolated, in 1978, 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 the TSO I or II. Schmer 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 [36].

L-tryptophan depletion in the blood of a patient with cancer by extracorporeal enzymatic degradation of L-tryptophan in the pheresed blood has long been perceived to have therapeutic benefits [34,37]. For example, blood levels of L-tryptophan modulate synthesis and synaptic release of the neurotransmitter serotonin. Varying L-tryptophan blood levels provides and means to affect brain serotonin levels. The human kidneys eliminate the metabolites, which are producing by the L-tryptophan degrading enzymes.

The extracorporeal bioreactor system containing TSO type I was developed by *Schmer, et al*. [30]. The bioreactor is based on silica. The amino groups containing silica beads were activated with 25 % glutaraldehyde. The activated amino silane 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 and/or using an endotoxin removing kit. 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.

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. The TSO-bioreactor was tested in different animals [22,30,34,36,37]. In sheep and rabbits, Schmer tested the TSO-bioreactor 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 excellent, because the L-tryptophan was effectively eliminated [30].

The investigations in immune supprimized 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, too. This sug-

gests that the treatment with TSO-bioreactor can be combined with other cancer therapies especially vascular inhibiting substances [38,39].

In 20 different tumor cell lines, there were some different results. Brest 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 in doleamine 2.3-dioxygenase [22,40].

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 [35]. L-tryptophan as an essential amino acid cannot be produced by human or animal cells [41]. Removal of this nutrient from blood cannot be overcome by a higher production in the cells, there-fore 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.

To design a so-called bioreactor for removing the potential cancer nutrient L-tryptophan from blood, the L-tryptophan degrading enzyme tryptophan side chain oxidase III was chemically bound to glutaraldehyde activated gamma amino silane silica and to Zetaffinity microcolumns consisting of a glutaraldehyde activated polyacrylic-cellulose copolymer [42]. 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. A hollow fiber membrane separates the patient blood 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 the metabolites of L-tryptophan are then pumped back to the patient. A further point could be the whole blood perfusion through the bio-reactor. The human kidney can eliminate the metabolites.

The treatment with the TSO-bioreactor will be daily 4 to 5 hours and 5 days per week over three to maximum of 4 weeks. This is one treatment cycle (15-20 treatments). One cycle can be repeated every 2 to 3 months until a remission is reached or the cancer can be effectively treated with surgery, 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 phase, 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. To the next treatment, the blood level of L-tryptophan in-creased. L-tryptophan is probably removed from cells to increase the blood levels [43]. This can be found in an example from the United States Patent for TSO I and II in 1993 as shown in Table 2 [35].

Table 2: Blood level of L-Tryptophan [35]

Treatment Days	Pre-L-Tryptophan (µg/ml)	Post-L-Tryptophan (µg/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

Note*: ND: Indicates No Data

Yang, 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) [44]. *De novo* sequencing provided them the complete amino acid sequence of TSO protein. The results of CCD-8, colony formation, trans-well and angiogenesis confirmed that TSO inhibitory effects on the proliferation, migration of human hepatocarcinoma cell line M3 (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, *Yang, et al*. found that 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 its basic information was obtained by extraction/purification and amino acid sequencing; then a preliminary analysis of its anticancer effects was performed [44].

The bioreactor for degrading Tryptophan from blood was used in a 58 years old female patient with metastatic uterus cancer. The previous therapy of surgery, radiation and chemotherapy failed. The cancer showed a rapid progression with a lymphedema of the left leg and liver metastases [45]. Over 3 weeks, 15 treatments with bioreactor were performed. The columns with the TSO enzymes were introduced in the filtrate line of a therapeutic plasma exchange unit. Per session, 5-8 L plasma were treated by the bioreactor. Measurements of L-tryptophan pre and post the bioreactor showed a depletion rate of 21 to 43 % of L-tryptophan per session. After 10 treatments the lymphedema disappeared, and the tumor markers decreased significantly. The patient was after 15 treatment in a significantly better condition. All the treatments were tolerated well. Only in three treatment side effects such as shivering and fever were observed, and these could be stopped by reducing the filtrate rate of the blood separation and the application of steroids and fluid. The first treatment of a metastatic cancer patient showed the blood L-tryptophan depletion by TSO resulted in an antineoplastic activity against cancer without severe side effects.

Discussion

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. Different cancers utilize L-Tryptophan to promote their growth and evade host defenses [46]. Cancers can down-regulate enzymes of serotonin synthesis, thereby removing competition for L-Tryptophan from the serotonin pathway. A new bioreactor TSO III for degrading L-tryptophan 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. Zetaffinity bioreactors degraded L-tryptophan in animals to more than 95 % in a single pass [34]. 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 [47].

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 oxidase III, is developed to treat cancer disease successfully [48]. 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 [34]. 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 [42,45]. 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 different washing procedures, or using an endotoxin removing kit a less number 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 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 could 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, which is developed by *Schmer, et al* [30], then the sterilization of the bioreactor material and the sterile production of the columns with all necessary tests like sterilization or stability tests. At the end of this first step is the industrial production of the TSO III-bioreactor [48].

A second step will be the clinical studies after the revised Declaration of Helsinki in different cancer diseases and 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 two 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 [48]. Here, the implementation of the TSO containing columns in the double filtration system of INUSpherese would be excellent [49,50]. Between the hollow fiber membrane, which separate plasma from blood cells, and the adsorber membrane TKM 58 the TSO bioreactor could be inserted the bioreactor. The adsorber membrane could eliminate cell debrides, alpha-2 macroglobulin, TNF autoantibodies, and other toxins from plasma after the bioreactor. This apheresis system would be very effective and minimize side effects.

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 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 TSO III enzyme has a high anti neoplastic effect in breast cancer, medulloblastoma and other metastatic cancers. Tryptophan side chain oxidase III enzyme has low side effects, which are not comparable with those of chemo- or radiation therapy or other modern cancer therapies. 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. A L-tryptophan free diet is not necessary. However, it could be applicated during the treatment time with the bioreactor. 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 must be clarified with different washing procedures before the introduction of this therapy in humans. Endotoxins are available only won by Pseudomonas sources. Different washing procedures or an endotoxin removal kit can eliminate them. 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 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 the cancer. The therapeutic measures to date have very different results in view point of healing or quality of life, etc. [48].

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. 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. In conclusion, TSO extracted and purified from Pseudomonas or fungal source had 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 drugs.

Acknowledgments

None.

Conflicts of Interest

None.

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