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Anticancer Potential of L-Asparaginase: An Overview

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Abstract

Asparaginase, derived from microbial origin hydrolyses L-asparagine to L-aspartic acid. The enzyme finds principal use in the treatment of Acute Lymphoblastic Leukemia during childhood that primarily occurs between two to ten years of age. L-Asparaginase finds its use in management of haemopoietic disorders especially in pediatrics that is caused due to proliferation and enlargement of lymphoblast in bone marrow and in blood as well as other part of the body. L- Asparaginase from bacterial sources exhibit quaternary and tertiary structural forms. However for using it in therapeutic and clinical application it should not generate any fatal allergic reaction to the patient. Such effects can occur due to the enzyme associated L-Glutaminase activity and also due to the endotoxins from bacteria in enzyme preparations. Therefore, with the recent development in biotechnology with respect to production and purification techniques it is possible to get pure L- asparaginase from microbial origin. The present article provides an insight into the mechanism of action of L-Asparaginase as an anticancer agent and its industrial applications.

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INTRODUCTION

The most common type of cancer in childhood is ALL, reports have also indicated that about half the cases out of every 10 cases occur in adults (Nguyen *et al.*, 2018). According to a recent estimate, approximately 53,000 cases are expected worldwide (Solomon *et al.*, 2017). The enzyme under study is an amino hydrolase. Because of its importance in the treatment of a certain biological disorder, it forms the remedy of choice which is used together with other drugs in the management

of disorders like acute lymphoblastic leukaemia and various other similar disorders (Wierzbowska *et al.*, 2008). It is assumed that the demand for L – asparaginase will improve soon due to their possible therapeutic applications as well in food processing (Arif and Hussain, 2014). However, for using it in therapeutic and clinical application, it should not generate any fatal allergic reaction to the patient. Such effects can occur due to the enzyme associated L-Glutaminase activity and also due to the endotoxins from bacteria in enzyme preparations. Therefore, with the recent development in biotechnology for production and purification techniques, it is possible to get pure L- asparaginase from microbial origin. The enzyme catalyzes the hydrolysis of endogenous amino acid asparagine (amide of 2-aminosuccinic acid) into L-aspartic acid as well as ammonia. In most of the cells, regression of L-asparagine can be taken up by alternate production pathway by which L- asparagine is generated form amino acids like aspartic acid and glutamine by the help of enzyme asparagine synthetase. Reduction of L- asparagine from the blood through L- asparaginase leads to impairment of RNA and DNA synthesis with successive blastic cell apoptosis (Leventhal and Henderson, 1971). To combat lymphoma cells, most common and fundamental techniques is to shoot up the levels of L-asparaginase through intravenous route to present levels of L- Asparagine in the body which lead to selective degradation of cancerous cell. Because of this unique anticancer mechanism of action, L- asparaginase is used as multidrug chemotherapy in children and adults with leukaemia. *Erwinia carotovora* and *Escherichia coli* have found their use in the management of critical lymphoblastic leukemia (Verma *et al.*, 2007). Extracellular L-asparaginase enzymes have more advantage than intracellular enzyme because they can grow in great quantity in the culture medium in a familiar environment, and it can be purified with economical methods. This enzyme is mainly found in different bacterial species, animals and plants (Pronk *et al.*, 2008).

Experimental Studies With L-Asparaginase

In the early 1950′s it was observed that guinea pig serum had very high potential, which inhibits the growth of lymphoma cells. In an experiment, the mouse was transplanted with lymphoma cells. Repeated intraperitoneal injection of serum from guinea pig was given to them. In this condition, the mice with lymphoma survived while in control mice lymphoma cells grew continuously and died by a month (Kidd, 1953). In previous studies, it was observed that the serum of guinea pig has anti- carcinoma activity and they reported that serum is rich in enzyme L- asparaginase (Broome, 1968). Around the 1960s, Lymphoma cells susceptible to guinea pig serum along with inadequate L-asparagine were made to grow in cell culture media. This led to a reduction in cancer cell proliferation at a rapid rate in this initial phase. Still, later some of the cells survived and followed by its proliferation under a limited quantity of L- asparagine. Limiting the L-asparagine leads to a reduction of the cell population, however certain cells managed to stay alive which was caused due to lymphoma cells losses its susceptibility after transplantation where original lymphoma cells are inhibited by normal serum of guinea pig after transplantation. Susceptibility lost to guinea pig serum was limited to L-asparagine restriction, although certain amino acids, purines as well as pyrimidines were not able to match this outcome. It can thus be said that L- asparaginase presence in serum of guinea pig were capable of antineoplastic action. Other animal's sera such as that of horse serum, rabbit serum also tested but failed to demonstrate any significant result. Broome was the scientist who experimented to find out the extent and exact role of the L- asparaginase in anticancer activity in the guinea pig. Dolowy carried out the First clinical trial based primarily on L-asparaginase in 1966 (Dolowy *et al.*, 1966). Currently, formulation present in the market has been widely used for acute lymphoblastic leukaemia. The formulations are, however, not entirely free from adverse responses. Thus, there is ample opportunity for researchers to find out safety and effectiveness index of this particular enzyme (Delage *et al.*, 1971).

Structure Of L-Asparaginase

Many experiments have been performed by Scientists to understand the structural confirmation of L- asparaginase right into the molecular level. The enzyme is usually found in Tetrameric form. It has also been reported to be found in hexameric, monomeric and dimeric systems when are isolated from altered sources (LN *et al.*, 2011). L-Asparaginase from bacterial sources exhibit quaternary and tertiary structural forms. Molecular structure of *Erwinia* and *E.coli* species investigated wonderfully with similar 3D structures. *Erwinia carotovora* contains two tetramers made up of 4 identical monomers each (Aghaiypour *et al.*, 2001). 327 amino acid gather in monomers through 14 α-strands, 8 β-helices double domains, a giant N-terminal domain and a lesser C-terminal domain. Between two head-to-head monomers is the active spot present (Swain *et al.*, 1993). The following amino acids contribute to the active location: Thr15, Tyr29, Ser62, Glu63, Thr95, Asp 96, Ala120, and

Lys168, whereas only one residue that is, Ser254 exists next to monomer. The residues leading to the catalytic activity of the enzyme include Thr15 and Thr95 (Janin *et al.*, 2007).

Mechanism Of Action

The enzyme L-Asparaginase finds its use in the management of haemopoietic disorders especially in paediatrics that is caused due to proliferation and enlargement of lymphoblast in bone marrows also in blood as well as other parts of the body. 80 % of childhood leukaemia occurs due to this condition, and some adult leukaemia was also reported in 20% population (Shrivastava *et al.*, 2016). Anti-lymphoma activity is due to reduction of L-asparagine present in blood by L-asparaginase, causing inhibition of protein synthesis, which causes arrest of the cell cycle in the G1 phase. In most of the cells regression of the L-asparagine can be taken up by alternative synthesis pathway by which L- Asparagine is generated from amino acids like L-aspartic acid and glutamine by the help of enzyme asparagine synthetase. Reduction of L- asparagine from the blood by the enzyme L- asparaginase leads to impairment of RNA and DNA production with successive blastic cell apoptosis (Killander *et al.*, 1976).

The main disadvantage of using L- asparaginase therapy is the inactivation of the enzyme before reaching the target. Action sustain for a brief duration also shows high plasma clearance, which required continuous administration of the enzyme at a particular time interval to maintain the therapeutic level (Keating *et al.*, 1993). Also having allergic reaction associated with Glutaminase activity of the enzymes causes depletion in plasma glutamine level. Hydrolysis of amino acid L- asparagine occurs in 2 steps with beta acyl-enzyme produced as an intermediate. L-Glutaminase causes a certain side effect, which can also contribute to the destruction of cancer cells (Distasio *et al.*, 1982). The decline of L-asparagine and Glutamine level was induced by L –asparaginase which is connected to the mTOR Pathway, which leads to subsequent suppression of the pathway. mTOR pathway contains a specific mammalian molecule which acts as a target of Rapamycin (Krall *et al.*, 2016).

mTOR function disturbance leads to inhibition of various other functions related to this pathway which includes serine phosphorylation, threonine kinase and factor 4E binding protein1 which is eukaryotic initiation factor which inhibits the ribosomal synthesis of protein at mRNA translation (Iiboshi *et al.*, 1999). Inhibition of mTOR pathway contributes to the anti-leukemic activity of L-Asparaginase. In other studies, it was found that L- asparaginase has induced autophagic process by inhibiting mTOR1 (two types of mTOR has been reported which are different in structure mTORC1 and mTORC2). mTOR1 regulates the average cell growth and protein synthesis functions, and it inhibits the cell death by a mechanism called autophagy which is regulated by autophagy-related proteins Agt1/ULK and ATG13. L-asparaginase enzyme initiated the reduction of L-asparagine amino acid level, which leads to the death of lymphoma cells (Jacque and Bouscary, 2014).

Role of L-Asparaginase

In leukaemia treatment

L- Asparaginase used as an injectable drug, plays a significant role in the clearance of L-Asparagine from bloodstreams, which has made it a popular and effective therapeutic drug. Treatment of leukaemia with L- asparaginase requires constant monitoring on the level of L- asparagine in the blood, which can lead to recurrence if neglected· L -asparaginase act on leukemic cells by inhibiting enzyme asparagine synthetase which is required for the synthesis of L-asparagine and due to scarcity of L-asparagine, growth of leukemic cell is suppressed significantly making an effective management method for acute lymphoblastic leukaemia.

The enzyme is used for the treatment of leukaemia in all paediatrics and also in the treatment of adults. Various other therapies including the use of steroids, radiation therapy, bone marrow or stem cell transplants etc. available for the treatment of leukaemia. They, however, comprise of a more complex procedure compared to enzyme therapy.

In Amino Acid Metabolism

The enzyme is a significant player in the biosynthesis of amino acids such as lysine, methionine and threonine. Precursor for lysine and threonine is Aspartic acid, and it is also formed by L-asparaginase enzyme.

In Food processing industry

L- Asparaginase finds wide usage as a food processing aid. With present advances in food processing technology, colourless, odourless, crystalline solid acrylamide is formed due to Millard reaction when any starch-containing food was fried or baked at more than 120°C. Acrylamide is toxic and can be carcinogenic to humans (Tareke *et al.*, 2002).

The enzyme was utilized in the food industry because of its capability to transform L-asparagine into aspartate, thus reducing the number of precursors for Millard reactions by pretreating the starch-containing food with L-asparaginase.

In Biosensor technology

With the recent development of technology, L-asparaginase has been used as a biosensor to analyze the level of L-asparagine in leukaemia or food industry. Various spectroscopic methodologies such as XPS, XRD, SEM and TEM are available which are presently being utilized for analyzing L -asparagine level. However, because of the higher cost and tedious procedure, it makes them

unfavourable. For this reason, biosensor technology can be beneficial, reliable, economical and user friendly. It detects ammonia which is released by the effect of L- asparaginase and leads to variation in pH ensuing colour change.

CONCLUSIONS

Research modules have emphasized their focus on asparaginase from bacterial origin. It is, however, imperative to observe and search for the source of enzymes from fungal and actinomycetes origin. The enzyme has well-documented evidence in terms of its usage in the treatment of ALL; the focus should also dwell upon its role in the treatment of other types of cancer. Microorganisms are a vast source of a new identity, especially concerning antibiotics and enzymes. Optimization and purification of the enzyme also need to rich attention to attain reasonable, cost-effective, and best treatment regimen to the ones affected.

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Conflict of Interest

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