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Review

L-asparaginase as a critical component to combat Acute Lymphoblastic Leukaemia (ALL): A novel approach to target ALL

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ABSTRACT

L-asparaginase, an anti-leukaemic drug that has been approved for clinical use for many years in the treatment of childhood Acute Lymphoblastic Leukaemia (ALL), is obtained from bacterial origin (*Escherichia coli* and *Erwinia carotovora*). The efficacy of L-asparaginase has been discussed for the past 40 years, and an ideal substitute for the enzyme has not yet been developed. The early clearance from plasma (short half-life) and requirement for multiple administrations and hence frequent physician visits make the overall treatment cost quite high. In addition, a high rate of allergic reactions in patients receiving treatment with the enzyme isolated from bacterial sources make its clinical application challenging. For these reasons, various attempts are being made to overcome these barriers. Therefore, the present article reviews studies focused on seeking substitutes for L-asparaginase through alternative sources including bacteria, fungi, actinomycetes, algae and plants to overcome these limitations. In addition, the role of chemical modifications and protein engineering approaches to enhance the drug's efficacy are also discussed. Moreover, an overview has also been provided in the current review regarding the contradiction among various researchers regarding the significance of the enzyme's glutaminase activity.

Contents

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1. Introduction

Acute Lymphoblastic Leukaemia (ALL) is a blood cancer in which enormous numbers of immature white blood cells or lymphocytes are produced in the bone marrow. Globally, 3 in 100,000 individuals may acquire the disease, mostly at ages under six years (Grigoropoulos et al., 2013) with the highest likelihood of developing the disease occurring between the ages of 2 and 4 years. The anti-proliferative properties of L asparaginase on leukaemic cells were first identified and characterised in human clinical trials in 1970s. Since then, the enzyme has become a key component in the treatment of ALL. Biochemically, it catalyses the hydrolysis of asparagine into aspartic acid and ammonia. Leukaemic cells require large amount of asparagine to maintain their malignant growth. To meet this demand, they obtain the non-acidic, hydrophilic amino acid both from blood serum as well as synthesising the amino acid themselves in limited amounts (Narta et al., 2007). L-asparaginase exploits the high demand of leukaemia cells for asparagine by depleting the circulating pool of asparagine from blood serum. The depletion of the circulating pools of asparagine from the blood serum results in the death of the leukaemia cells. Various organisms including animals, bacteria, fungi, actinomycetes, algae and plants have been reported to synthesise L-asparaginase, but the anti-neoplastic action of the *E. coli* and *Erwinia caratovora* forms of L-asparaginase have been studied most extensively (Howard and Carpenter, 1972; Narta et al., 2007; Oza et al., 2011; Paul, 1982; Sarquis et al., 2004; Sudhir et al., 2012)

To use L-asparaginase as a key element in the treatment of ALL, it should be devoid of glutaminase activity. However, some investigators such as Parmentier et al. (2015) have provided evidence that the glutaminase activity of L-asparaginase is essential for its cytotoxicity against leukaemic cells. Other key factors for successful use of the enzyme include its high affinity for its substrate asparagine (low K_m value), no side effects, no immunogenic complications and delayed clearance from plasma (prolonged half-life) to minimise the required frequency of administration (Nagarethinam et al., 2012; Parmentier et al., 2015). L-asparaginase from bacterial sources contains up to 10% L glutaminase activity. However, the *Pyrococcus furiosus* L asparaginase demonstrates no glutaminase activity (Bansal et al., 2010; Campbell et al., 1967).

The glutaminase activity of L-asparaginase is probably due to the structural similarity of asparagine and glutamine. The structural formulae show a similarity in the amide groups while the difference between these amino acids is the presence of one additional methyl group in glutamine (Ramya et al., 2012). Furthermore, during the biosynthesis of asparagine, transamidation of aspartate occurs in which glutamine serves as the amide group donor (Nagarethinam et al., 2012). The L-asparaginase enzymes from *E. coli* and *Erwinia carotovora*, which have K_m values of 1.15×10^{-5} M and 1.8×10^{-5} M, respectively, have been shown to have value in clinical applications against ALL (Cedar and Schwartz, 1967; Ho et al., 1970a, 1970b).

The present article reviews various attributes that are considered to be important for effective ALL therapy, including the K_m values, optimal pH, optimal temperature, glutaminase activity and blood plasma clearance rates for enzymes derived from alternative sources including bacteria, fungi, actinomycetes, algae and plants. In addition, strategies to enhance the drug's efficacy including chemical modification, protein engineering approaches and site-directed mutagenesis are also discussed. Because the enzymes from alternative sources including plants, fungi and algae have not been extensively characterised, data regarding pre-clinical and clinical trials for blood plasma clearance and glutaminase activity are not included in this article.

2. Historical background of L-asparaginase (L-ASP)

Asparaginase activity was first detected by Lang in beef tissues in 1904 (Lang, 1904). In 1922, Clementi provided experimental evidence for Lang's observation and reported that the amido-hydrolytic activity of the enzyme was exhibited in all tissues of herbivores, only in the livers of omnivorous animals, and was not present in the organs of carnivorous animals, amphibians, or reptiles (Clementi, 1922). The potency of the enzyme as an anticancer drug was reported by Kid in 1953, who observed the antitumor properties of guinea pig serum (Kidd, 1953). Later, Neuman and McCoy in 1956 demonstrated the metabolic differences between normal and malignant cells *in vitro* in the presence and absence of the amino acid, asparagine (Neuman and McCoy, 1956). Based on insights from these studies, Broom linked the anti-tumour activity of guinea pig serum to asparagine depletion by the enzyme L-asparaginase (Broome, 1963). Despite the acknowledgement of the theory behind the utilisation of the enzyme in malignancies, there were a few challenges in its clinical use because at the time guinea pigs were the main source and the extraction of enzyme from the pig serum in large quantity was difficult. It was only in 1964 that Mashburn and Wriston investigated an alternative source of the enzyme, and L-asparaginase from *E. coli* became of primary interest. Two asparaginases were isolated from *E. coli*, i.e., EC-1 (periplasmic) and EC-2 (cytoplasmic) (Mashburn and Wriston, 1964). However, only EC-2 exhibited antitumor activity. These findings provided a practical basis for the production of enzyme in large quantities for pre-clinical and clinical studies.

The potential of L-asparaginase as an anticancer drug was first shown by Oettgen et al. in 1967 (Oettgen et al., 1967). Parenteral therapy with foreign proteins in humans is limited by the key issue of the drug's immunogenicity, and hypersensitivity reactions were found to be associated with the enzyme extracted from bacteria. Since then, several attempts have been made to reduce the immunogenicity of the drug while preserving its enzymatic activity. One such attempt involved coupling of the enzyme to polyethylene glycol (PEG) which resulted in reduced immunogenicity without altering the antineoplastic property of the enzyme (Abuchowski et al., 1979). When tested in an animal model, this modified version showed reduced antibody formation compared to native form and a significant prolongation of its half-life (Yoshimoto et al., 1986). Recently, a protein engineering approach resulted in significant stability and higher cytotoxicity of the enzyme against cancer cell lines (Mehta et al., 2014). For these reasons, L-asparaginase has been established as an indispensable component in modern chemotherapeutic procedures.