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**Mini Review**

# Hepatotoxicity Issues Associated with Antineoplastic Drug Asparaginase

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**ABSTRACT:**
With the advancement of time, it was proposed to develop an improved approach that makes use of human enzymes for the treatment of various cancers. Asparaginase is one of those enzymes which work by depriving the cancer cells of a vital amino acid and causing apoptosis. Asparaginase has been known for treating acute lymphoblastic leukemia in children and adults since 40 years. But the use of asparaginase has been restricted due to its adverse potential for hepatotoxicity. The present review deals with the hepatotoxic effects of asparaginase and few of the compounds which ameliorates its associated toxic effects. Hepatotoxicity cited by asparaginase may include hyperglycemia, pancreatitis, hyperlipidemia and hyperbilirubinemia. These disorders may get reversible with the use of L-Carnitine, vitamin B complex, mitochondrial superoxide dismutase or eukaryotic initiation factor. Apart from reversibility, some fatal hepatic disorders caused by asparaginase are also discussed.

**Keywords:** Asparagine, hepatotoxicity, cancer, antineoplastic drug, anticancer agent.

## 1. INTRODUCTION

Asparaginase is an enzyme that catalyzes L-asparagine to L-aspartic acid and ammonia [1,2]. It has multiple names like Crisantaspase and Erwinase. L-asparaginases have been used as an anticancer agent to treat acute lymphoblastic leukemia [2] since 40 years whereas in food industry they act as an additive which reduces the production of acrylamide, a carcinogenic compound, present in baked and fried foods [1]. Asparaginase as a hydrolase tends to deplete surrounding asparagine and glutamine causing abruptions in the process of protein synthesis [3]. Asparaginases are mainly derived from some bacterial forms like L-asparaginase from *Escherichia coli* and PEGylated or non-PEGylated asparaginase from *Erwinia chrysanthemi* [4].

Asparagine possess two types of structures viz. chemical structure and crystal or protein structure. While acting as a drug, its crystal structure plays a vital role. Both asparaginases, whether derived from *E. coli* or *E. chrysanthemi* are active as homo-tetramers with 222 symmetry [5]. Each has a monomer of 330 amino acids with two domain fold. Both domains belong to class of alpha/beta proteins. The larger N-terminal domain contains an unusual left-handed beta-alpha-beta crossover which forms a cradle for the active site. The tetramer consists of a pair of dimers, each with an extensive intimate dimer interface which contains two active sites, and each active site contains some residues from both monomers. The crystal structure of L-asparaginase derived from *E. coli* (EcA) contains four molecules of L-aspartate, one bound into each active site. Two threonine residues - T12 and T89 which are conserved throughout the L-asparaginase family, and are known to be essential for activity, lie close to the bound aspartate.

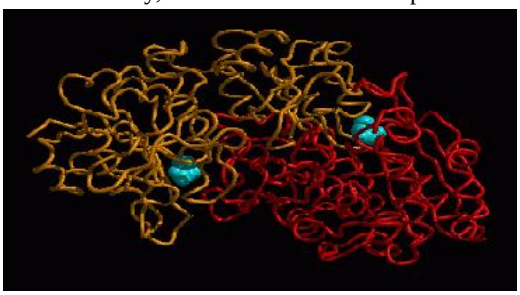

**Fig 1: Intimate dimer of L-asparaginase derived from *E. coli* (EcA)**

Asparaginase works to hydrolyse the asparagine to aspartate and ammonia. Asparagine and aspartate are the most significant non-essential amino acids which are responsible for synthesis of glycoproteins and also play roles in several metabolic reactions. Similarly, the oxaloacetate production is also facilitated by the reactions catalyzed by the enzyme. Being a non-essential amino acid, asparagine is present in normal cells in sufficient amount so that it can carry the processes smoothly. Whereas in cancerous cells, they are

unable to form asparagine by their own as they lack an enzyme called asparagine synthetase which is required to proceed with DNA or RNA synthesis process [3]. Thus it may cause death due to starvation of asparagines by the respective cells. However, Asparaginase enzyme helps those cells to break down the asparagine so that they can carry their wear and tear mechanisms without any hindrances and can make their proteins easily.

## 2. TOXICITY ASSOCIATED WITH ASPARAGINASE

Chemotherapeutic drugs may pose some toxic effects which may restrict their normal application. Hepatotoxicity is like a sin to asparaginase. The adverse hepatotoxic reactions associated with L-asparaginase therapy may include pancreatitis, allergic reactions, thrombotic events and hyperlipidemia [3]. Asparaginase is known not only for its advantages in the field of anti-cancer drugs but also for its several lethal or deleterious effects. Asparaginase can be derived from various sources like plants, fungi and bacteria. Despite the advancements in the field of chemotherapy, the aspect of toxicity of the various chemotherapeutic drugs is also prevalent in the scientific community. Though in cases of acute lymphoblastic leukemia, asparaginase plays a critical role to treat patients but its use has been limited due to several side effects including hepatotoxicity. Hepatotoxic side effects of this drug may include hyperglycemia, hyperlipoproteinemia, hypoalbuminemia, coagulation factor deficiencies [4] and pancreatitis [6]. These effects may lead to liver failure and even death of the patient [7]. Apart from these effects, hypersensitivity reactions are also caused by the polyethylene glycol (PEG) linked asparaginase [8]. Nausea, vomiting, facial swelling, loss of weight and skin rashes are some other side effects caused by PEGylated asparaginase. With respect to toxicity, it has been evaluated that PEGylated asparaginase is less toxic than the native L-asparaginase. Thus, severe hepatotoxic effects are mostly viewed under the influence of native L-asparaginase. Table 1 reveals the studies related to the asparaginase associated toxicity.

**Table 1: Studies related to the asparaginase associated toxicity**

| Sr. No. | Author | Year | Type of Asparaginase used | Model used | Toxic Effects | Reference |
|---|---|---|---|---|---|---|
| 1 | Raja *et al.* | 2012 | L-Asparaginase | Human | Pancreatitis in children | [9] |
| 2 | Christ *et al.* | 2018 | L-asparaginase + PEGylated asparaginase | Human | 3-4 grade hepatotoxicity leading to pancreatitis and thrombosis | [10] |
| 3 | Patel *et al.* | 2016 | PEGylated asparaginase | Human | Sepsis with hepatotoxicity | [11] |
| 4 | Earl | 2009 | Asparaginase | Human | Thrombosis, Pancreatitis and Hyperglycemia | [12] |
| 5 | Shrivastava *et al.* | 2016 | L-asparaginase | Human | Thrombosis, Pancreatitis, and Hyperglycemia | [13] |
| 6 | Radke *et al.* | 1988 | L-asparaginase | Human | Alopecia, nausea, vomiting and hepatotoxicity | [14] |
| 7 | Jenkins and Perlin | 1987 | L-asparaginase | Human and mice | Severe hepatotoxicity | [15] |
| 8 | Koprivnikar *et al.* | 2017 | L-asparaginase | Human | Hypersensitivity reactions, pancreatitis, liver dysfunction and thrombosis | [16] |
| 9 | Rausch *et al.* | 2018 | PEGylated asparaginase | Human | Multivariability in bilirubin content | [17] |
| 10 | Helbig *et al.* | 2018 | PEGylated asparaginase | Human | 3-4 grade hepatotoxicity | [18] |
| 11 | Tsutsui *et al.* | 2001 | L-asparaginase | Human | Hepatitis and anaphylaxis | [19] |

The above table shows the prevalence of several toxic side effects in patients upon treatment with asparaginase, L-asparaginase and PEGylated asparaginase. In a report, a diabetic woman when treated with L-asparaginase developed severe hepatotoxicity. Though, glutamine free asparaginase has been proved to be less toxic in case of diabetic patients [15]. A dose of 1000 IU/$m^2$ L-asparaginase when given to patients suffering from acute lymphoblastic leukemia, 31% of patients were found to have increased bilirubin content and some of the patients also developed pancreatitis [18]. Elevation in the levels of liver transaminases and bilirubin, leading to fulminant hepatitis has also been reported in a 44-year-old man suffering from acute lymphoblastic leukemia [19]. Several allergic reactions are also reported associated with the treatment by L-asparaginase [16]. Anaphylaxis, nausea, vomiting, face swelling and skin rashes are another reported side effects [14, 19]. When L-asparaginase was given with sequential high-dose of cytosine arabinoside to acute non-lymphocytic leukemia sufferers, hepatotoxicity with some allergic reactions was observed [14].

Asparaginase therapy is an essential component of the treatment protocol for acute lymphoblastic leukemia. The effect of asparaginase on protein synthesis may result in a number of toxicities [12]. Thrombosis, pancreatitis, hyperglycemia, liver dysfunction [16], hyperlipidaemia [9] and hepatitis are the common side effects of asparaginase therapy. When the UK National Research Centre evaluated

tolerability of PEG-asparaginase (1000 IU/$m^2$) among 90 patients, it was found that eight patients died because of hepatotoxicity associated with sepsis [11]. PEG-asparaginase and L-asparaginase are always found to be compared for their extent of toxicities. In a study, Forty eight patients receiving PEGylated asparaginase and nine patients receiving L-asparaginase were observed and it was found that the rates of the toxicities were hepatotoxicity (60% vs. 33%), pancreatitis (17% vs. 22%,), thrombosis (19.0% vs. 0%,), or any grade 3-4 toxicity (71% vs. 44%) respectively [10]. The fluctuations in the level of bilirubin and several hepatic disorders suggested omitting asparaginase from the treatment regimen of acute lymphoblastic leukemia as it caused more of hepatotoxicity than curing effect on the respective disease [17].

## 3. AMELIORATION OF HEPATOTOXICITY ASSOCIATED WITH ASPARAGINASE

It has been noted that hepatotoxicity related to asparaginase may get reversed or lowered by treating the hepatotoxic patients with some ameliorative drugs. There are a number of drugs to show this effect. Table 2 highlights some of the studies that revealed ameliorative effects of some drugs to hepatotoxicity of asparaginase. Levo-carnitine (L- Carnitine) is one of those drugs which has been found to have a potential ameliorative effect against asparaginase associated hepatotoxicity. L-Carnitine is a mitochondrial co-factor that can potentially ameliorate the mitochondrial toxicity of asparaginase [20]. Asparaginase associated hyperbilirubinemia [20] and trans aminitis were also found to be reversed by the treatment with L-carnitine [21]. When non-steatotic and steatotic rats were treated with L-carnitine, it was observed that there was a decline in portal venous pressure, oxygen consumption and mitochondrial damage in fatty livers [22]. Even when lymphoblastic leukemic patients (administered with asparaginase)with Grade3-4 hyperbilirubinemia were treated with L-Carnitine, a subsequent improvement of hyperbilirubinemia was seen [20]. The potential of L-carnitine was further enhanced when it was administered in the hepatotoxic patients along with Vitamin-B complex. This combined therapy of vitamin B complex and L-carnitine proved beneficial in treating the hepatotoxicity caused by PEGylated asparaginase to the acute lymphoblastic leukemia sufferers [23].

**Table 2: Studies related to amelioration of hepatotoxic effect of asparaginase with different compounds**

| Sr.No. | Author | Year | Compounds used | Model used | Ameliorative effect | References |
|---|---|---|---|---|---|---|
| 1 | Schutle *et al.* | 2018 | L-asparaginase L-Carnitine | Human | Hyperbilirubinemia improved with L-carnitine | [20] |
| 2 | Alsheikh-Nasany and Douer | 2016 | PEG-asparaginase + L-Carnitine | Human | Hyperbilirubinemia and transaminitis recovered with the use of L-carnitine | [21] |
| 3 | Roesmann *et al.* | 2013 | L-Asparaginase + L-Carnitine | Rats | High hepatic toxicity in asparaginase treated rats and lower toxicity with L-carnitine | [22] |
| 4 | Alachkar *et al.* | 2017 | L-asparaginase + Mitochondrial superoxide dismutase | Human | Hepatotoxicity mitigated with superoxide dismutase | [23] |
| 5 | Wilson *et al.* | 2013 | Eukaryotic factor 2 kinase $GCN_2$ + Asparaginase | Rats | Hepatic stress mitigated by activating amino acid stress response | [24] |
| 6 | Blackman *et al.* | 2013 | L-carnitine + Vitamin B complex + L-asparaginase | Human | Recovery of hepatic function with L-Carnitine and Bcomplex | [7] |

Apart from L-carnitine, there are several other factors which also helps to cure the asparaginase associated hepatotoxicity including superoxide dismutase (SOD) [23] and initiation factor 2 kinase $GCN_2$ [24]. $GCN_2$ tends to mitigate the hepatic stress by activating the amino acid response (AAR). It was evaluated that mice with or without $GCN_2$ always found to deplete the surrounding asparagine but the loss of $GCN_2$ promotes oxidative stress and inflammatory-mediated DNA damage during asparaginase therapy, suggesting that patients with reduced or dysfunctional AAR are found at risk of developing hepatic complications [24]. Thus presence of $GCN_2$was found to be protective against several hepatic complications. As we know oxidative stress mediated by excessive reactive oxygen species (ROS) causes enhanced mitochondrial permeabilization and subsequent cell apoptosis and is considered as a plausible mechanism for drug-induced hepatotoxicity, a common toxicity of asparaginase in adults with acute lymphoblastic leukemia was seen [23]. A potential association between variant rs4880 in $SOD_2$ gene and mitochondrial enzymes acted as a protective factor against the hepatotoxicity during asparaginase-based therapy in 224 patients [23].

## 4. CONCLUSION

Asparaginase is mainly known to treat acute lymphoblastic leukemia. While treating the patients with different strains, asparaginase was found to cause some serious hepatotoxic disorders. PeGylated asparaginase has been found to cause sepsis with hepatotoxicity whereas L-asparaginase results in thrombosis, pancreatitis, hyperglycemia and

hyperbilirubinemia. Near to 1000 IU/$m^2$ dosage of L-asparaginase is found to be hepatotoxic to acute lymphoblastic leukemic patients. It has been revealed that asparaginase associated hepatotoxicity is reversible and its extent can be lowered with some ameliorative drugs including L-carnitine, superoxide dismutase and initiation factor 2 kinase $GCN_2$. Thus asparaginase must be explored more to reveal its potential health effects on leukemic patients. It should be administered in appropriate dozes keeping in mind its associated hepatotoxic effects. Future studies should also be focused on finding new and effective ameliorative agents against negative health impacts associated with the use of asparaginase.

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