A Review on Azathioprine

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Abstract:
Azathioprine (AZA) is an immunosuppressive drug used in organ transplantation and autoimmune diseases and belongs to the chemical class of purine analogues. Synthesized originally as a cancer drug and a pro-drug for mercaptopurine in 1957, it has been widely used as an immunosuppressant for more than 50 years. Azathioprine acts as a pro-drug for mercaptopurine, inhibiting an enzyme required for the synthesis of DNA. Thus, it most strongly affects proliferating cells, such as the T cells and B cells of the immune system. The main adverse effect of azathioprine is bone marrow suppression, which can be life-threatening, especially in people with a genetic deficiency of the enzyme thiopurine-S-methyltransferase. It is also listed by the International Agency for Research on Cancer as a group 1 carcinogen (carcinogenic to humans). This review covers the properties, toxicity, metabolism, mode of action of azathioprine in a brief manner.

Key words: Azathioprine; properties; toxicity; metabolism; mode of action

INTRODUCTION
Azathioprine (AZA) a synthetic purine analogue has been extensively used as used as an immunosuppressive agent and indicated as an adjunct for the prevention of rejection in organ transplantation like renal, cardiac, hepatic and pancreatic transplantations.¹⁻²

AZA also used in various diseases, like inflammatory bowel disease³, rheumatoid arthritis⁴, autoimmune hepatitis⁵, systemic lupus erythematosus⁶, and Crohn’s disease.⁷

TOXICITY PROFILE
AZA leads to serious adverse drug reactions⁸,⁹ such as:

✓ Hepatotoxicity
✓ Hepatobiliary carcinomas
✓ Pancreatitis
✓ Gastrointestinal disturbances
✓ Bone marrow suppression

METABOLISM OF AZATHIOPRINE¹⁰
Azathioprine (AZA) undergoes a rapid non-enzymatic conversion in yielding 6-mercaptopurine (6-MP). During this process GSH is consumed. 6-MP is metabolized by three enzymes (xanthine oxidase, thiopurine methyltransferase (TPMT) and hypoxanthine phosphoribosyl transferase (HPRT)). Xanthine oxidase and TPMT catalyse the reaction of 6-MP to 6-thiouric acid (6-TU) and 6-
methylmercaptopurine (6-MMP), respectively. The TPMT enzyme system is responsible for the formation of 6-thioinosine-monophosphate (6-TIMP) which may ultimately be transformed into the pharmacologically active 6-thioguaninenucleotides: 6-thioguanine-monophosphate (6-TGMP), 6-thioguanine-diphosphate (6-TGDP) and 6-thioguanine-triphosphate (6-TGTP).

Figure 1: Structure of Azathioprine

Table 1: Properties of Azathioprine

<table>
<thead>
<tr>
<th></th>
<th>Molecular formula</th>
<th>C9H7N7O2S</th>
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<tbody>
<tr>
<td>2</td>
<td>Relative molecular mass</td>
<td>277.3</td>
</tr>
<tr>
<td>3</td>
<td>Melting point</td>
<td>244° C</td>
</tr>
<tr>
<td>4</td>
<td>Colour</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>5</td>
<td>State/form</td>
<td>Powder</td>
</tr>
<tr>
<td>6</td>
<td>Odour</td>
<td>Odourless</td>
</tr>
<tr>
<td>7</td>
<td>Solubility</td>
<td>Insoluble in water and very slightly soluble in ethanol.</td>
</tr>
</tbody>
</table>

MODE OF ACTION:

The exact mechanism of immunosuppressive activity of azathioprine has not been determined. AZA primary metabolite 6-MP is readily converted by the enzyme HPRT and TPMT into a number of active purine thionucleotide metabolites. These purine metabolites act to inhibit purine synthesis. Azathioprine which is an antagonist to purine metabolism may inhibit RNA and DNA synthesis. The drug may be incorporated into nucleic acids resulting in chromosome breaks, malfunctioning of the nucleic acids. The drug may also inhibit coenzyme formation and functioning, thereby interfering with cellular metabolism. Mitosis may be inhibited by the drug. These nucleotide-dependent processes provide azathioprine with both immunosuppressive and cytotoxic properties.

MECHANISM OF AZATHIOPRINE INDUCED HEPATOTOXICITY

Oxidative Theory

Oxidative stress is the main factor involved in Azathioprine induced hepatotoxicity which is caused due to the consumption of GSH during the conversion of AZA to 6-MP. Depletion of GSH (a ubiquitous antioxidant of the cell) leads to accumulation of superoxide anion, which is predominant ROS generated through the mitochondrial respiratory chain. This elevates levels of superoxide anion hampers the activities of CAT and GPx which are the major enzymes involved in the detoxification of peroxides like hydrogen peroxide, peroxynitrates. Besides, the increased level of hydrogen peroxide precludes the activity of SOD, which is concerned with the dismutation of superoxide anion. Also, due to depleted GSH levels, the enzymic activities of GST and GR are also decreased. This is due to the fact that GSH is the substrate for these...
enzymes. The enzyme GST catalyzes the detoxification of toxic metabolites through their conjugation with GSH, while GR is responsible for the reduction of GSSG (oxidized glutathione), formed during GPx catalyzed anti-peroxidative activity. Thus, there is an impressive repertoire of antioxidants in the cells which work in a harmonious manner. GSH depletion in AZA metabolism thus diminishes the antioxidant enzymes, thus imposing an oxidative insult over the hepatocytes, thereby leading to increased ROS generation, which consequently leads to the membrane lipid peroxidation, DNA and protein damage and also mitochondrial and other subcellular damages. These intracellular degenerative cascades culminate in the hepatocellular injury and death, finally leading to liver damage.

Figure 2: Metabolism of azathioprine

Molecular Theory

When AZA enters the cell, it is transformed into 6-mercaptopurine by an unknown cytosolic isofrom of GST using GSH as a cosubstrate, which depletes the cytosolic GSH pool. 6-MP is oxidized to thiouric acid by xanthine oxidase (XO), which could generate ROS and subsequently trigger mitochondrial swelling. This ROS production and GSH depletion imposes oxidative stress on the hepatocyte, thereby activating MAPKs (ERK, JNK, and p38 kinase), leading to necrotic cell death. Besides, literature data suggest that the purine moiety (present in AZA) is able to bind to an MPTP domain, increasing the transition frequency between their open and close states leading to transient mitochondrial swelling. Transient opening of the MPTP seems to be its physiological mode of behavior, as demonstrated previously. Studies show that AZA shows a dramatic effect on mitochondria, which is consistent with the necrotic state and is characterized by the appearance of giant mitochondria showing a loss of mitochondrial cristae and outer membrane disruption. It has been suggested that ROS production by mitochondria could damage membranes and macromolecules at this level.

REFERENCES

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