

The Intervention and Treatment based on the Poisoning Mechanism of Alpha-amanitin

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Abstract. Alpha-amanitin (AMA) are eight-amino-acid cyclic peptide with polar branches, which is produced in some specific genera of mushrooms. AMA is extremely toxic to humans, with LD50 (0.1 mg/kg). Due to AMA's special structure, it's resistant to hot and cold. AMA's structure and function cannot be destroyed and solved by high temperature cooking or refrigeration. For decades, scientists put effort and finding the fundamental mechanism that causes AMA's lethal and incurable. AMA in cell will bind to RNA polymerase and stop transcription. Recent research has shown that new biotechnologies have the potential to serve as new methods for screening drugs that control conditions caused by AMA. This article will focus on the recent diagnosis and treatment of AMA. There are some methods that have been used for long time and do slow down the rate of development caused by AMA. This article will talk about their advantage to use and the problem of these method. This article will also talk about the technology used to research AMA. With the development of genetic tools, scientists could determine the genetic level reason for AMA function in human body and do research on treatment and drugs based on the genetic result provided by biotech. Nowadays, the research on AMA still needs further work since now there is not a systematic method to diagnose AMA. The development of drugs for AMA is still in its early stages, which these factors need scientists to do more clinical tests on the drugs.

Keywords: Alpha-amanitin, biological technology, diagnosis, drug.

1. Introduction

Fungus is integral to human daily lives, which encounter and use fungi in various aspects, from pharmaceutical testing to everyday dining tables. Humans need to get further knowledge of fungi. Not only the positive function of fungus working on humans but also the negative situation. Some mushrooms are extremely toxic to human beings. AMA is a toxic which causes 70–90% of mushroom poisoning deaths in China [1]. α -amanitin also occurs all over the world and harmfully influences humans, which can cause RNA polymerase II (RNAP II) inhibition, then trigger further body mechanism shutting down. It is mainly found in the genus *Amanita*, which highly looks like the edible fungi *Agaricus bisporus* (product name: white mushroom). *Agaricus bisporus* is eaten all over the world, especially in traditional agricultural countries, like China [2, 3]. In the genus *Amanita*, the *Amanita phalloides*, which is also called “death cap”, causes about 90% of deaths of mushrooms in China [4]. It is important to get effective treatment for α -amanitin. For decades, scientists tried to develop new effective drugs to reduce toxicity and decrease the death rate. In other words, with more understanding of AMA, doctors and paramedics will gain further research and clinical information to identify if the toxic in human body is α -amanitin and more effective treatment to cure α -amanitin poisoning.

This article will focus on the treatment of AMA both in intervention and drug methods. AMA is extremely toxic. However, with interventions such as ganoderic acid, the liver damage could be reduced by mechanism intervention [5]. This article will mention the actual toxicity and lethality showing up in the mice test and the identification or screening method of AMA toxicity. The liver transcriptomics analysis, CRISPR screen and other methods will help scientists to target poisoning of AMA in body for further treatment determination for clinical. This article will also talk about the detailed toxic mechanism of AMA and what's the most recent effect of antidotes. For example, ICG STT3B pathway decrease the negative effect of α -amanitin. STT3B has been studied this enzyme has the main role of the function of α -amanitin toxicity in human body. ICG could inhibit the toxic effect

caused by STT3B [4]. This article will mention the actual toxicity and lethality showing up in the mice test and the identification or screening method of AMA toxicity. The Liver transcriptomics analysis, CRISPR screen and other methods will help researchers target poisoning of AMA in body for further treatment determination for clinical. This article with introduction of AMA and the function of AMA in human body with RNAP II recent research conclusion focuses on genetic pathway research, providing a new angle of view to research medicine and approach academic progress with the new development of biological technology.

2. Toxicity Mechanism for AMA

The research on toxicity mechanism of AMA began in the 1960s. The crucial mechanism of AMA getting into human cells is by organic anionic transporter peptide 1B3 (OATP-1B3). In general, AMA into the human body through food causes liver and kidney injuries. AMA crosses below the helix bridge and warp triggering loop, Rpb1 and Rpb2 to inhibit the action of RNA polymerase II to replicate mRNA [6]. The liver is a very active organ for processing mechanisms. So, the normal function of liver relies on RNA polymerase II. The inhibition of RNA polymerase II, the presence of AMA, in this case, will cause liver cells to work abnormally. Then the liver cell will die [7]. Avoiding AMA to inhibit RNA polymerase II is the main focus for scientists to build up research and find drugs to decrease the poisoning of AMA.

3. Toxicokinetics of AMA

AMA is a lethiferous molecule that exists mostly in genus *Amanita*. Research suggests that the lethal dose of the mice is between 30-60mg/kg [1]. The main reason for human death from AMA poisoning is that the poison of *Amanita* looks like edible mushrooms, which is closely related to human eating habits. In many countries, people used to go to the mountains and harvest wild mushrooms. And genus *Amanita* with AMA is usually white or brown, it is believed that the fresh mushroom in their basket is delicious and innocuous. Once AMA into human body, it causes apoptosis of liver cells and body loss [1]. AMA is easy to dissolve in water and has high resistance. If the taken dose doesn't reach lethal dose (LD), the liver maintains its normal metabolism after the body gets 21 days. If the mice do not die after 3-9 days, they will get better, and the body routine will back to normal [1]. The research results prove that the earlier to treatment of AMA poisoning, the more effective the medicine or other treatment will do their job in human body [1]. Therefore, keeping level of AMA low in human body and avoiding AMA causes more cells in human apoptosis in the early stage of toxicosis will decrease the rate of death. In that case, fast and effective identification and determination methods of AMA in patients will help paramedics provide treatment and increase the livability of patients.

According to the "Chinese Expert Consensus on Clinical Diagnosis and Treatment of Goose Gastrointestinal Peptide Mushroom Poisoning" [8], the clinical manifestations of AMA are according to four stages after AMA in human body. The four stages would be incubation period, acute gastroenteritis phase, convalescent period and acute hepatic failure phase [9-12]. The incubation period of most patients would be 6-12 hours. Some patients don't have this stage, while some patients could have over 20 hours to get into next stage. The acute gastroenteritis phase is where the patients have upper abdominal pain, Nausea, vomiting, severe diarrhea, and watery stools. The alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum bilirubin levels are recent clinical tests that determine the toxic type. In this stage, maintained at the normal level of humans. Convalescent period happens in patients who accept symptomatic treatment of gastrointestinal symptoms, which relieves the gastrointestinal symptoms. However, the ALT, AST and serum bilirubin levels continue to rise and reach to peak at 60-72h. Some patients with less AMA acceptance in body will back to normal. Some patients will be directed into convalescent period without the acute gastroenteritis phase. In convalescent period, after 2-4 days, the patients have a surge of ALT, AST,

lactate dehydrogenase (LDH) and creatine kinase (CK). Patients who have mild symptoms will get appetite loss, nausea, and mild upper abdominal discomfort. Liver failure will happen in worse situations in patients with bleeding from the skin, mucous membranes and digestive tract, ascites and enlarged liver or liver atrophy. Once patients have serious hepatic insufficiency in this stage, the symptoms will show up as progressive elevation of serum bilirubin, significant decrease in serum albumin, which serious liver dysfunction causes more complications. In conclusion, In the four stages of AMA toxication, the clinical test results that can determine AMA come from the last stage acute hepatic failure phase. The difficulty of diagnosing AMA is that the symptom for doctors determine the symptoms caused by the AMA rather than acute gastroenteritis in the last stage of AMA toxication, which is the stage in which the death rate after treatment is 30%-60%. In that case, an earlier and more effective method to diagnose AMA is the further research focus [13].

4. Clinical identification of AMA

The most common way for doctor to diagnose an AMA is by asking relative diet in two days. The common question for doctors to determine: Have patients eaten mushrooms or not; What kind of mushrooms they ate caused patients uncomfortably [8,14]. Through the oral explanation from patients and identification of the mushroom, if patients have information about the mushroom they eat (pictures, place to get mushroom, shape and color of mushroom), doctors could do further treatment for patients to alleviate the progression of the symptoms caused by AMA. This method with high practicality and is easy to accomplish in clinical. However, with the incubation period, the patients won't realize they are poisoning themselves until 6-20 hours later. They won't be aware that their uncomfortable comes from a meal eaten long time ago, which causes inaccurate medical diagnoses. Also, the patients in the acute gastroenteritis phase will have nausea, upper abdominal pain, diarrhea, and other symptoms that might cause wrong diagnosis. Also, this method needs the conscious patient to identify what type of mushroom they eat, which is necessary for doctor to recognize AMA. A more accurate diagnosis is needed since AMA poisoning is extremely toxic.

Nowadays, laboratory results based on testing human cells, blood and other tissues to get information about human body are the more accurate ways to help doctors gain information about patients' situations and do treatment. Fecal and urine examinations are preferred. About 65% of AMA into the body was out of human body by excrement. The scientists come up with a new method of detecting AMA in mushrooms and urine. AMA is a cyclic peptide, which causes its divertive properties. It's polar and easy to solve in water. Therefore, it could not be detected in blood after 4 hours of exposure. AMA also has 4-20-hour latency stage so that the medical assayer cannot detect AMA in blood when the patients have symptoms and go to hospital [8]. The new method has less limited dose to detect and higher accuracy and practical applicability [13]. They use DNA aptamer with a specific DNA sequence that will form a stem-loop structure. Once they meet AMA, they will bind to it. The AuNPs with NaCl could test if there is AMA could bind with DNA aptamer or not. AuNPs first bind with DNA aptamer or aptamer-AMA complex. The percent of NaCl with aptamer-AMA complex will turn AuNPs from red to blue, which proves that there is AMA in the sample. This method uses magnetic bead-based enzyme-linked immunoassay (MELISA) to detect and analyze that the results is specifically AMA. This method is tested in mushroom and urine samples and proved that it's more accurate and specific than traditional ways to detect AMA.

However, it's not always available for nurses and doctors to get these samples. The blood test and other serum tests are easier to get. For example, the standard liver panel in liver function tests will provide a concentration of different molecules in the blood. With these data, the doctor could have a mathematical presentation of liver situation. AMA will stimulate liver cells to apoptosis, which makes the value of ALT, AST, direct bilirubin and other factors in an abnormal range in convalescent period and acute hepatic failure phase [8]. AMA is a polar molecule that easily dissolves in blood and goes into the cell. The AMA in the blood could be determined by liquid chromatography (LC)-high resolution tandem mass spectrometry (HRMS/MS) method in blood [15]. LC can separate molecules

into small pieces. It works with high resolution tandem MS and detector and computer as analysis to get the mass spectrum, which can be used to determine AMA in blood. It could also measure the concentration of AMA in the blood in the unit of pg/mL. The laboratory result shows that the AMA tested negative in the plasma of the mice after 4 hours of injection [16]. The researchers set up the experiment and put plasma with AMA-RNAP II complex and Trypsin hydrolysis to break the RNAP II as protein. Then without binding with RNAP II, AMA will be free again and back to plasma. Then the laboratory results of patients would be positive for AMA. The effectiveness of this method could be extended to 14 days.

Laboratory result is a strong tool for clinical purposes and research exploration. However, the laboratory result, especially for the blood test, have their limitation which are not solved right now as the previous paragraph said. The incubation period and acute gastroenteritis phase might make a wrong diagnosis due to the long incubation time and syndrome and symptom similarity compared to other diseases [8]. Blood tests show up abnormal starting in the third stage when the patient's symptoms seem to be cured. The real stage that the laboratory result will determine if it's AMA is the fourth stage which most patients are already on the brink of death. In the same dose injured in the mice body, the 24-hour exposure to AMA had mild symptoms, compared to the mice who were exposed to AMA for 48 hours [1]. Also, if mice have ingested a small or insufficient amount of the mushroom, there is a possibility of recovery and survival. Therefore, an effective lab detecting method of AMA in the acute gastroenteritis phase will increase livability. An earlier stage identification needs to be explored.

5. Treatment of AMA poisoning

Now there are two main ideas on treatment of AMA. The first list of methods mainly focuses on conservative therapy, which focuses on liver rebuild which will be used to stimulate regeneration of liver, which is the place α -amanitin function [1]. In the earlier stage of AMA poisoning, combined blood purification and blood perfusion can remove AMA and maintain function of organs by adding medical molecules and nutrients in the replaced plasma. The clinical results prove the feasibility of this method. Ganoderic acid (GA) is found in *Ganoderma lucidum*. Scientist proves that ganoderic acid could decrease the toxicity of AMA in human body and protect liver [5]. The mechanism will be that when the liver cell is irreversible damaged, the immune cells will release biomolecules, which then leads to cell apoptosis. GA treatment will decrease the number of biomolecules and decrease the oxidative stress in liver cells. Also, AMA will disturb the normal production of some protein and fatty acids that could regulate metabolic activity and vitamin A metabolism. This situation will increase oxidative stress. The cell will go cell apoptosis. Vitamin A is used to control intracellular lipid body metabolism. AMA in this case broke the function of vitamin A. GA could solve this problem by increasing the concentration of protein and fatty acid that could regulate metabolic activity and regulate vitamin A to reduce oxidative stress. These two methods of AMA conservative therapy can stave off illness and increase of self-rebuilding of the liver and other organs. Overall, it didn't cure the effect of AMA [17].

For decades, scientists tried to find an effective drug to deal with the problem of AMA. With the technological development in recent years, the research in AMA poisoning and detoxing has significantly improved. The ICG and STT3B mechanism is that the scientists first use CRISPR screen to identify which genes are responsible for the function of AMA in HAP1 [4]. What they did was they put AMA solution in the culture with mutated HAP1 and see which cell did not die. The result has 559 genes which are relative to function of AMA. In these genes, the researchers found that the STT3B pathway has a significant influence on AMA function in human body. Then the researcher uses silico screen to figure out which molecule could effectively inhibit the STT3B pathway. The result is ICG dye. Finally, they used mice to do vivo test to prove the drug effectiveness, which the ICG could inhibit STT3B and prevent cell death. The researchers prove that that ICG will be removed from the plasma and partially and selectively picked by liver, which is same as previous observations.

ICG could protect liver or other organs. The ICG treatment reduced inflammation cell infiltration and necrosis in the liver of AMA-treated mice. The mice that deal with treatment early will have better medical results. Overall, ICG is a useful antidote for treating AMA toxicity. Since ICG is a fluorescent iodide dye, researchers can detect the intracellular localization of ICG. ICG decreased N-glycosylation of the modified luciferase in the experiment. Also, the researchers found that ICG and STT3B were in the same direction. So, this proves that the ICG could effectively inhibit STT3B.

6. Conclusion

In general, the development of AMA analysis and detoxication has huge breakthrough in recent years. With the advancement of modern technology and treatment methods, the toxicity of AMA to humans has been effectively treated and alleviated. The mechanism of AMA in human body and the toxicokinetics of AMA have a complete picture of AMA function. This article analyzes the most recent research progress in AMA, which has advancements in laboratory research and clinical diagnosis, specifically in the medicine of AMA. Also, with the health improvement, the detection of AMA has shown promising development with new laboratorial testing method and technology. With the development of molecular biology and sequencing technologies, genetic identification relative to AMA mechanism in human body could be used in medicine. Therefore, there are more effective identification and treatment, such as ICG inhibiting STT3B. However, AMA poisoning still be a big challenge for researchers, doctors and patients since AMA is still has poisoning unclear, hard to diagnose for doctors and serious body damage to patients. With further research of AMA and technology method development, there will be more diagnoses and research accomplishments. AMA is famous for its poisoning in human bodies, and it causes most of the deaths from mushroom poisoning. Due to further research on AMA, the function of genetic pathways that are associated with AMA will have more detailed research results to benefit humans. AMA enters the cell by organic anionic transporter peptide 1B3. Avoiding AMA binding to OATP1B3, which might block AMA into the cell and bind with RNA polymerase II and leading to more serious body function loss. This could be started as drug research.

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