Review Article: Amygdalin as Anti-Cancer Agent

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Abstract :

Amygdalin (d-Mandelonitrile 6-O-β-d-glucosido-β-d-glucoside) and its semi synthetic product is Laetrile (also called vitamin B17): a natural cyanogenic glycoside occurring in the seeds of some edible plants, such as bitter almonds and peaches. Early in the 19th century, Amygdalin was first isolated in 1830 by two French chemists, Robiquet and Boutron-Charlard, as active components in various fruit pits and raw nuts. However, the systematized study of vitamin B17 started when chemist Bohn (1802) discovered that a hydrocyanic acid is released during distillation of the water from bitter almonds. The various pharmacological effects of Laetrile include anti-atherogenic, activity in renal fibrosis, pulmonary fibrosis, immune regulation, anti-tumor, and anti-inflammatory activities. Despite numerous contributions to the cancer cell lines, the clinical evidence for the anti-cancer activity of Amygdalin is not fully confirmed. Moreover, high dose exposures to Amygdalin can produced cyanide toxicity. In the presented work, pharmacological activity, antitumor activity, and toxicity of Amygdalin have been summarized, focusing primarily on advanced research on Amygdalin and its anti-tumor effects, providing fresh perspectives for the creation of new anti-cancer drugs, the examination of natural antitumor mechanisms, and the search for new targets.

مقالة بحثية :الاميغدالين كعامل مضاد للسرطان

جميلة كاظم طاهر العيساوي كوثر عبد الواحد عبد الحميد ايمان عبد علي عباس

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الخلاصة :

الاميغدالين (د–مانديلونتريل– 6–او–بيتا–د–كلوكوسايدو–بيتا–د– كلوكوسايد)ومنتوجه الشبه صناعي اللاتريل (ويسمى ايضا فيتامين ب 17) هو مركب طبيعي من كليكوسيدات السيانوجين يتواجد في جذور بعض النباتات الصالحة للاكل ,كاللوزالمر والخوخ.في بداية القرن التاسع عشر , تم عزل الاميغدالين اولا في عام 1830 من قبل اثنان من العلماء الفرنسيين وهما روبيكيت وبوترون شارلاد كاحد المكونات الفعالة في بذور الفاكهة والمكسرات النيئة. ان الدراسة المنهجية لفيتامين ب 17 بدأت عندما اكتشف الكيميائي بون عام 1803 تحرر سايانيد الهايدروجين عند تقطير اللوز المر.ان التاثيرات الدوائية للاتريل متنوعة وتتضمن انه يعتبر مضاد لتصلب الشرايين,تليف الكلية ,التليف الرئوي,تنظيم المناعة,مضاد للاورام بالاضافة الى نشاطه في كونه مضادا للالتهابات.بالرغم من مشاركات المركب العديدة في تثبيط خط الخلايا السرطانية الا ان الادلة السريرية لفعالية الاميغدالين ضد السرطان ليست مؤكدة كليا . بالاضافة الى ذلك , يمكن أن ينتج عن التعرض لجرعات عالية من الأميغدالين التسمم بالسيانيد. في العمل المقدم ، تم تلخيص النشاط الدوائي ، والنشاط المضاد للأورام وسمية الأميغدالين ، مع التركيز بشكل أساسي على الأبحاث المتقدمة حول الأميغدالين حول آثاره المضادة للورم مؤخرًا ، مما يوفر وجهات نظر جديدة لإنشاء عقاقير جديدة مضادة للسرطان ، واختبارآليات مضادة الأورام الطبيعية ، والبحث عن أهداف جديدة في هذا المجال.

كلمات المفتاحية : سرطان , لاتريل , فيتامين ب17 , اميغدالين .

Introduction

The WHO has designated malignant tumors as the top disease which poses serious threats to human well-being because they are the most common cause of substantial harm to human health. Antitumor drug development has changed recently from cytotoxic medications to drugs with improved selectivity, overcoming multidrug resistance, drugs with high specificity and low toxicity, and new targeted drugs (1). One of the most contentious vitamins in the past thirty years is vitamin B17 (Amygdalin). It has the chemical formula C20H10NO11, and its molecular weight is supposed to be 457.42 Dalton with the chemical name d-mandelonitrile 6-*O*-β-d-glucosido-β-d-glucoside. Early in the 19th century, Amygdalin was first isolated in 1830 by two French chemists, Robiquet and Boutron-Charlard, as one of the active components in various fruit pits and raw nuts (2). A glycoside called Amygdalin is extracted from natural foods such as apricot and apple seeds, clover, lima beans, and sorghum. Laetrile and Amygdalin are two distinct names for vitamin B17; furthermore, vitamin B17, also called Amygdalin, is a cyanogenic glycoside generated from aromatic amino acid phenylalanine. It is semi-synthetic. Vitamin B-17, also called the mandelonitrile beta-D-gentiobioside in science, is regarded as a nitriloside (a natural cyanide-containing substance). The hydrolysis reaction of the Amygdalin and extract out as vitamin B17 produces Laetrile, a semi-synthetic substance. Figure 1 illustrates how the cyanide group in Amygdalin gives it its primary anticancer properties. The enzyme and Amygdalin, located in different places, are activated upon tissue damage and begin a hydrolysis reaction, which, in turn, offers a natural defensive mechanism (3).

A cyanogenic glycoside plant component called vitamin B-17, also named "Amygdalin," is present in kernels of several fruits and many plants from the Prunus genus (4). Gentiobiose, a disaccharide that has been made up of two units of D-glucose, and Mandelonitrile make up Amygdalin (5).

The Cancer Association of South Africa (CANSA) recommends that one should not replace conventional cancer treatment with any alternative cancer therapy, such as Laetrile. Laetrile can cause serious side effects because of its cyanide content. It is also not recommended that Laetrile be used alongside or instead of conventional cancer treatment. (6)

History of Amygdalin

Two French chemists, Boutron-Charlard and Robiquet, discovered Amygdalin for the first time in 1830 (7). In Russia, it was originally applied as an antineoplastic agent in 1845 (8). Dr. Ernst Krebs discovered in the 1920s that apricot kernel extracts reduced tumors in rodents, whereas he was researching ways to improve the flavor of bootleg whiskey (9). In the US, the earliest records on the use of Amygdalin originate from the 1920s. (10) Yet, oral formulation was not employed at the time since it was deemed to be too hazardous (11). Amygdalin has been one of the most common alternative antineoplastic treatments in the 1970s, and by 1978, it had been taken through about 70,000 cancer patients in the US (12). Amygdalin transport within the US was subsequently prohibited. Yet, 23 states in the USA allowed using Amygdalin for cancer patients who were close to death (13). Amygdalin therapy has been the subject of debate. Therefore, the National Cancer Institute (NCI) decided to examine its effectiveness. With the approval of FDA, NCI conducted a clinical trial, but it has been unsuccessful in demonstrating the anti-cancer effectiveness of Amygdalin (14). In addition, Amygdalin was then prohibited by the FDA from being sold as a pharmaceutical medication. It's still created and utilized as anti-cancer agent despite this global ban (15).

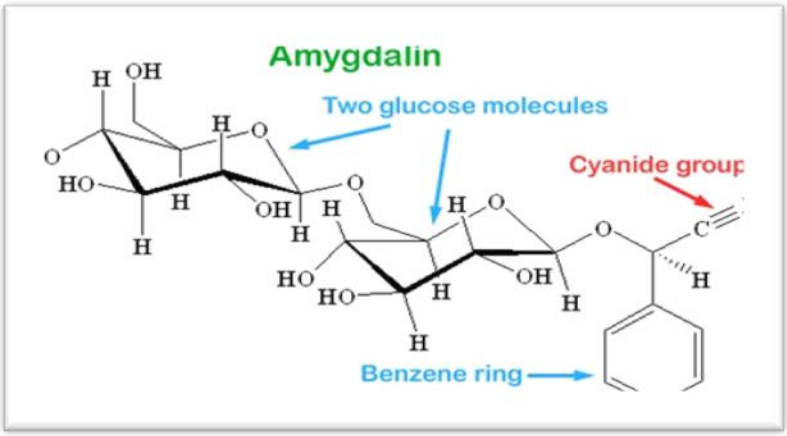

Figure 1: Chemical structure of Amygdalin (16)

Laetrile

Laetrile (D-Mandelonitrile-β-glucuronide), which is derived from Amygdalin, has been used as a complementary and alternative natural medicine (CAM) in the treatment of cancer for over 30 years (17)**.** It is a purified form of chemical Amygdalin, which is a cyanogenic glucoside that has been detected in pits of various fruits and uncooked nuts as well as other plants like clover, lima beans, and sorghum. The term Laetrile has been formed from the terms mandelonitrile and laevorotatory (18). At the physiological pH, hydrogen cyanide dissolves in order to create cyanide anion in body fluids. Laetrile has been given the vitamin B-17 name by E.T. Krebs Jr., yet Committee on Nomenclature of American Institute of Nutrition Vitamins has not recognized it. Laetrile became well-known as an anticancer medication in the 1970s. No less than 70,000 people were said to have received treatment with it in the US by the year 1978. Both a single agent and in conjunction with a metabolic therapy regimen, which includes a specific diet, pancreatic enzymes, and high-dose vitamin supplementations, Laetrile was utilized as a cancer treatment (19).

The lack of Laetrile's effectiveness and the risk of side effects from cyanide poisoning led the Food and Drugs Agency (FDA) in the US and the European Commission to ban its use. However, it is possible to buy Laetrile or Amygdalin via the Internet. As there is no government control of these markets, preparations may not only come from questionable sources, but they may also be contaminated. Cancer patients should be informed about the high risk of developing serious adverse effects due to cyanide poisoning after Laetrile or Amygdalin, especially after oral ingestion. This risk could increase with concomitant intake of vitamin C and in vegetarians with vitamin B12 deficiency (20).

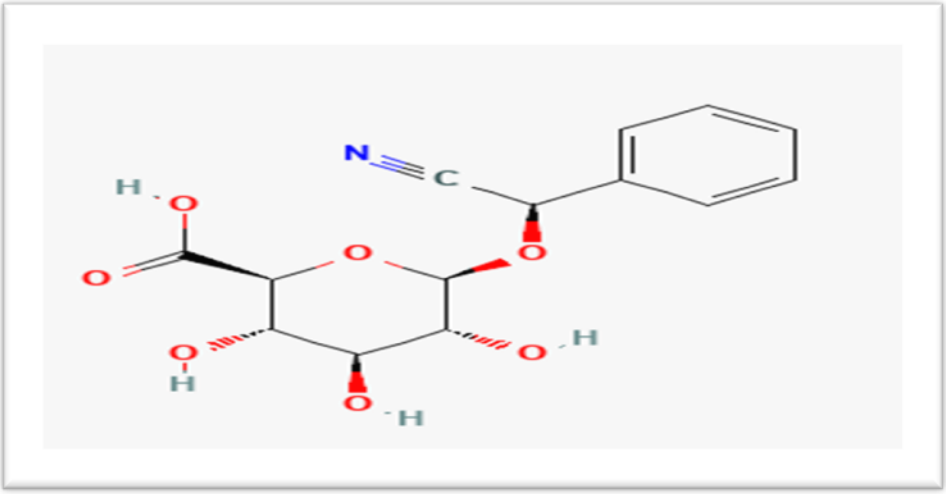

Figure2: Chemical structure of Laetrile

Metabolism of Amygdalin:

Knight and Heismann originally reported on the full enzymatic and acid hydrolysis regarding Amygdalin in 1967 (21). Amygdalin's interaction with beta-glucosidase results in the formation of prunasin and glucose. Glucose and another substance known as mandelonitrile are produced by further hydrolyzing prunesin. Without the use of any enzymes, such substance is converted into hydrocyanic and benzaldehyde acids (22). Amygdalin is hydrolyzed by acid to produce a single genetic product (i.e. disaccharide with 6-6 beta-binding compounds). Enzymatic mechanisms could be determined using Michaelis-Menten kinetics. Prunasin lyase, amylase lyase, and hydroxyl lyase are three enzymes that were identified and catalyzed in three different steps. The two main factors that contribute to Amygdalin for inducing apoptosis and preventing the proliferation of cancer cells are glucosidase and hydrocyanic acid (23). Glucosidase activity is markedly improved with the existence of lactate created by cancer cells throughout anaerobic respiration. By increasing the acidity of cancer cells and generating lysosomes, which release their enzyme contents and cause cell lysis, HCN could also destroy cancer cells. Five minutes following injection, mice have Amygdalin in their blood. According to pharmacokinetic. The first happens prior to the liver or the first transit in the proximal intestine when Amygdalin is converted to prunasin (25). Amygdalin is first broken down to prunasin and subsequently to mandelonitrile, according to the metabolism of Amygdalin in the gastrointestinal cell culture. Beta-glucosidase then hydroxylates it to hydroxy sem-andononitrile in the small intestine. As neither cyanide nor benzaldehyde is produced at this stage, cyanide will most likely form in the lower intestine, where bacteria are abundant (26).

Toxicity of Amygdalin

Although Amygdalin is not hazardous, its product HCN is broken down by certain enzymes (27). Current research has revealed that the HCN has been released in normal cells, suggesting that it might not be safe for human consumption (28). Cyanide compounds released following Amygdalin degradation have harmful side effects (29). Cyanide inhibits cellular respiration by preventing the conversion of oxygen to water through binding reversibly to ferric ions in cytochrome oxidase a3 in mitochondria. The aerobic cell metabolism cessation and the cellular hypoxia that result in dysfunctions regarding the central nervous system and the cardiovascular system are chiefly responsible for cyanide's toxicity (30). Natural plant toxins like cyanogenic glycoside and Amygdalin could be harmful to animals' health. Prior to the onset of the lipid peroxidation process, protein oxidation could be triggered by Amygdalin-induced reactive oxygen species (ROS) generation and subsequent overproduction of benzaldehyde. Even though oral Amygdalin at low and medium dosages (50mg/kg and 100mg/kg) does not cause toxicity in mice, Amygdalin at high doses (200mg/kg) can cause toxicity and has a

detrimental impact on oxidative balance regarding hepatic tissues, which in turn has a clear impact on the histopathology in mice. A lethal dose of cyanide was considered to be between 5 to 3.5 mg/kg of body weight, according to the Committee on Toxicity (2006) (31). Despite this, animal studies do not offer any reliable evidence for assessing acute risks to human health. However, according to Bolarinwa et al. (2015), Amygdalin levels in commercially available apple juices are not expected to pose health risks to consumers (32). The panel on Contaminants in Food Chain of the European Food Safety Authority (CONTAM Panel, 2016) concluded that the lethal dosage is between 0.50 and 3.50mg/kg of body weight. Using a 0.105mg/kg exposure related to a non-toxic blood cyanide level of 20M and a 1.50 uncertainty factor to allow for toxicokinetic and 3.16 to account for the toxico-dynamic inter-individual variability, an acute reference dosage (ARfD) of 20g/kg has been determined (33). The maximum amount of Amygdalin that may be administered to rabbits, mice, and dogs without causing any unfavorable side effects is 3 g/kg when administered intramuscularly and intravenously and 0.075g/kg when administered orally. Moreover, 0.07 g/kg of Amygdalin is the maximum tolerable dose for intravenous administration in humans. (34)

Extraction and Identification of Amygdalin

Amygdalin was removed from apricot kernels in boiling water with ethyl alcohol. Amygdalin extraction becomes more effective at boiling temperatures without the assistance of β-glucosidases, the enzymes responsible for Amygdalin hydrolysis (35). Moreover, the extraction with the existence of citric acid could stop Amygdalin from epimerizing into neoAmygdalin (36). The chemical properties of Amygdalin powder have been identified as being crystalline, white, relatively soluble in cold water, odorless powder, soluble in acetone and alcohol, considerably soluble in hot water, and insoluble in the ether following drying, filtration, and crystallization regarding the extract to form a powder. (37)The dried solid samples are pressed into pellets (with KBr), and the spectrum of Amygdaline in pure form and samples is scanned using Shimadzu FTIR-8201A (Japan) single beam laser Infrared Spectrometer. In a saturated aqueous solution, it has a pH of 7. OF FRUIT: Apart from bitter almonds, fruit contains the highest amount of vitamin B-17 in nature (Berries, nuts, flax, Chia, peach, sesame, nectarine, pear, pears, plums, and prunes) (38).

Anti-Cancer Effects of Amygdalin

Studies of Amygdalin on various cancer cell lines demonstrated their anticancer activity (39). Still, the statements related to a patient study by the U.S. Food and Drug Administration (FDA) in the late 1970s (40) did not confirm this. Since then, however, many publications have been presented confirming both the toxicity occurring with excessive consumption of Amygdalin in bitter almonds and the therapeutic, especially anticancer, properties of Amygdalin(41). Amygdalin may exert its anticancer effects by inhibiting tumor cell growth and metastasis through apoptosis (42,43). According to certain studies, vitamin B17 helps prevent the spread of cancer cells and keeps their levels at a minimum, preventing cancer and limiting the spread of already-existing cancer cells. At the same time, other studies demonstrate no impact of vitamin B17 on the cancer cells. In addition, vitamin B17 has a greater capacity for killing cancer cells by releasing cyanide than it does in killing normal, healthy cells (44). The delivery of enzymes coupled to antibodies against tumor antigens via the systemic route is known as antibody-directed enzyme prodrug treatment (ADEPT). The prodrug has been locally transported to the tumor with the existence of the enzyme, in which it has been changed into a cytotoxic agent (45). Sweet almond β-glucosidase might degrade the prodrug Amygdalin, which treats bladder cancer, to release free cyanide. If such a substance has been triggered at the tumor site, malignant tumor cells could be destroyed locally without doing any systemic harm. It was believed to be an antibody and cytotoxic drug combination that would kill cancer cells. The ADEPT system, in combination with β-glucosidase and Amygdalin, was one of the promising targeted cancer medicines (46).

Mechanism of action of Amygdalin:

Previous studies suggested that Amygdalin can impact numerous signaling pathways, which play pivotal roles in various physiological and/or pathological processes, including aberrant regulation, as identified in multiple human diseases. Amygdalin inhibits the adhesion of breast cancer cells, lung cancer cells, and bladder cancer cells by decreasing the expression of integrins, reducing catenin levels, and

inhibiting the Akt-mTOR (mammalian target of rapamycin) signaling pathway, which may consequently lead to the inhibition of metastases of cancer cells (47). Moreover, β-glucosidase can accelerate the hydrolysis of Amygdalin into hydrogen cyanide, which can effectively kill tumor cells by inhibiting cytochrome C oxidase in mitochondria, resulting in a significant increase in the cell mortality rate (48).

According to a study that has been carried out by Ernest T. Krebs Jr., the action mechanism depends on the presence of one particular enzyme called rhodanese, found everywhere in the body except in cancer cells, and the enzyme Beta-Glucosidase found in vast quantities only in the cancer cell but not found anywhere else in the body. If there is no cancer in the body, there is no enzyme Beta-Glucosidase (49). Vitamin B17 is made up of 2 parts glucose, 1-part Hydrogen Cyanide and 1-part Benzaldehyde. When B17 is introduced to the body, it is broken down by the enzyme rhodanese, which breaks the hydrogen cyanide and benzaldehyde down into two products, thiocyanate and benzoic acid, which are beneficial in nourishing healthy cells and forms the metabolic pool production for vitamin B12. When the B17 comes into contact with the cancer cells, there is no rhodanese to break down and neutralize vitamin B17, but instead, only the enzyme beta-gucosidase is present in vast quantities, which interacts with the vitamin B17 by a chemical reaction to produce hydrogen cyanide and benzaldehyde that combine synergistically to produce a poison which destroys and kills the cancer cells(50). This whole process is known as selective toxicity. Only the cancer cells are specifically targeted and destroyed(51).

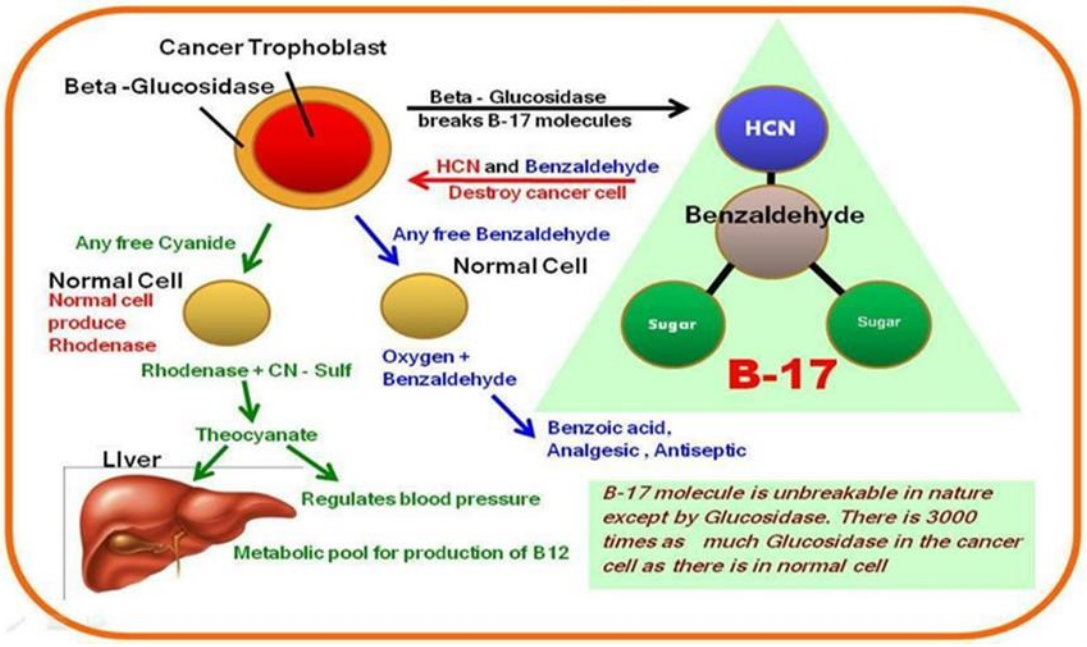

Figure3: Mechanism of Action of Amygdalin/ Laetrile (52)

Conclusions

Research on cyanogenic glycosides has increased dramatically over the past 10 years. Much of this interest centers on the cytotoxic effect of Amygdalin on cancer cells in vitro and understanding the distribution of Amygdalin in plants that are commonly consumed in the human diet. Research into the use of Amygdalin in the treatment of cancer continues. There is evidence confirming the cytotoxic effect of Amygdalin on cancer cells in vitro. However, these results have not yet been demonstrated in clinical studies. Nevertheless, there is still a need to quantify the levels of Amygdalin in plant materials in order to support the clinical trials and to understand their intake in the human diet better. To date, there have been numerous cases of cyanide poisoning resulting from the ingestion of too many seeds containing Amygdalin. As a result of the Laetrile treatment, there are no

reports of people cured of cancer by consuming the seeds containing Amygdalin. The assertions that Amygdalin or Laetrile are helpful for cancer patients aren't backed up by reliable clinical evidence. Amygdalin or Laetrile have high risks of major side effects from cyanide poisoning, particularly when taken orally. Amygdalin or Laetrile, as a cancer treatment, clearly has a poor risk-benefit ratio. Amygdalin/laertile cannot be marketed as an antitumor medication until randomized controlled clinical trials demonstrate both its efficacy and tolerable adverse effects. Before considering Laetrile as a pure medication, dosage, formulations, and administration techniques must be standardized. Therefore, this topic requires more studies to prove the therapeutic properties with the least side effect of its use.

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