

The Effect of Amygdalin in the Treatment of Squamous Cell Carcinoma induced in the Buccal Pouch of Golden Syrian Hamster

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Abstract:

Background: Scientific studies have recently focused on the alternative and complementary medicine as a wide spread method in treating a lot of diseases, including cancer. Cancer that is considered as a life-threatening disease affect patients all over the world. Traditional medicine still does not provide patients with permanent and absolute solution to their suffering, especially in terminal cases of cancer. Many people go for using alternative and natural methods to reduce as possible as they can the pain and the side effect of this awful disease. Amygdalin, extracted from the seeds of apricot and almond, is under debate as a true cure of cancer and many other diseases. Therefore, we wanted through this study to evaluate the efficiency of Amygdalin in the treatment of squamous cell carcinoma, the most common carcinoma in the oral cavity, induced in Syrian Hamster and approach in which pathway this substance does arrest the cancer cells.

Materials and methods: 20 Hamsters were divided into two groups, cases group (10 Hamsters), that has been treated with Amygdalin, and control group (10 Hamsters) that has not received any treatment. The carcinogenic material that has been used was (DMPA) and the immunohistochemistry stains were P53 and Ki67.

Results and conclusions: There is a significant statistic difference between the two groups as to the P53 as well as to the Ki67. In conclusion, amygdalin has a therapeutic effect in the treatment of squamous cell carcinoma, by inducing apoptosis in the cancer cells.

Key words: Squamous cell carcinoma, Amygdalin, alternative therapy, Syrian Hamster, cell cycle, P53, Ki67.

I. Introduction:

Malignant tumors are the major diseases that cause serious damage to human health, and have been listed as the primary disease which seriously threatened human health by World Health Organization (WHO).

Oral squamous cell carcinoma (OSCC) represents 95% of all forms of head and neck cancers, and over the last decade its incidence has increased by 50%. Oral carcinogenesis is a multistage process, which simultaneously involves precancerous lesions, invasion and metastasis. Degradation of the cell cycle and the proliferation of malignant cells results in the loss of control mechanisms that ensure the normal function of tissues (1).

Squamous cell carcinoma of the head and neck (SCCHN) arises as a consequence of multiple molecular events induced by the effects of various habits such as tobacco and the use of alcoholic beverages, influenced by environmental factors, possibly viruses in some instances, against a background of heritable resistance or susceptibility. Oral squamous cell cancers have a similar aetiology. Genetic damage affects many chromosomes and genes, including oncogenes and tumour suppressor genes, and it is the accumulation of such genetic damage, possibly along with an impaired ability to repair this damage - an inherited trait in some cases - that appears to lead to carcinoma in some instances, sometimes via a clinically evident pre-malignant or potentially malignant lesion. This communication reviews the advances in the understanding of this complex and rapidly developing area of research over the past decade (2).

Oral squamous cell carcinoma (SCC) is considered as a primarily localized disease, and distant metastasis is not common. An increasing number of case reports involving unusual sites for distant metastasis from oral carcinomas have been reported in the literature. This is likely due to the improved control of cancer at the primary site, increasing the chance of developing a delayed metastasis. One article presents a case of a 58-year-old woman who refused surgical treatment for a very aggressive SCC on the mandibular alveolar ridge. The tumor did not respond to chemotherapy or radiotherapy, and the patient developed metastasis in the skull

bone approximately 1 year after the initial diagnosis. The site of the primary tumor (next to the bone) as well as the patient's refusal of the proposed treatment, may have led to the hematological spread of the malignant cells, resulting in the distant metastasis (3).

Nowadays, many studies suggest that 4-6% of oral cancers occur at an age younger than 40 years. When examining the risk factors in this age group, it has been referred that it is not related to smoking or consuming alcohol, which constitutes the major risk factors in older ages groups. The predisposition to genetic instability has been hypothesized as a likely cause(4).

Head and neck squamous cell carcinoma (HNSCC) is a disease of middle-aged to elderly adults. However, an increased incidence of HNSCC in young people under 45 years of age has been reported recently. In our search in the literature, we focused on the epidemiology and aetiology of HNSCC in adults under 45 years of age.

HNSCC in young adults is associated with a higher incidence rate in nonsmokers, lower female-to-male ratio, a higher percentage of oral cavity and oropharynx tumours, and fewer second primary tumours. However, aside from traditional risk factors of tobacco and alcohol exposure, the causes of these cancers in young adults remain unclear. Agents that might contribute to risk include infection with high-risk human papillomavirus subtypes as well as genetic factors or immunodeficiency status. The expected increase in incidence and mortality of the young with HNSCC may become a major public health concern if current trends persist, particularly lifestyle habits that may contribute to this disease (5).

A study on Human papilloma virus and its expression in normal oral mucosa and some oral diseases has shown that Human papilloma virus was identified with increasing frequency in normal oral mucosa, benign leukoplakia, intraepithelial neoplasia, squamous cell carcinoma, and verrucous carcinoma. It was detected in oral squamous cell carcinoma significantly more often in studies that used a high sensitivity assay (polymerase chain reaction) than studies that used moderate sensitivity assays (e.g., Southern blot hybridization) and low sensitivity assays (e.g., immunohistochemistry, in situ hybridization). Human papillomavirus DNA was detected significantly more often in frozen oral squamous cell carcinoma than paraffin-embedded tissue. In studies that analyzed the use of chemical cofactors, the use of tobacco and alcohol was associated more often with oral squamous cell carcinoma than the presence of human papillomavirus. However, the difference was not significant. High-risk human papillomavirus genotypes have a significant association with oral squamous cell carcinoma (6).

Mutation, deactivation and disregulated expression of oncogenes and tumour-suppressor genes may be involved in the pathogenesis of oral squamous cell carcinoma (SCC). Deactivation of the p53 tumour-suppressor gene allows cell proliferation and blocks apoptosis of malignant oral keratinocytes. Mutation in the ras oncogene results in persistent mitogenic signalling. Upregulated c-Myc expression, in the presence of growth factors, provides an additional proliferative signal. Loss of retinoblastoma tumour-suppressor gene (Rb) function may contribute to oral keratinocyte hyperproliferation and recent evidence suggests that simultaneous deactivation of both p53 and Rb is required for tumorigenesis. Enhanced Bcl-2 and reduced Fas expression inhibit tumour cell apoptosis and may convey resistance to cytotoxic drugs and T cell-mediated cytotoxicity, respectively. Exogenous mutagens such as tobacco, alcohol and viral oncogenes may cause altered expression of oncogenes and tumour-suppressor genes in some cases of oral SCC. The impact of these mechanisms on future therapies for oral SCC is highlighted (7).

In recent years the development of antitumor drugs has been gradually transformed from cytotoxic drugs to improving the selectivity of drugs, overcoming multidrug resistance, development of new drugs with low toxicity and high specificity.

Amygdalin is also called bitter apricot, laetrile, almond. It is a cyanogenic compound and belongs to the aromatic cyanogenic glycoside group. Its molecular formula is: $C_{20}H_{27}NO_{11}$, the molecular weight is 457.42. The chemical structure is D-mandelonitrile- β -D-glucoside-6- β -glucoside. Amygdalin is widely distributed in plants, especially in the rosaceous plant seed, for example, apricot, peach, cherry, plum etc (8, 9).

Amygdalin itself is non-toxic, but when decomposed by some enzymes, it produces HCN, which is a poisonous substance (9). Numerous studies have documented that amygdalin has antitussive and antiasthmatic effects, as well as an effects on the digestive system. Moreover, the pharmacological effects also include antiatherogenic, inhibition of renal interstitial fibrosis, prevention of pulmonary fibrosis, resistance to hyperoxia induced lung injury, immune suppression, immune regulation, antitumor, antiinflammatory and antiulcer (10) (11) (12) (13)

It has been used for the treatment of asthma, bronchitis, emphysema, leprosy, colorectal cancer and vitiligo (11) Amygdalin were decomposed to hydrocyanic acid, which is an antitumor compound, and benzaldehyde, which can induce an analgesic action, therefore it can be used for the treatment of cancer and relieve pain(14).

Scientists indicate using amygdalin in the treatment of cancer. A new research has studied the correlation between amygdalin and prostate cancer. It has shown that amygdalin induces apoptotic cell death in

human DU145 and LNCaP prostate cancer cells by caspase-3 activation through down-regulation of Bcl-2, an anti-apoptotic protein, and up-regulation of Bax, a pro-apoptotic protein (14).

Another research investigated how amygdalin could induce apoptosis in human cervical cancer cell line HeLa cells. Amygdalin-treated HeLa cells developed typical apoptotic changes. The development of apoptosis in the amygdalin-treated cells were confirmed by double staining of amygdalin-treated cells with annexin V-FITC and propidium iodide (PI) along with an increase in caspase-3 activity in these cells (15).

The effect of amygdalin have been studied in diseases other than cancer, such as pulmonary fibrosis. In a study on rats, study groups were treated with intraperitoneal injection of amygdalin (15 mg / kg / day). The rats were sacrificed 7, 14 and 28 days after bleomycin administration. Amygdalin may reduce the differentially expressed protein peak intensities in rat serum (10).

Amygdalin is effective at alleviating inflammatory pain and thus can be used as an analgesic with anti-nociceptive and anti-inflammatory activities. A study was conducted on the antinociceptive effect of amygdalin isolated from *Prunus armeniaca* on rats. The intramuscular injection of amygdalin significantly reduced the formalin-induced tonic pain in both early (the initial 10 min after formalin injection) and late phases (10-30 min following the initial formalin injection). During the late phase, amygdalin reduced the formalin-induced pain in a dose-dependent manner in a dose range less than 1 mg/kg (16).

A study evaluated the anti-inflammatory and analgesic activities of amygdalin, using an in vitro lipopolysaccharide (LPS)-induced cell line and a rat model with carrageenan-induced ankle arthritis. Amygdalin significantly inhibited the expression of TNF-alpha and IL-1beta mRNAs in LPS-treated RAW 264.7 cells. Amygdalin (0.005, 0.05, and 0.1 mg/kg) was intramuscularly injected immediately after the induction of carrageenan-induced arthritic pain in rats, and the anti-arthritic effect of amygdalin was assessed by measuring the weight distribution ratio of the bearing forces of both feet and the ankle circumference, and by analyzing the expression levels of three molecular markers of pain and inflammation (c-Fos, TNF-alpha, and IL-1beta) in the spinal cord. The hyperalgesia of the arthritic ankle was alleviated most significantly by the injection of 0.005 mg/kg amygdalin. At this dosage, the expressions of c-Fos, TNF-alpha, and IL-1beta in the spinal cord were significantly inhibited. However, at dosage greater than 0.005 mg/kg, the pain-relieving effect of amygdalin was not observed. Thus, amygdalin treatment effectively alleviated responses to LPS treatment in RAW 264.7 cells and carrageenan-induced arthritis in rats, and may serve as an analgesic for relieving inflammatory pain (17).

An in vitro study has investigated the influence of amygdalin on the growth of a bladder cancer cell lines (UMUC-3, RT112 and TCCSUP). Tumor growth, proliferation, clonal growth and cell cycle progression were investigated. The cell cycle regulating proteins studied were cdk1, cdk2, cdk4, cyclin A, cyclin B, cyclin D1, p19, p27. Amygdalin dose-dependently reduced growth and proliferation in all three bladder cancer cell lines, reflected in a significant delay in cell cycle progression and G0/G1 arrest. Amygdalin, therefore, may block tumor growth by down-modulating cdk2 and cyclin A (18).

In another study, one hundred seventy-eight patients with cancer were treated with amygdalin plus a "metabolic therapy" program consisting of diet, enzymes, and vitamins. The great majority of these patients were in good general condition before treatment. One third had not received any previous chemotherapy. No substantive benefit was observed in terms of cure, improvement or stabilization of cancer, improvement of symptoms related to cancer, or extension of life span. This study advised that patients exposed to this agent should be instructed about the danger of cyanide poisoning, and their blood cyanide levels should be carefully monitored, since amygdalin is a toxic drug that is not effective as a cancer treatment (19).

A new research has revealed that amygdalin inhibits genes related to cell cycle in SNU-C4 human colon cancer cells. Microarray showed that amygdalin down regulated especially genes belonging to cell cycle category. Real time PCR (RT-PCR) analysis revealed that mRNA levels of these genes were also decreased by amygdalin treatment in SNU-C4 human colon cancer cells. Thus, it suggests that amygdalin have an anticancer effect via down regulation of cell cycle-related genes in human colon cancer cells, and might be used as a therapeutic anticancer drug (20).

An advanced new research on anti-tumor effect of amygdalin showed that amygdalin is a natural product that owns antitumor activity, less side effects, widely sourced and relatively low priced. All these features make the amygdalin a promising antitumor drug, if combined with conditional chemotherapy drugs, which can produce synergistic effect, providing new insights for the development of new anticancer drug (9).

II. Materials and methods:

Study sample: it consisted of 10 Golden Syrian Hamsters, which were divided into two groups, cases group, in which cancer was induced in the buccal pouch and amygdaline was provided as a treatment. The second was the control group, in which cancer was induced, and no treatment received.

Amygdalin: It was extracted drug in the laboratory of chemistry collage, Damascus university from bitter apricot kernel, then the powder obtained was dissolved in distilled water to be ready for injection in the peritonea.

Carcinogenic agent: it is DMBA, polycyclic aromatic hydrocarbon carcinogen, which was applied in the buccal pouch mucosa of hamsters using a painting brush.

Scarifying: hamsters in the second group were sacrificed after 14 weeks, the estimated period to induce cancer in the buccal pouch. While hamsters in the case group, started to receive amygdalin injected in peritonea after that 14 weeks, after ulcers of carcinoma were observed in their pouch. It lasted for 21 days, the recommended period in different studies.

Sample staining: The specimens were obtained and stained using traditional stains (hematoxillin & eosin), then were stained using two immunohistochemicals stains, P53 and Ki67.

Statistics:

T-student test was used for two separated groups, to compare the expression of P53 and Ki67 between the vitamin and the cancer group.

III. Results:

Data that is shown in table 1, clarifies the descriptive statistics that is related to both P53 and Ki67 in order to the two groups of cancer and vitamin.

Table 1 descriptive statistics for IHC in order to the vitamin and cancer group

Groups	IHC	Median	Standard deviation	Lowest value	Highest value
Vitamin	P53	0.00	0.00	0.00	0.00
	Ki67	0.00	0.00	0.00	0.00
Cancer	P53	519.65	76.22	405.25	619.00
	Ki67	184.15	41.53	138.50	236.75

T-student test was used to compare between the two separated groups. The results are illustrated in table 2.

Table 2 t-student test to compare between the vitamin ad cancer group

Variables	Mean value	t-test	P.value
P53	519.65	15.244	0.000 *
Ki67	184.15	9.914	0.000 *

As table 2 shows, there is a significant difference in the expression of P53 and Ki67 between the cancer and the vitamin group. The vitamin group was always negative.

IV. Discussion:

Amygdalin, as a complementary and natural substance, is suggested to be a therapeutic agent of many diseases. A lot of studies have been conducted to improve its efficiency in curing inflammatory diseases as well as different kinds of cancers. However, it is still a potential poisonous material, due to its related release of cyanide from its degradation. In addition, there is not enough proven data in the literature to declare the very absolute mechanism of this substance inside the cancerous cells.

We performed this study by inducing squamous cell carcinoma, as the most common carcinoma in the oral cavity, in the buccal pouch of Golden Syrian Hamster by using DMBA as a carcinogenic agent. Then the induced cancer was treated by injection of amygdalin in the peritonea.

Amygdalin is composed of two molecules of glucose, one benzaldehyde, which is an analgesic agent, and one hydrocyanic acid, which is considered as an antineoplastic compound.

In the present study, the antineoplastic effects of amygdalin were investigated by detecting the mitotic index and the P53 pathway after treating the induced carcinoma, comparing the results with that of control group.

Ki67 was almost negative in the hamsters treated with amygdalin, while it was elevated in the cancer group.

P53 was also negative in the treated group, compared to its high value in the cancer group.

There are no articles in the literature, that mentioned the mitotic index while studying the effect of amygdalin, nor the P53 pathway was studied.

Prostate cancer cells treated with amygdalin exhibited several morphological characteristics of apoptosis. The study declared that amygdalin increased expression of Bax, a pro-apoptotic protein, decreased expression of Bcl-2, an anti-apoptotic protein, and increased caspase-3 enzyme activity in prostate cancer cells(14).

The same pathway of BAX proteins was studied in the cervical cancer cells(HeLa cells) , showing that amygdalin-treated HeLa cells developed typical apoptotic changes. (15)

Another study explained the effect of amygdalin in blocking bladder cancer through diminishing cyclin A and cyclin- dependent kinase (cdk2), the cell cycle regulating proteins in cancer cells.

V. Conclusion:

To conclude, we found that amygdalin induces apoptosis in the cells by down regulating the expression of P53. Additionally, amygdalin reduces the rate of mitotic index (Ki67) in these cells.

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