BACKGROUND INFORMATION ON LAETRILE

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

NCI TESTING OF LAETRILE IN ANIMALS

The National Cancer Institute (NCI) has conducted a series of tests of Laetrile in rodents with transplanted and spontaneous tumors. These systems are used routinely to screen compounds for anticancer activity. "Screening" is a commonly used term in new drug development. It is the process by which a large number of materials are evaluated in experimental test systems to quickly eliminate the inactive compounds and to identify those materials having the greatest potential for activity in man. Six different samples of Laetrile have been used in the NCI tests. These tests began in 1957 and have continued as new animal tumor systems have become available. All tests have failed to demonstrate that Laetrile has any anticancer activity.

Laetrile is variously described as a form of Amygdalin, a nitriloside, Vitamin B_{17}, or l-mandelonitrile-beta-glucuronide. Amygdalin is a cyanogenic glucoside that occurs in the seeds of many plants. It is not necessary for the normal metabolic functioning of the body and is therefore not regarded as a vitamin. Supporters of the drug have used the names Laetrile and Amygdalin synonymously. The compound actually used in all tests at NCI and elsewhere has been Amygdalin.

Chemically, Amygdalin is a derivative of a molecule called mandelonitrile. Specifically, it is a mandelonitrile-beta-gentiobioside, in which mandelonitrile is linked with a chain of two glucose units. The enzyme beta-glucosidase, used in some of the tests, can break the link between mandelonitrile and the one or more sugar derivatives. Mandelonitrile may decompose further, releasing highly toxic cyanide.

Ernst T. Krebs, Jr., who claimed the synthesis of Laetrile in 1952, proposed that the compound acted through the release of cyanide in cancer cells. He suggested that normal cells contain an enzyme called rhodanese that detoxifies cyanide by converting it to thiocyanate. Assays of this enzyme have shown no such differences between normal and tumor cells.

The NCI tests of Amygdalin either alone or in combination with beta-glucosidase are described below. Because these tests have failed to produce evidence of anticancer activity, there is no basis for predicting that Amygdalin might act against cancer in humans.

1957: Amygdalin obtained from Dr. Emil White of Johns Hopkins University, Baltimore, was tested in the three transplanted mouse tumor systems used at the time by the NCI Cancer Chemotherapy National Service Center. The tests were conducted at WARF (Wisconsin Alumni Research Foundation) Institute, Inc., Madison, Wisconsin. Drug was administered by intraperitoneal injection:

Leukemia L1210 - 6 mice were treated at a dose of 500 mg drug/kg mouse body weight/day; beginning the day after implantation of the tumor (day 1) until death.

Carcinoma 775 - 10 mice were treated at a dose of 450 mg/kg/day; days 1 to 11.

Sarcoma 180 - 6 mice were treated at a dose of 250 mg/kg/injection twice daily, days 1 to 7.

The compound failed to show anticancer activity by increasing the lifespan of the animals with leukemia L1210. It also failed to inhibit growth of carcinoma 775 or sarcoma 180.

1960: Amygdalin obtained from the General Chemical Company, Portland, Oregon, was tested against the same three mouse tumors at Microbiological Associates, Inc., Bethesda, Maryland.
Drug was administered intraperitoneally:
Leukemia L1210 - 6 mice were treated with 400 mg/kg/day;days 1 to death;
Carcinoma 775 - 10 mice were treated with 400 mg/kg/day; days 1 to 11;
Sarcoma 180 - 6 mice were treated with 250 mg/kg/twice daily, days 1 to 7.
The compound failed to show anticancer activity.

1969: Amygdalin obtained from the Aldrich Chemical Company, Waukegan, Illinois, was tested alone and in combination with the enzyme beta-glucosidase in mice with leukemia L1210. The tests were performed at Microbiological Associates, Inc., Bethesda, Maryland. Doses ranged from 25 mg/kg/day to 400 mg/kg/day, varying by successive doubling. Thus the following daily doses were administered intraperitoneally, days 1 to death: 25 mg/kg; 50 mg/kg; 100 mg/kg; 200 mg/kg; and 400 mg/kg. Ten mice were used at each of the five dose levels. No anticancer activity of Amygdalin was demonstrated, alone or in combination with the enzyme.

Toxic side-effects were observed when 10 mg/kg of the enzyme was combined with doses of Amygdalin in excess of 100 mg/kg.

1969: The test was repeated at Hazelton Laboratories, Vienna, Virginia, using the same sample of Amygdalin, alone or in combination with beta-glucosidase in mice with leukemia L1210. Doses of 100 mg/kg/day, 200 mg/kg/day, or 400 mg/kg/day, days 1 to 9, failed to demonstrate anticancer activity. Six mice were tested at each dose level. Toxic side-effects increased when the drug and enzyme were given together.

1973: A clinical sample of Mexican Amygdalin was obtained for NCI by the Food and Drug Administration from the McNaughton Foundation, Montreal, Canada. This material was tested alone and in combination with beta-glucosidase at the Southern Research Institute, Birmingham, Alabama, against the Lewis lung carcinoma implanted subcutaneously into mice. Amygdalin in doses ranging from 0.75 to 800 mg/kg/injection was given intraperitoneally for nine days. The same doses and schedule of Amygdalin were used in combination with either 5 or 10 mg/kg of beta-glucosidase. Each treated group consisted of 10 mice, and each control group contained approximately 30 mice. Sporadic increases in lifespan were observed at one dose level of the drug alone and one dose of the drug-enzyme combination. A group of mice which received the enzyme alone also showed an increase in lifespan. The compound had no significant effect on growth of the tumors. Because the increase in lifespan could not be confirmed when the

experiment was repeated three more times, this study was reported as showing no anticancer activity of Amygdalin alone or in combination with beta-glucosidase.

Tests of Amygdalin are continuing at the Southern Research Institute. Scientists there are evaluating the effect of the compound on development of metastases (tumors that spread from the original tumor) in mice harboring the Lewis lung carcinoma.

1973: The same Mexican sample of Amygdalin was tested alone and in combination with beta-glucosidase at Arthur D. Little, Inc., Cambridge, Massachusetts, in the following transplanted rodent tumor systems:

Leukemia L1210 - Amygdalin was administered daily for 9 days intraperitoneally in doses ranging from 6.25 mg/kg/day to 3200 mg/kg/day. Doses of 10 mg/kg/day of beta-glucosidase were given one-half hour prior to Amygdalin in doses ranging from 6.25 mg/kg/day to 800 mg/kg/day. Approximately 10 mice were used for each dose level. No anticancer activity was observed with the compound, alone or with the enzyme. Toxicity occurred when the enzyme was combined with Amygdalin at doses of 100 mg/kg/day and higher.

P388 Leukemia - Amygdalin was tested alone at daily doses of 100,200 or 400 mg/kg/day for 9 days. It was also tested in combination with beta-glucosidase (5 or 10 mg/kg/day) at doses ranging from 6.25 to 800 mg/kg/day. Ten mice were used in each test group. No anticancer activity was observed. Toxicity occurred when more than 200 mg/kg of Amygdalin was combined with the enzyme.

B16 Melanoma - Doses of Amygdalin ranging from 6.25 mg/kg/day to 800 mg/kg/day were administered intraperitoneally for 9 days to mice bearing the B16 melanoma. Doses of 5 or 10 mg/kg of beta-glucosidase also were combined with Amygdalin (dose range from 6.25 to 100 mg/kg/injection). Ten mice were used for each dose level. No anticancer activity (increase in lifespan) was observed. Only the combination of 100 mg/kg of Amygdalin and 10 mg/kg of the enzyme was toxic for mice bearing this tumor.

Walker 256 Carcinoma - Rats harboring this transplanted tumor were treated with doses of Amygdalin ranging from 31.3 to 1000 mg/kg/injection. The drug was administered intraperitoneally on days 1, 3 and 6. The drug also was tested at doses from 1.95 to 500 mg/kg/injection in combination with beta-glucosidase (doses from 1.25 to 10 mg/kg). Ten rats were used at each dose level. No activity was observed, as measured by effect on lifespan. There was no toxicity.

Reference: Isidore Wodinsky and Joseph K. Swiniarski, Antitumor Activity of Amygdalin MF as a Single Agent and with Beta-Glucosidase on a Spectrum of Transplantable Rodent Tumors. Cancer Chemotherapy Reports, Volume 59, September-October 1975, pages 939-950.

1973: The same Mexican sample of Amygdalin was tested alone and in combination with the enzyme beta-glucosidase in three transplanted rodent tumors by scientists at the Southern Research Institute, Birmingham, Alabama:

Ridgway Osteogenic Sarcoma (ROS) - Amygdalin was administered intraperitoneally for 9 days in daily doses of 220 mg/kg/day,

335 mg/kg/day or 500 mg/kg/day. Ten mice of the AKD_2 F_1 strain were used per dose. No anticancer activity, as measured by an increase in lifespan, was observed. To test for toxicity, normal (nontumor-bearing) AKD_2 F_1 mice were given the same dose of Amygdalin alone or in combination with the enzyme. Toxicity, as measured by a decrease in lifespan, was observed when doses of Amygdalin exceeded 335 mg/kg/day. This toxicity was increased (observed at lower doses of Amygdalin) when the enzyme was given in combination with Amygdalin.

Lewis Lung Carcinoma - Amygdalin in the same doses and schedule as used for the ROS assay was tested against mice of the BDF_1 strain bearing the Lewis lung carcinoma. Tumors were implanted subcutaneously in one experiment. In a second experiment, they were implanted intravenously. A control group of normal BDF_1 mice was assayed for toxicity, as measured by a decrease in lifespan and a change in body weight. Amygdalin either alone or in combination with the enzyme did not increase the lifespan or decrease the size of tumors in any of the treated groups. Toxicity of Amygdalin alone was observed in doses exceeding 220 mg/kg/injection. This effect was increased when Amygdalin was combined with the enzyme.

P388 Leukemia - Amygdalin was administered in the same dose and schedule as stated for the ROS system, to BDF_1 mice harboring P388 leukemia. No anticancer activity was observed.

Reference: W. R. Laster, Jr. and F. M. Schabel, Jr., Experimental Studies of the Antitumor Activity of Amygdalin MF Alone and in Combination with Beta-Glucosidase. Cancer Chemotherapy Reports, Volume 59, September-October 1975, pages 951-965.

1977: A sample of Amygdalin obtained from the Aldrich Chemical Company, Waukegan, Illinois, was tested for activity against two human cancers, of the colon and breast, grown as xenografts in athymic (nude) mice. This mouse model is a new tool for screening anticancer drugs. Due to a mutation, the mouse is born hairless and lacking a thymus gland. The latter defect allows these mice to accept foreign tissue transplants, including those from other species (xenografts). Scientists have found that some human cancers will grow in athymic mice and that administration of active anticancer drugs will cause the tumor to shrink. In the tests, performed at the Battelle Memorial Institute, Columbus, Ohio, Amygdalin was administered in doses of 400, 800 or 1600 mg/kg/injection intraperitoneally once every 4 days for 4 courses. Six mice were used in each test group. Amygdalin showed no anticancer activity, as measured by a decrease in tumor size, in mice bearing either the human breast or the human colon cancer.

In another series of tests, Amygdalin was administered at doses ranging from 100 to 800 mg/kg/injection for 9 days beginning 14 days after implantation of the human colon tumor under the mouse's skin. Doses of 200, 100 or 50 mg/kg/day of Amygdalin were combined with either 5 or 10 mg/kg of beta-glucosidase. Approximately four mice were used in each of the test groups. No effect of Amygdalin on tumor size was noted. Amygdalin in doses of 200 mg/kg/day when combined with the enzyme was toxic to all mice in the test group.

1973-1977: Scientists at Memorial Sloan-Kettering Cancer Center, New York City, and the Catholic Medical Center of Brooklyn and Queens, Inc., Woodhaven, New York, tested Amygdalin in rodents with a series of transplanted and spontaneous tumors. The study was sponsored in part by grants and in part by a contract from the National Cancer Institute.

Two samples of Amygdalin were tested in these studies. One was prepared in Mexico for the McNaughton Foundation; the other was obtained from Sidus Arzneimittel GmbH in West Germany.

Transplanted Tumors

- Sarcoma 180 - Doses of Amygdalin ranging from 25 to 500 mg/kg/day given orally were ineffective in inhibiting growth of this transplanted tumor in mice. Similarly doses of Amygdalin ranging from 500 to 100 mg/kg/day administered intraperitoneally were inactive. Approximately 10 mice were used at each dose level. Amygdalin was toxic at 500 mg/kg/day when given orally.
- Amygdalin at doses of 500 mg/kg/day or 100 mg/kg/day given intraperitoneally was inactive in mice with the following transplanted tumors: Mecca lymphosarcoma, sarcoma T241, mammary carcinoma E0771, Taper liver tumor, Ehrlich carcinoma (solid and ascites). It was also inactive against Walker 256 carcinoma in rats.
- Amygdalin was inactive when administered alone in doses of 100 mg/kg/day to mice bearing either the mouse plasma cell

tumor LPC-1 or leukemia L1210, Amygdalin at 500 mg/kg/day was inactive against Ridgway osteogenic sarcoma in mice. It also failed to inhibit growth of DMBA (dimethylbenzanthracene)-induced mammary tumors in rats. When Amygdalin was administered along with anticancer drugs known to be active in these systems, it did not influence the toxicity of the drugs, nor did it impair their efficacy.

Reference: C. Chester Stock, George S. Tarnowski, Franz A. Schmid, Dorris J. Hutchinson, and Morris N. Teller, Antitumor Tests of Amygdalin in Transplantable Animal Tumor Systems. Journal of Surgical Oncology, in press.

Spontaneous Tumors

- Amygdalin at doses of 2,000 mg/kg/day was ineffective in either treating or preventing the development of spontaneous leukemia in the AKR strain of mice. The leukemia, which is caused by a virus, develops in 80 percent of AKR mice within the first year of life. Amygdalin was administered intraperitoneally to AKR mice six days a week until death. Thirty mice in the prevention experiment began receiving Amygdalin at 6 months of age, before they had developed spontaneous leukemia. Thirty-eight mice with advanced spontaneous leukemia received Amygdalin in the treatment experiment.
- In further tests of Amygdalin, the hybrid CD_8F_1 strain of mice was used. Nearly 80 percent of females develop mammary tumors at an average age of 10 months. In a manner similar to human cancers, the mammary tumors in CD_8F_1 mice metastasize (spread) to the lungs.

Thirty female CD_8F_1 mice began receiving Amygdalin (1000 mg/kg/day 6 days a week for life) at 5 months of age. The compound was unsuccessful in preventing the development of breast cancer in the treated mice.

Amygdalin at doses of 1000 or 2000 mg/kg/day intraperitoneally 6 times a week for life was ineffective in destroying the primary tumors in CD_8F_1 mice with established breast cancer.

In a series of 6 experiments by one investigator, Amygdalin in doses of 1000 or 2000 mg/kg/day 6 days a week inhibited the number of lung metastases compared to animals receiving only injections of a salt solution (saline). By visual observation, the scientist noted an average of 22 percent of mice with lung metastases in the Amygdalin-treated group, compared to 91 percent in the control group. Three independent investigators repeated the experiment and found no significant difference in the number of mice with metastases between the Amygdalin-treated and saline-treated groups. Furthermore, a blind study (coded samples of Amygdalin or saline unknown to the participating investigators) in which the first scientist collaborated, failed to show a benefit of Amygdalin in preventing metastases in CD_8F_1 mice.

Based on these findings, the scientists concluded that Amygdalin possesses no activity in preventing or curing cancer, or in shrinking tumors or in blocking the spread of cancer by metastasis

Reference: C. Chester Stock, Daniel S. Martin, Kanematsu Sugiura, Ruth A. Fugman, Isabel M. Mountain, Elisabeth Stockert, Franz A. Schmid, and George S. Tarnowski, Antitumor Tests of Amygdalin in Spontaneous Animal Tumor Systems. Journal of Surgical Oncology, in press.

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