Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6513–6516, October 1981
Medical Sciences

Amygdalin (Laetrile) and prunasin β-glucosidases: Distribution in germ-free rat and in human tumor tissue

[cyanogenic glucosides/neutral β(1→6)- and β(1→1)-glucosidases/gentiobiose]

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ABSTRACT **Amygdalin, the gentiobioside derivative of mandelonitrile commonly referred to as Laetrile, is presently under intensive investigation as a potential cancer chemotherapeutic agent. Because of this interest, we investigated the activity of β-glucosidases that cleave glucose from amygdalin and from prunasin (mandelonitrile monoglucoside) in tissues from germ-free rats and in normal and neoplastic human tissues. Rat and human small intestinal mucosa contain high levels of activity of glucosidases that act on both of these cyanogenic glucosides. Release of glucose from these compounds was not detected in any of the human neoplastic tissues examined in the present study. These observations are consistent with reports of cyanide toxicity through the oral use of amygdalin or prunasin and pose serious questions concerning the alleged tumoricidal effect of amygdalin.**

Amygdalin (D-mandelonitrile-β-D-glucosido-6-β-D-glucoside) has been promoted (1) and, in 23 American states, legalized as an oral chemotherapeutic agent for "terminal" cancer. These endeavors were endorsed despite documented heterogeneity of amygdalin and "Laetrile" preparations (2, 3), a dearth of evidence of the effectiveness of amygdalin on animal (4–6) and human neoplasms (7, 8), and serious questions regarding potential cyanide toxicity.

Schmidt *et al.* (9) demonstrated that oral administration of amygdalin in doses equivalent to the recommended human tumoricidial doses along with the sweet almond preparations containing the amygdalin-hydrolyzing enzyme complex emulsion produced high levels of HCN in serum, clinical signs of cyanide toxicity, and death of 6 of 10 experimental animals. Khandekar and Edelman (10) administered amygdalin to rats intraperitoneally and demonstrated dose-dependent mortality and clinical evidence of cyanide toxicity without the concurrent use of almond β-glucosidases. However, Ames *et al.* (11) found that parenteral administration of amygdalin resulted in excretion of this compound in the urine primarily in an unchanged form. In humans, Moertel *et al.* (12) found that intravenous infusion of amygdalin produced neither cyanidemia nor signs of toxicity but that oral administration resulted in significant blood cyanide levels and that, in one case, oral amygdalin plus almond extract produced transient symptoms of cyanide intoxiciation and further increase of blood cyanide. These studies seemed to indicate that amygdalin was probably metabolized in some portion(s) of the gastrointestinal tract.

Freese *et al.* (13) isolated and partially purified a neutral β(1→6)-glucosidase that catalyzes the hydrolysis of the terminal glucose moiety of amygdalin and clearly differentiated this enzyme from previously known mammalian β-glucosidases. The enzyme was unable to hydrolyze gentiobiose, the disaccharide component of amygdalin, suggesting a requirement for an aryl or alkyl aglycone residue for enzymatic activity. The natural occurrence of this enzyme was unusual, being particularly plentiful in feline kidney but also present in rat and rabbit kidney and rodent intestine. It was notably absent in human kidney preparations. This enzyme and the subsequent activity of a β(1→1)-glucosidase on the prunasin produced by the β(1→6)-glucosidase were considered to be important in the metabolism of exogenous amygdalin because mandelonitrile can spontaneously release cyanide *in vivo*. Thus, it was suggested that, if amygdalin exerts a tumoricidal effect by release of cyanide, a tissue susceptible to amygdalin should contain the required β-glucosidases. In order to better characterize the occurrence of amygdalin and prunasin glucosidases, we have extended the previous study (13) to an examination of tissues from germ-free rats and normal and neoplastic human tissues.

MATERIALS AND METHODS

Amygdalin was obtained from Aldrich. Prunasin was prepared as described (13). Gentiobiose was purchased from Sigma.

Germ-free male rats (200 g) were obtained from Charles River Breeding Laboratories, Wilmington, MA. Upon arrival, the rats were sacrificed by asphyxiation with solid CO_2; the tissues were removed and homogenized by hand in 10 vol of cold distilled water. The mixtures were centrifuged at 500 × *g* for 10 min, and the supernatant suspensions were assayed for enzyme activity. The quantity of glucose enzymatically released from amygdalin, prunasin, and gentiobiose was determined with glucose oxidase reagent (Sigma) as described (13). β-Glucosidase activity was also measured with 4-methylumbelliferyl-β-D-glycopyranoside (Sigma) as substrate at pH 4.5 in acetate buffer and at pH 7.5 in phosphate buffer (14). Glucocerebrosidase activity was also determined in the tissue specimens by using [^{14}C]glucocerebroside as described (15). Protein was determined by the procedure of Lowry *et al.* (16).

Human tissues from 19 patients were obtained at operation from the Department of Pathology, Suburban Hospital, Bethesda, MD. Patients were unselected. The tissues were refrigerated until homogenization 2–16 hr postoperatively. Tissues were processed in the same manner as before, and the tissue diagnosis on each specimen was subsequently confirmed microscopically. Two specimens of human small intestine were obtained at autopsy. Postmortem diagnosis for each patient was cardiac disease, and no gross pathology was seen in the abdomen. The autopsies were performed the day after death, and the bodies had been refrigerated for an unstated time before autopsy.

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RESULTS

Enzymes that catalyze the cleavage of glucose from amygdalin and prunasin were present in the small intestine and intestinal contents of germ-free rats and in the kidney (Table 1). These β-glucosidases were practically absent in the stomach and in the large intestine. Both proximal and distal halves of the small intestine mucosa contained both amygdalin- and prunasin-cleaving activity. The colon contents showed significant activity "downstream" from the site of high small intestinal mucosal activity. This observation is consistent with shedding of mucosal elements or secretion of these enzymes, or both, into the lumen of the small intestine. The possibility of a bacterial source of these enzymes is remote because the rats were germ-free.

The data obtained with human tissue confirm the localization of these β-glucosidases to the small intestine (Table 2). None of the human neoplastic tissues examined exhibited either amygdalin- or prunasin-hydrolyzing activity. All of the tissues contained significant glucocerebroside-β-glucosidase activity as well as glucosidase(s) that catalyze the hydrolysis of 4-methylumbelliferyl-β-D-glucopyranoside. Catalytic activity with the latter substrate was much greater at pH 4.5 than at pH 7.5 in all tissues except rat kidney and rat and human small intestine. The pH optimum of rodent kidney β(1→6)-glucosidase is 7.5 (13). The comparatively high activity with the fluorogenic substrate at this pH in these tissues is consistent with the presence of additional glucosidases that act on the cyanogenic glucosides. The presence of enzymes that catalyze the hydrolysis of glucocerebroside and 4-methylumbelliferyl-β-D-glucopyranoside in all of the tissue specimens examined in this study indicates that the samples had been processed in a manner so that the activity of these glucosidases was retained. The hydrolysis of gentiobiose at pH 7.5 was not detected in any of the enzyme preparations that showed amygdalin and prunasin β-glucosidase activities. Kobayashi and Suzuki (17) have extended earlier observations of Brady *et al.* (18) that rat intestinal tissue contains a nonspecific glycosidase with an acidic pH optimum that catalyzes the hydrolysis of both glucocerebroside and galactocerebroside. The purified enzyme is active with gentiobiose as substrate. Because of the difference in pH optimum and substrate specificity, it can be distinguished from the enzymes that catalyze the hydrolysis of amygdalin and prunasin.

Even in our small sample, substantial variations were observed in levels of amygdalin- and prunasin-hydrolyzing glucosidases in human intestine. This apparent inconsistency could reflect variations between individuals or differences in the processing of the samples, or both. Evidence that the latter was at least partially responsible was found by allowing aliquots of the two most active samples in 1:1 dilution with 5.6 mM sodium azide to stand at room temperature for 24 hr before assaying; this caused a 33% reduction in amygdalin-hydrolyzing activity and a 50% reduction in prunasin-hydrolyzing activity.

DISCUSSION

The biological roles of mammalian β-glucosidases that hydrolyze amygdalin and prunasin are not understood. The high level of β(1→6)-glucosidase activity in kidney from several species (but not human renal tissue) (13) may be related to the nephritogenic glycopeptide in rat glomerular basement membrane examined by Shibata and Nakanishi that contains the unusual β(1→6)-glucosylglucopyranosidic bond (19, 20). Metabolic turnover of this component would presumably require the activity of such a glucosidase. The presence of the polypeptide portion of this molecule could satisfy the apparent requirement for an alkyl or aryl aglycone moiety for the renal β(1→6)-glucosidase. Human feces exhibit a wide variety of glycoside-splitting enzyme activities (21) that are presumed to be largely of bacterial origin. The natural substrates of the intestinal enzymes that catalyze the hydrolysis of glucose from amygdalin and prunasin remain to be identified.

Laetrile advocates have argued that glycosidically substituted nitriles exert their tumoricidal effect by releasing cyanide into susceptible (presumably tumor) cells, causing cell death through cyanide toxicity. Amygdalin is stable at physiological temperatures, but mandelonitrile dissociates into cyanide and benzaldehyde at 25°C. Therefore, it would seem that the diglucosidic component of amygdalin tends to protect users from cyanide toxicity. It would also seem that signs of cyanide toxicity might appear in tissue containing enzymes that cleave this disaccharide moiety. It would be logical to suspect that, if amygdalin is tumoricidal by the proposed mechanism, it would preferentially work in tissues containing β-glucosidases that cleave glucose from amydgalin and prunasin. Our data indicate that small intestine (rat and human) is rich in amygdalin- and prunasin-hydrolyzing activities. This finding is consistent with the observation of Moertel *et al.* (12) that amygdalin administered intravenously caused no signs of cyanide toxicity, but that oral administration caused toxic effects and cyanidemia. No amygdalin or prunasin glucosidase activity was detected in the 17 human neoplasms examined in this study. If, as Laetrile advocates claim, the tumoricidal effect depends on the cleavage to mandelonitrile from which cyanide is spontaneously or pos-

Table 1. β-Glucosidase activities in tissues from germ-free rats

		Substrate				
					4-Methylumbelliferyl-β-D-glucopyranoside	
Tissue	N	Amygdalin	Prunasin	Glucocerebrosidase	pH 4.5	pH 7.5
Kidney	6	19 ± 2.4	8.6 ± 1.5	42 ± 6.9	152 ± 42	329 ± 38
Stomach	6	ND	ND	17 ± 11	3.7 ± 0.8	2.9 ± 1.1
Whole small intestinal mucosa	3	36 ± 8.9	7.1 ± 1.8	30 ± 8.5	58 ± 28	32 ± 10
Proximal small intestinal mucosa	3	48 ± 29	6.4 ± 3.2	135 ± 45	126 ± 26	50 ± 13
Distal small intestinal mucosa	3	30 ± 19	7.9 ± 3.6	55 ± 25	85 ± 4.8	31 ± 1.3
Small intestinal contents	6	68 ± 7.7	7.7 ± 0.8	11 ± 3.2	51 ± 10	29 ± 5.9
Colonic mucosa	3	ND	ND	20 ± 10	8.5 ± 1.2	5.1 ± 3.8
Colonic contents	5	27 ± 4.3	6.4 ± 1.7	6.4 ± 1.2	17 ± 5.4	17 ± 9.0

Activity is expressed as mean ± SD nmol of glucose cleaved per mg of protein hr^{-1}. ND, nondetectable (<0.1); N, number of rats.

Table 2. β-Glucosidase activities in normal and neoplastic human tissue

Patient				Substrate				
							4-Methylumbelliferyl-β-D-glucopyranoside	
No.	Preoperative diagnosis	Specimen	Histological diagnosis	Amygdalin	Prunasin	Glucocerebrosidase	pH 4.5	pH 7.5
1	Colon cancer, recurrent	1A	Normal colon	ND	ND	46	31	11
		1B	Villous adenoma, colon	ND	ND	111	63	7
		1C	Adenocarcinoma of colon	ND	ND	53	22	3
2	Colon cancer	2A	Normal colon	ND	ND	32	14	2
		2B	Adenocarcinoma of colon	ND	ND	105	50	6
3	Colon cancer	3A	Normal colon	ND	ND	51	26	10
		3B	Villous adenoma, colon	ND	ND	77	46	17
		3C	Adenocarcinoma of colon	ND	ND	33	8	1
4	Vaginal mass, history of colon cancer	4A	Adenocarcinoma of colon, metastatic to vagina	ND	ND	46	7	0.6
5	Colon cancer	5A	Normal appendix	ND	ND	25	29	4
		5B	Normal ileum	2	1	34	54	86
		5C	Normal colon	ND	ND	18	4	5
		5D	Adenocarcinoma of colon	ND	ND	29	15	2
6	Colon cancer	6A	Normal colon	ND	ND	14	4	5
		6B	Adenocarcinoma of colon	ND	ND	52	3	1
6	Colon cancer	6C	Adenocarcinoma of colon, metastatic to mesenteric lymph node	ND	ND	19	7	2
7	Chest nodule, history of mastectomy for breast cancer	7A	Ductal carcinoma of breast	ND	ND	74	61	1
8	Breast cancer	8A	Ductal carcinoma of breast	ND	ND	29	3	5
9	Breast mass	9A	Ductal carcinoma of breast	ND	ND	18	86	17
10	Metastatic breast cancer	10A	Poorly differentiated carcinoma metastatic to axillary lymph node	ND	ND	20	8	0.5
11	Breast mass	11A	Ductal carcinoma of breast	ND	ND	20	5	0.3
12	Lung mass	12A	Squamous cell carcinoma, metastatic to supraclavicular lymph node	ND	ND	42	14	0.7
13	Enlarged lymph node	13A	Poorly differentiated epidermoid carcinoma metastatic to supraclavicular lymph node, primary unknown	ND	ND	73	7	2
14	Thyroid nodule	14A	Normal thyroid	ND	ND	7	1	0.9
		14B	Benign follicular adenoma of thyroid	ND	ND	31	6	0.4
15	Thyroid nodule	15A	Lymphocytic thyroiditis	ND	ND	25	10	8
		15B	Papillary carcinoma of thyroid	ND	ND	14	5	3
16	Trauma to abdomen	16A	Normal spleen	ND	ND	8	15	15
17	Brain abscess or hematoma	17A	Meningotheliomatous meningioma	ND	ND	35	19	1
18	Malignant melanoma	18A	Malignant melanoma, metastatic to inguinal lymph node	ND	ND	134	123	3
19	Gunshot wound to abdomen	19A	Normal ileum	72	54	51	199	140
	Autopsy 1	A1A	Normal ileum	6	0.3	14	59	68
	Autopsy 2	A2A	Normal ileum	25	8	10	87	85

Activity is expressed as mean ± SD nmol of glucose cleaved per mg of protein hr^{-1}. ND = nondetectable (<0.1).

sibly enzymatically liberated (22), we are unable to substantiate this rationale for the use of amygdalin (or prunasin) as a cancer chemotherapeutic agent.

A theoretical limitation of the present study was its reliance on surgical pathology specimens from a general hospital. Specifically, during the period of this investigation, no small intestinal tumors were obtained. It is possible that neoplasms of this organ might be suitable targets for amygdalin (or prunasin). However, we found no evidence in the more common tumors (colon, breast, lung) to support the contention that amygdalin or prunasin would be expected to exert a therapeutic effect.

Note Added in Proof. Since submission of this manuscript, we had an opportunity to examine β-glucosidase activity in a papillary carcinoma of human kidney. Although there was significant hydrolysis of glucocerebroside and 4-methyl-β-D-glucopyranoside, neither amygdalin nor

prunasin was cleaved by this preparation or by adjacent histologically normal renal tissue.

J.N. is the recipient of a National Institutes of Health [NRSA] Postdoctoral Research Fellowship.

1. Holden, C. (1976) *Science* **193**, 982–985.
2. Levi, L., French, W. M., Bickis, I. J. & Henderson, I. W. D. (1965) *Can. Med. Assoc. J.* **92**, 1057–1061.
3. Davignon, J. P., Trissel, L. A. & Kleinman, L. M. (1978) *Cancer Treat. Rep.* **62**, 99–104.
4. Wodinsky, I. & Swiniarski, J. K. (1975) *Cancer Chemother. Rep.* **59**, 939–950.
5. Laster, W. R. & Schabel, F. M. (1975) *Cancer Chemother. Rep.* **59**, 951–965.
6. Hill, G. J., Shine, T. E., Hill, H. Z. & Miller, C. (1976) *Cancer Res.* **36**, 2102–2107.
7. Ellison, N. M., Byar, D. P. & Newell, G. R. (1978) *N. Engl. J. Med.* **299**, 549–552.
8. Sun, M. (1981) *Science* **212**, 758–759.
9. Schmidt, E. S., Newton, G. W., Sanders, S. M., Lewis, J. P. & Conn, E. E. (1978) *J. Am. Med. Assoc.* **239**, 943–947.
10. Khandekar, J. D. & Edelman, H. (1979) *J. Am. Med. Assoc.* **242**, 169–171.
11. Ames, M. M., Kovach, J. S. & Flora, K. P. (1978) *Res. Commun. Chem. Path. Pharm.* **22**, 175–185.
12. Moertel, C. G., Ames, M. M., Kovach, J. S., Moyer, T. P., Rubin, J. R. & Tinker, J. H. (1981) *J. Am. Med. Assoc.* **245**, 591–594.
13. Freese, A., Brady, R. O. & Gal, A. E. (1980) *Arch. Biochem. Biophys.* **201**, 363–368.
14. Ginns, E. I., Brady, R. O., Stowens, D. W., Furbish, F. S. & Barranger, J. A. (1980) *Biochem. Biophys. Res. Commun.* **97**, 1103–1107.
15. Furbish, F. S., Blair, H. E., Shiloach, J., Pentchev, P. G. & Brady, R. O. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3560–3563.
16. Lowry, O. M., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
17. Kobayashi, T. & Suzuki, K. (1981) *J. Biol. Chem.* **256**, 7768–7773.
18. Brady, R. O., Gal, A. E., Kanfer, J. M. & Bradley, R. M. (1965) *J. Biol. Chem.* **240**, 3766–3770.
19. Shibata, S. & Nakanishi, H. (1980) *Carbohydr. Res.* **81**, 345–348.
20. Shibata, S. & Nakanishi, H. (1980) *Carbohydr. Res.* **86**, 316–320.
21. Tamura, G., Gold, C., Ferro-Luzzi, A. & Ames, B. N. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4961–4965.
22. Haisman, D. R. & Knight, D. J. (1967) *Biochem. J.* **103**, 528–534.