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Pharmacology of Amygdalin (Laetrile) in Cancer Patients

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Summary. *Plasma and urine concentrations of amygdalin, whole-blood concentrations of cyanide, and thiocyanate concentrations in serum and urine were determined in cancer patients following intravenous (4.5 g/m²) and oral (500-mg tablet) administration of amygdalin. To measure low plasma concentrations of amygdalin following oral administration a GC/MS assay was developed. Following intravenous administration, concentrations of parent drug as high as 1,401 μg/ml were observed, with no increase in plasma concentrations of cyanide or serum concentrations of thiocyanate. Plasma elimination of amygdalin was best described by a two-compartment open model with a mean distributive phase half-life of 6.2 min, mean elimination phase half-life of 120.3 min, and mean clearance of 99.3 ml/min. Following oral administration of amygdalin, plasma concentrations were much lower, with peak values of less than 525 ng/ml. Cyanide concentrations increased to values as high as 2.1 μg/ml whole blood. Thiocyanate concentrations did not increase for several days, plateauing at values as high as 38 μg/ml serum. Ingestion of almonds by two patients taking oral amygdalin increased cyanide concentrations compared with values obtained after oral amygdalin alone.*

Introduction

Amygdalin (D-mandelonitrile-β-D-glucosido-6-β-D-glucoside) is a cyanogenic glucoside found in a variety of plant species, notably in the seeds of apricots and bitter almonds. Amygdalin, commonly referred to as laetrile, has been proposed to be effective in both the prevention and the treatment of cancer in humans [12, 16] although reports in the scientific literature do not show significant effect of amygdalin on experimental tumor systems [14, 17, 26]. The National Cancer Institute is currently sponsoring a clinical trial of amygdalin. Clinical and preliminary pharmacological findings in an initial toxicology study have recently been reported [20].

Pharmacology studies of amygdalin and related cyanogenic glycosides have focused on toxicity related to liberation of cyanide. β-Glucosidases, found predominantly in nuts and seeds containing the glycosides, hydrolyze amygdalin to two molecules of glucose and one molecule of mandelonitrile [13]. The cyanohydrin is unstable, and readily decomposes to benzaldehyde and hydrocyanic acid [13]. This decomposition may also be catalyzed by nitrile lyase, an enzyme found in glycoside-containing plants [13]. Amygdalin toxicity has been demonstrated in dogs by feeding amygdalin and a sweet almond paste (sweet almonds contain β-glucosidases and nitrile lyase, but not the cyanogenic glycosides) [22]. Toxicity was clearly related to formation of cyanide [22]. High blood concentrations of cyanide and cyanide toxicity have also been observed in rats following administration of amygdalin PO [6]. β-Glucosidases are found in gut microflora, and toxicity was not observed in germ-free rats [6].

Literature reports on the disposition of amygdalin are limited. In mice, 96-h urinary recovery of parent drug (measured as benzaldehyde after enzymatic hydrolysis) following IV or PO administration (600 mg/kg) was 70% and 20%, respectively [10]. We reported plasma and urinary concentrations of amygdalin following IM (6 g) and PO (500 mg) self-administration of amygdalin in two patients [1]. The benzaldehyde assay revealed high plasma concentrations after IM injection, while no amygdalin was detected following oral administration [1]. Urinary recovery (24 h) was 71% and 55% for IM and PO administration, respectively [1]. We now report detailed pharmacologic studies on patients receiving

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amygdalin PO and IV as part of the initial toxicology clinical trial.

Materials and Methods

Materials. Oral and IV dosage forms of amygdalin were provided by the National Cancer Institute. D-Amygdalin was purchased from Aldrich Chemical Company (Milwaukee, WI, USA), prunasin and emulsin from Sigma Chemical Company (St Louis, MO, USA), Tri-sil from Pierce Chemical Company (Rockford, IL, USA), Porapak Q resin from Dupont Company (Wilmington, DE, USA), and chloramine-T from Fisher Chemical Company (Pittsburgh, PA, USA). Other solvents and chemicals were of the highest grade obtainable.

Patient Selection and Treatment Program. Details of this aspect of the study have been described elsewhere [20]. Patients had malignant disease beyond any hope of cure or significant palliation by any known method of therapy. The experimental nature of the treatment program was fully explained to patients, and all provided their written informed consent. The amygdalin and 'metabolic therapy' program was based on published recommendations of practitioners who prescribe laetrile. Similarly, the dietary regimen was designed to be representative of recent laetrile practice.

Patients were treated with 4.5 g amygdalin/M^2 by rapid IV injection daily for 21 consecutive days, followed by 0.5 g amygdalin PO three times daily, administered 1 h before meals. Daily oral therapy was continued until progression of disease was observed. For pharmacologic studies, three patients were initially treated with amygdalin at 0.5 g three times daily PO for 7 days. They then began the standard regimen of 21 days IV amygdalin followed by oral maintenance therapy.

Blood and Urine Sample Collection. Venous blood was collected in heparinized glass tubes via an indwelling catheter.For measurement of amygdalin, blood was centrifuged and frozen until analysis. For cyanide determination, whole blood was analyzed within 3 h of collection. Venous blood was also collected into an evacuated tube, and serum collected for thiocyanate analysis. Analysis was performed within 3 h of specimen collection. Spontaneously voided urine was collected and aliquots frozen until analysis.

Cyanide Analysis. Cyanide was determined in whole blood or urine by a modification of the method of Feldstein and Klendshaj [8]. In a sealed Conway diffusion cell, 1 ml whole blood was mixed with 1 ml 3.6 N sulfuric acid in the outer chamber. Hydrocyanic acid was allowed to diffuse across the barrier for 2 h, and was absorbed by 0.5 ml 0.1 N sodium hydroxide in the center well. Separately, a color reagent was prepared by combining 3 g barbituric acid, 3.0 ml concentrated hydrochloric acid, and 15 ml pyridine. This mixture was diluted to a volume of 30 ml with water, vigorously mixed for 1 min, and allowed to stand at room temperature during specimen incubation. Just prior to the termination of incubation, the color reagent was centrifuged at 3,000 rpm to yield a clear yellow supernatant. Duplicate 0.2-ml aliquots of specimen diffusate were transferred from the center well of the Conway diffusion cell to 12 × 75 mm glass tubes and combined with 1 ml 1 *M* monobasic sodium phosphate and 0.5 ml 0.25% (w/v) chloramine-T. The mixture was vortexed, and mixed with 1.5 ml color reagent, vortexed again and allowed to stand at room temperature for 5 min. Absorbance at 580 nm was determined with the aid of a Perkin-Elmer Model 200 dual beam spectrophotometer. The instrument was set at zero by means of an aqueous blank carried throughout the entire procedure. Standards of potassium cyanide containing 0.25, 0.5, 1.0, and 2.0 μg/ml were analyzed with each set of specimens to define a standard curve. In addition, whole blood containing cyanide at 1.0 μg/ml was analyzed with each unknown sample as a control. Standard curves were linear from 0.05 μg/ml to 2.0 μg/ml. The principal disadvantage of the procedure is the time required for analysis (3 h). A specific ion electrode assay [7] is no more rapid, because a diffusion step is required for specificity. Atomic absorption spectroscopy [15] gave unacceptable variability with biological samples.

Thiocyanate Analysis. Thiocyanate was determined by a modification of the procedure of Bowler [3]. A protein filtrate of blood, when combined with acidified ferric nitrate, yields a chromophore of iron thiocyanate (λ max 455 nm). However, at that wavelength the procedure is subject to interference. To eliminate interference, the procedure was modified by setting the spectrophotometer to zero at 610 nm for each sample, and determining the absorbance at 550 nm. When performed in this manner, the method is specific for quantitation of thiocyanate in blood serum. A mixture of 2 ml blood serum and 3 ml 10% trichloroacetic acid was centrifuged at 3,000 rpm for 5 min. Simultaneously, two standards containing 10 and 30 μg thiocyanate/ml were treated with 10% trichloroacetic acid. Of each supernatant, 3 ml was transferred to a 16 × 125 ml glass tube, mixed with 1 ml 5% ferric nitrate in 0.5 N nitric acid, and kept at room temperature for 10 min. Absorbance of samples was determined against a water blank that had been carried through the entire procedure. The relative absorbance was set to zero at 610 nm, the wavelength changed to 550 nm, and the absorbance difference between the reference beam and the sample beam recorded. The concentration of thiocyanate was determined with the aid of a standard curve consisting of 0, 10, and 30 μg/ml thiocyanate in H_2O in blood.

Spectrophotometric Analysis of Amygdalin. Amygdalin was measured in plasma following IV administration of laetrile, and in urine excreted after IV and PO administration of laetrile by an assay based on enzymatic hydrolysis of amygdalin and spectrophotometric determination of benzaldehyde [1, 10]. Urine (0.01−1.0 ml) and plasma (0.1−1.0 ml) were diluted to a final volume of 2 ml with distilled water. Of a 1.5 u/ml solution of emulsin in 0.1 *M* sodium acetate buffer, 1 ml at pH 5.0 was added. After brief agitation, samples were kept at room temperature for 60 min. Concentrated ammonium hydroxide (100 μl) was added and the samples agitated. For urine samples, 4 ml methylene chloride was then added and the sample vortexed for 30 s. After centrifugation (5,000 *g*, 30 min), the organic layer was removed and ultraviolet absorbance determined at 243 nm with a Zeiss Q3 spectrophotometer. For plasma samples, 0.7 ml 3 N perchloric acid was added after the ammonium hydroxide. A mixture of methylene chloride/heptane (95/5, v/v, 3 ml) was added and the sample vortexed for 20 s. Following centrifugation, ultraviolet absorbance of the organic phase was determined at 243 nm. Standard curves were determined in control plasma and urine by the addition of known amounts of amygdalin in aqueous solution. Absorbance was determined against control samples from which emulsin was omitted. No free benzaldehyde was detected by this assay in any plasma or urine samples.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Amygdalin. Plasma samples (1 ml) were diluted to 2 ml with distilled water containing 750 ng prunasin as internal standard and placed on Porapak Q columns (0.9 × 3.5 cm) that had been washed sequentially with water and methanol, 10 ml each. After two washings with 3 ml water, materials bound to columns were eluted with 9 ml methanol. The methanolic eluate was lyophilized. The residue was derivatized by the addition of 200 μl Tri-sil reagent

and heating for 20 min at 70° C. The liquid phase was evaporated under a stream of nitrogen with gentle heating, and the residue was taken up in hexane (50–200 µl) for GC/MS analysis.

GC/MS analyses were carried out with a LKB 2091 GC/MS/DS system. GS/MS conditions were as follows: Column, 3% OV-101, 0.3 meters; initial column temperature, 215° C and final column temperature, 265° C, programmed at 8 deg C/min; flow, 11 ml helium/min; injection temperature, 260° C; separator temperature, 265° C; ion source temperature, 250° C; electron energy, 70 electron volts; ionization mode, electron impact.

Hexane samples were injected and the GC temperature program initiated. Following a 1.5-min delay, the separator was opened to the mass spectrometer source and the computer clock started. TMS prunasin eluted from the gas chromatograph approximately 2 min after sample injection, TMS amygdalin approximately 9 min after sample injection. Based on electron impact mass spectra of the per trimethylsilyl (TMS) derivatives of amygdalin and prunasin obtained in our laboratory, ion 217.0 was monitored for TMS prunasin quantitation and ion 204.0 for TMS amygdalin quantitation. In some runs, ion 217.0 was also monitored for TMS amygdalin, to provide a further check on specificity by comparing the 204.0/217.0 ratio with authentic TMS amygdalin. Standard curves in plasma contained 750 ng prunasin and varying amounts of amygdalin (10–1,000 ng). Peak widths and baselines of appropriate peaks were determined by cross hair placement on the computer-generated ion chromatogram. Area under the curves was generated by LKG software. Ratios of area under the curve of ion 204.0 (TMS amygdalin) to area under the curve of ion 217.0 (TMS prunasin) were calculated manually.

Results

GC/MS Assay of Amygdalin

The spectrophotometric assay previously employed in our laboratory for determination of plasma and urine concentrations of amygdalin [1] was satisfactory for measurement of high plasma concentrations obtained following IV administration of amygdalin. Plasma concentrations following oral administration of amygdalin were much lower, and required a more sensitive assay. We developed a GC/MS assay for amygdalin, based on the per TMS derivative of amygdalin and per TMS derivative of prunasin, which lacks the terminal glucose moiety of amygdalin, as internal standard. The electron impact mass spectrum of TMS amygdalin has been described elsewhere [9]. Consistent with that report, analysis in our laboratory demonstrated intense ions at mass/charge (m/e) 204.0 and 217.0. These were also major ions in the electron impact mass spectrum of TMS prunasin. To obtain necessary sensitivity for plasma analysis of amygdalin, we monitored ion 204.0 for TMS amygdalin. Ion 217.0 was monitored for determination of TMS prunasin. Figure 1A shows an ion chromatogram of a control plasma sample to which 750 ng of prunasin was added, and Fig. 1B an ion chromatogram of a control plasma sample to which 750 ng prunasin and 50 ng amygdalin were added.

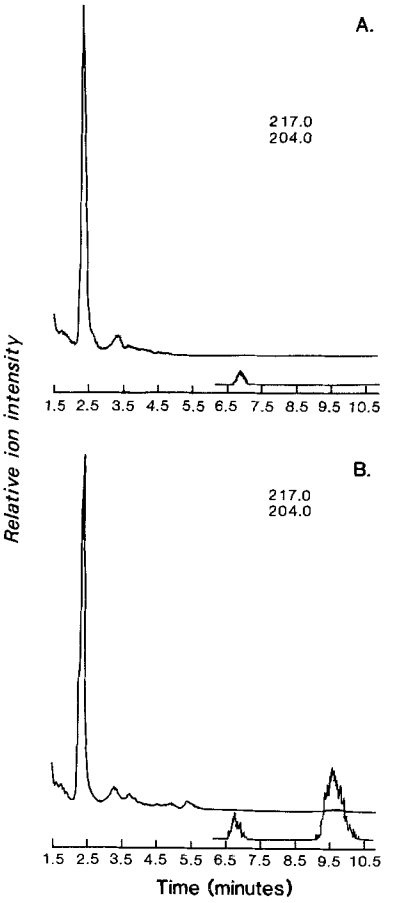

Fig. 1A and B. Selected ion-monitoring chromatograms of control human plasma samples following column isolation and derivatization to which 750 ng prunasin (**A**) or 750 ng prunasin and 50 ng amygdalin (**B**) was added

Isolation of parent drug was a major problem for analysis of amygdalin at the low concentrations encountered following oral administration of amygdalin. Because of their polar nature, amygdalin and prunasin cannot be isolated by solvent extraction. Protein precipitation with acetonitrile, acetone, or alcohol, or protein removal by membrane filtration or XAD column chromatography all resulted in low recoveries of amygdalin. Chromatography on Porapak Q provided protein removal with complete recovery of amygdalin at concentrations as low as 10 ng/ml plasma. Standard curves for amygdalin in human plasma to which prunasin (750 ng) was added as internal standard were linear ($r = 0.99$), and allowed detection at levels as low as 10 ng/ml plasma.

Plasma Concentrations of Amygdalin

Analysis of amygdalin concentrations in plasma following rapid IV administration (4.5 g/m^2) showed

peak values as high as 1,401 μg/ml. Figure 2 illustrates mean plasma concentrations ± standard error of the mean (+ SEM) over time for six patients receiving IV amygdalin on day 1 of their study. Three patients were sampled for 4 h following IV drug administration, and three patients for 24 h. No amygdalin was detected in any 24-h plasma samples. Data for the first three patients were best described by fitting a single exponential term to plasma concentrations from 15 min to 4 h. The mean plasma half-life (± SEM) was 97.3 ± 19.9 min. The more complete data from the second group of three patients were best described by a two-compartment open model. The mean plasma distribution phase half-life was 6.2 ± 2.1 min, the mean plasma elimination phase half-life was 120.3 ± 30.6 min, and the mean clearance 99.3 ± 10.4 ml/min. Three patients were sampled on day 7 as well as day 1 of IV amygdalin. There was no evidence of drug accumulation or change in the pattern of plasma elimination of amygdalin during the 7 days of treatment.

Plasma samples were obtained for 4 h following ingestion of the first of three daily 0.5-g amygdalin tablets. In marked contrast to plasma concentrations following IV administration of amygdalin, plasma concentrations after oral administration were very low, with peak levels less than 525 ng/ml (Fig. 3). Peak plasma concentrations occurred 30–60 min following administration of the tablet. Amygdalin was not detected in the plasma of a third patient after ingestion of the first tablet. These data were not subjected to pharmacokinetic analysis.

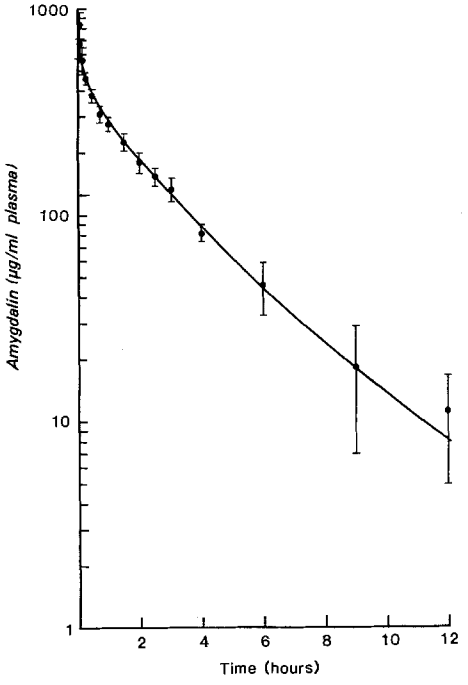

Fig. 2. Mean plasma concentrations of amygdalin in patients following IV administration of 4.5 g/m^2. Values are for six patients from 0 to 4 h, and for three patients for values beyond 4 h. *Error bars* represent ± standard error of the mean

Urinary Recovery of Amygdalin

Average 24-h urinary recoveries of parent drug during the 7-day course of IV treatment and during 4 days of oral therapy for three patients are shown in Fig. 4A. In contrast to relatively high recoveries following IV administration of drug (mean value 65.8%), recoveries were much lower following oral administration of amygdalin (mean value 19.1%) (Fig. 4B).

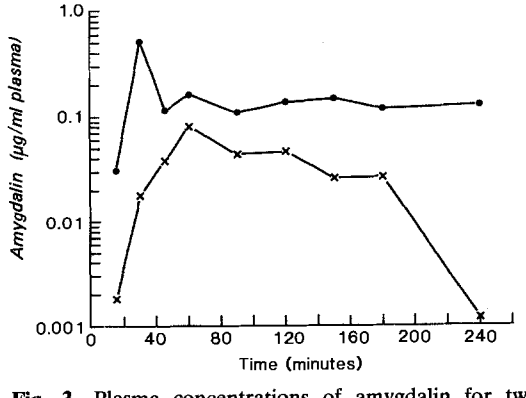

Fig. 3. Plasma concentrations of amygdalin for two patients following ingestion of the first of three daily 500-mg tablets

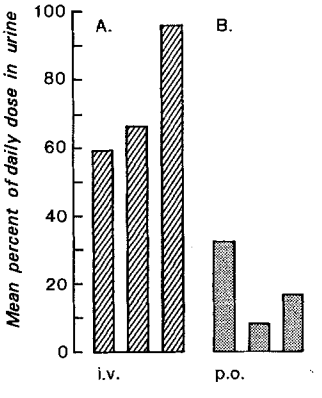

Fig. 4A and B. Mean daily recovery of amygdalin for three patients during (**A**) 7 days of IV (4.5 g/m^2) and (**B**) 4 days of oral (500 mg three times daily) amygdalin therapy

Whole-blood Cyanide and Serum Thiocyanate

Whole-blood samples were analyzed for cyanide in three patients following IV amygdalin. Mean concentrations of whole-blood cyanide for the group studied over 4 h on days 1, 3, and 7 of treatment were barely detectable (< 0.05–0.10 μg/ml) and did not change during the 14 days (data not shown). In contrast, whole-blood cyanide concentrations were markedly higher following oral administration of amygdalin. Figure 5 illustrates mean peak whole-blood cyanide concentrations ± SEM for three patients during their first 7 days of treatment. Peak concentrations (up to 2.1 μg/ml) were observed 30–120 min after administration of the tablet. The highest concentration in any patient always followed the first daily dose, with peak afternoon or evening concentrations usually less than 70% of that observed after the morning dose. Figure 5 (inset) also illustrates the wide variation of cyanide concentrations observed during the first 2 h compared with more consistent values after the first 2 h (decrease in SEM). Similar cyanide data were observed when patients started on IV amygdalin began taking amygdalin PO. When patients changed from oral to IV amygdalin, whole-blood cyanide concentrations decreased to near-detection limits within 1 day of the change (data not shown).

Two patients receiving oral amygdalin were also given 1 oz raw almonds at each meal following an initial 4-day period of observation during the standard amygdalin regimen. The first patient complained of light-headedness, weakness in the limbs, nausea and vomiting, and headache lasting 90 min [20]. This episode corresponded with a whole-blood cyanide concentration of 2.1 μg/ml, compared with a peak cyanide concentration of 0.7 μg/ml in the absence of almonds. Arterial blood gases were normal. Amygdalin and almonds were withdrawn, and the whole-blood cyanide concentration declined over 36 h to the detection limit. The second patient complained of gastrointestinal discomfort related to pre-existent diarrhea following ingestion of the initial dose of almonds, and refused to take additional doses. Peak cyanide concentration in the absence of almonds was 0.5 μg/ml, compared with 0.9 μg/ml after ingestion of the almonds.

Serum thiocyanate concentrations did not increase over the pretreatment values after IV administration of amygdalin (data not shown). Figure 6 summarizes data for serum thiocyanate in patients after oral administration of amygdalin. Peak thiocyanate concentrations (20–38 μg/ml) were not observed until 3–5 days after the initiation of therapy, and did not decline until 5–7 days after cessation of oral amygdalin therapy. At that time, serum thiocyanate concentrations declined slowly to pretreatment values (2–5 μg/ml). The mean half-life of thiocyanate clearance in the six patients studied was 6 days, with a range of 4–9 days.

Urinary Concentrations of Cyanide and Thiocyanate

Cyanide was not detected in urine samples collected from patients who had received amygdalin PO or IV. Urinary thiocyanate concentrations did not change significantly following IV administration of amygdalin. Marked increases in serum thiocyanate concentrations following oral administration of amygdalin were accompanied by small but statistically significant increases in urinary thiocyanate concentrations. The mean pretreatment urinary thiocyanate concentrations for three patients was 14.5 ± 2.0 mg/24 h. A peak concentration of 37.4 ± 14.9 mg/24 h was observed on day 6 of treatment, and the concentration remained constant until cessation of treatment.

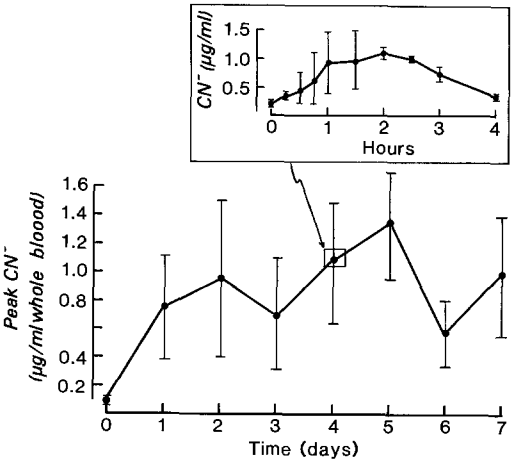

Fig. 5. Mean daily whole blood peak cyanide cocentrations in three patients during oral amygdalin (500 mg three times daily) therapy. The *insert* represents mean concentrations observed during the 4-h period following ingestion of the first tablet on day 4 of the study. *Error bars* represent ± standard error of the mean

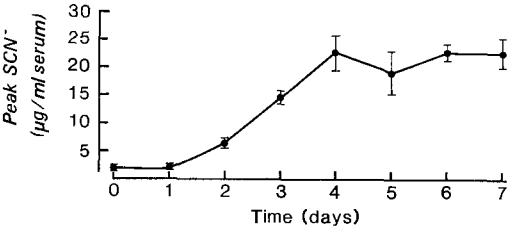

Fig. 6. Mean daily serum peak thiocyanate concentrations in three patients during oral amygdalin (500 mg three times daily) therapy. *Error bars* represent ± standard error of the mean

Clinical Observations

With exception of the single patient described above who had transient symptoms of cyanide toxicity after challenge with raw almonds, we observed no symptomatic toxicity that could be attributed to amygdalin [20].

Discussion

In an earlier study in two patients, we reported that amygdalin was present at high concentrations in plasma following IM injection, and that parent drug was not detectable in plasma following oral administration [1]. To determine concentrations of amygdalin in plasma following oral administration, it was necessary to develop new methodology. Methods published for qualitative analysis of amygdalin, including flame ionization gas chromatographic determination of derivatized amygdalin [5, 11, 14], determination of benzaldehyde following hydrolysis of amygdalin [1, 10, 23], and specific enzyme electrode assays [18], have limited sensitivity and/or specificity. HPLC and GC/MS methods [5] were not developed for analysis in biological samples. In our laboratory HPLC analysis provided acceptable chromatographic separation of amygdalin and prunasin from other plasma components. However, quantitation based on ultraviolet absorption was inadequate. Derivatization of amygdalin with fluorinated acylating reagents, previously employed for qualitative studies [5] was unsatisfactory for measurement of the low concentrations encountered in our studies. Hydrolysis of the acylated derivative after removal or dilution of the acylating reagent led to variable results.

Maximum sensitivity and specificity for amygdalin determination in plasma was achieved by means of a selected ion monitoring GC/MS assay of the per trimethylsilyl amygdalin derivative. The ions selected for monitoring (204.0, 217.0) are major ions in the electron impact mass spectra of both TMS prunasin and TMS amygdalin. They are associated with the derivatized glucose moiety and are frequently observed as major ions in electron impact mass spectra of TMS sugars [19]. Only by use of the base ion (m/e 204.0) could adequate sensitivity be achieved for determination of amygdalin in plasma following oral administration. Higher-mass ions are preferred for selectivity, but the long retention time of TMS amygdalin (approximately 9 min) provided separation from plasma consituents having ion intensity at m/e 204.0.

Following IV administration of amygdalin, plasma concentrations were very high. Cyanide was barely detectable in whole blood and thiocyanate concentrations did not increase. Our studies suggest that hydrolysis of amygdalin to cyanide did not occur even with extremely high plasma concentrations of the drug. While β-glucosidases have been reported in human red blood cell membranes [2] and several tissues [4], they are not thought to occur in appreciable concentrations in mammalian cells. Disposition of amygdalin following IV administration was similar in all patients, and there was no indication of accumulation of parent drug in plasma throughout the 7-day treatment course.

Following oral administration of amygdalin, plasma concentrations and urinary recovery of parent drug were markedly lower than those observed following IV administration. While these findings suggest that absorption may be inefficient, studies with radiolabeled drugs would be required to confirm this hypothesis. Significant concentrations of cyanide were observed in whole blood. The presence of cyanide in blood demonstrates metabolism of parent drug, probably occurring in the gut by microorganisms. Extensive metabolism of amygdalin by gut microflora has been reported in rats [6]. Analysis of serum thiocyanate did not correlate with clinical toxicity. This finding is consistent with previous data [25] suggesting that thiocyanate is rapidly distributed into the tissue anion pool. Peak concentrations of thiocyanate lagged behind peak cyanide concentrations by several days, making this test of little value in documenting acute cyanide toxicity secondary to oral amygdalin administration. As expected, higher concentrations of cyanide were observed after the ingestion of 1-oz doses of raw almonds (a known source of β-glucosidases) than with oral amygdalin alone. In one case this was accompanied by mild clinical symptoms consistent with cyanide toxicity and a whole-blood cyanide concentration consistent with cyanide toxicity [24]. Cessation of almonds and/or amygdalin administration resulted in prompt decreases of whole-blood cyanide. No serious acute toxicity has been encountered in an additional 50–60 patients receiving IV and oral amygdalin.

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