NOTES

High-dose ascorbic acid decreases detoxification of cyanide derived from amygdalin (laetrile): studies in guinea pigs[1]

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Cysteine, a sulphur-containing amino acid, is required to metabolize ascorbic acid (as ascorbate sulphate) and detoxify cyanide (to thiocyanate). In guinea pigs, conjoint use of laetrile (a cyanogenic glycoside) and ascorbic acid (in large doses) decreases the detoxification of cyanide derived from laetrile through diminishing the availability of cysteine, but not impairing hepatic rhodanese activity, which is involved in the detoxification of cyanide to thiocyanate. These results agree with the symptoms of a sublethal dose of KCN toxicity manifested by the animals. The studies, therefore, indicate that individuals taking megadoses of ascorbic acid concurrently with laetrile may be subject to self-poisoning.

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La cystéine, un aminoacide contenant du soufre, est nécessaire pour métaboliser l'acide ascorbique (tel que le sulfate d'ascorbate) et pour détoxiquer le cyanure (en thiocyanate). Dans de cobayes, l'utilisation simultanée de laetrile (un glucoside cyanogène) et d'acide ascorbique (à doses élevées) diminue la détoxication de cyanure dérivé du laetrile, en diminuant la disponibilité de la cystéine, mais non pas en altérant l'activité de rhodanèse hépatique qui est impliquée dans la détoxication du cyanure en thiocyanate. Ces résultats concordent avec les symptômes de toxicité d'une dose subléthale de KCN manifestés par les animaux. Par conséquent, les études indiquent que l'absorption simultanée de mégadoses d'acide ascorbique et de laetrile peut provoquer l'empoisonnement.

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Introduction

In recent years there have been a number of reports claiming that either laetrile (amygdalin), a cyanide-containing compound (Brown et al. 1960; Krebs 1970) or ascorbic acid in large doses (Cameron and Campbell 1974; Cameron and Pauling 1978), may have beneficial effects in malignant disease. As a consequence of these claims, these agents are taken by many individuals, and often in combination. Laetrile has recently been legalized as a drug in several states of the United States. In attempts to bypass any legal setbacks its proponents have tried to establish laetrile as the vitamin B_{17}, so that it may be made freely available. Although in Canada the use of laetrile is illegal, there is an easy access to laetrile-containing sources (Herbert 1979) such as kernels of apricot pits, peach pits, and other stone fruits, as well as a variety of nuts like almonds and macademia, which are available in many health food stores.

Hydrolysis of laetrile releases cyanide, benzaldehyde, and glucose, and this can be achieved by treatment with dilute acids and also by specific β-glucosidases present in cyanophoric plants and in the small intestine. The cyanide released from laetrile enters the small metabolically active pool of cyanide present in the body which is otherwise derived from both diet and tobacco smoke (Wilson and Langman 1966). Cyanide is mainly detoxicated by two enzymatic pathways in the liver, gut, and kidneys (Boxer and Rickards 1952; Osuntokun 1968). It reacts with both 3-mercaptopyruvate which is derived from cysteine, and thiosulphate, derived from 3-mercaptopyruvate as shown below:

[1] $CN^- + \text{3-mercaptopyruvate} \xrightarrow{\text{sulfur transferase}} SCN^- + \text{pyruvate}$

[2] $S_2O_3^{2-} + CN^- \xrightarrow{\text{rhodanese}} SO_3^{2-} + SCN^-$

The detoxification of cyanide may also occur via the conjugation of cyanide with cysteine resulting in the formation of 2-iminothiazolidine-4-carboxylic acid.

One study (Basu 1977) has revealed that the detoxification of cyanide to thiocyanate is impaired in healthy subjects following administration of ascorbic acid (3 g) daily for 1–5 weeks and that the effect is reversed by concomitant administration of 10 mg of cysteine. Furthermore, *in vitro* studies have shown that

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ascorbic acid causes a significant increase in the cyanide release of blood (Baker and Herbert 1979). Ascorbic acid is, in part, metabolized to ascorbic acid sulphate, where cysteine is believed to be involved as a sulphate donor (Baker et al. 1971). It is, therefore, possible that concomitant intake of laetrile and ascorbate in large doses, may further potentiate the toxicity of cyanide derived from laetrile by competing for the cysteine.

The present study was undertaken to investigate the *in vivo* effects of megadoses of ascorbic acid on the metabolism of cyanide derived from laetrile and the toxicity of KCN in guinea pigs, which like humans, do not synthesize ascorbic acid.

Animals and methods

Animals

Duncan–Hartley guinea pigs weighing 200–250 g were used throughout and maintained on a stock pellet diet (Wayne, Canada) and water *ad libitum*. The animals were divided into three groups: group A was given 10% sucrose solution and served as controls, groups B and C were treated with 10 mg of laetrile (amygdalin) obtained from Sigma Chemical Co. (St. Louis, MO) and in combination with ascorbic acid (100 mg) daily for 24 days, respectively. Ascorbate and laetrile were dissolved in 10% sucrose solution, and both the agents as well as the sucrose solution in control animals were given orally. All animals were kept in metabolic cages individually. Twenty-four-hour urine collections were carried out on days 4, 16, and 24. On day 24 the animals were anesthetized with chloroform and then killed by cervical fracture. The livers were quickly removed, excised, weighed, and placed in plastic beakers on ice.

A further experiment was carried out consisting of three groups of animals of at least eight in each group. Group D was given orally ascorbic acid (300 mg) alone, and group E was given ascorbic acid (300 mg) plus cysteine (10 mg) daily for 3 successive days. Both ascorbate and cysteine were prepared in 10% sucrose solution. Group F was administered orally 10% sucrose solution, and used as controls. Twenty-four hours later, they were given an oral dose of KCN at a concentration of 8 mg/kg body weight following overnight fasting. The rationale for choosing this level was based on the observation that the lethal dose of KCN for this strain of guinea pig was estimated to be 10 mg/kg, while 8 mg was nonlethal.

Methods

Thiocyanate was measured in urine following the method described by Pettigrew and Fell (1972), and cysteine in urine was determined by the method of Gaitonde (1967). All urinary values were expressed per unit of urinary creatinine. Ascorbic acid does not interfere with the determinations of either cysteine or thiocyanate (Basu 1977).

Rhodanese activity in the liver was determined according to the method of Cyril Vesey, St. Bart's Hospital, London (C. Vesey, unpublished observations). The procedure involved homogenization of 200 mg of liver in 10 mL 0.0125 *M* sodium thiosulphate at 0°C. The incubation mixture consisted of the homogenate (0.5 mL), 0.125 *M* sodium thiosulphate (5.0 mL), Tris–HCl buffer (5.75 mL), pH 8.3, and 9.5 *M* potassium cyanide (1.25 mL). Following the transfer of four 2.0 mL aliquots of the incubated mixture in tubes containing 40% formaldehyde (0.2 mL), at 30-sec intervals, thiocyanate concentrations were determined. The total liver protein was measured according to the method of Lowry et al. (1951).

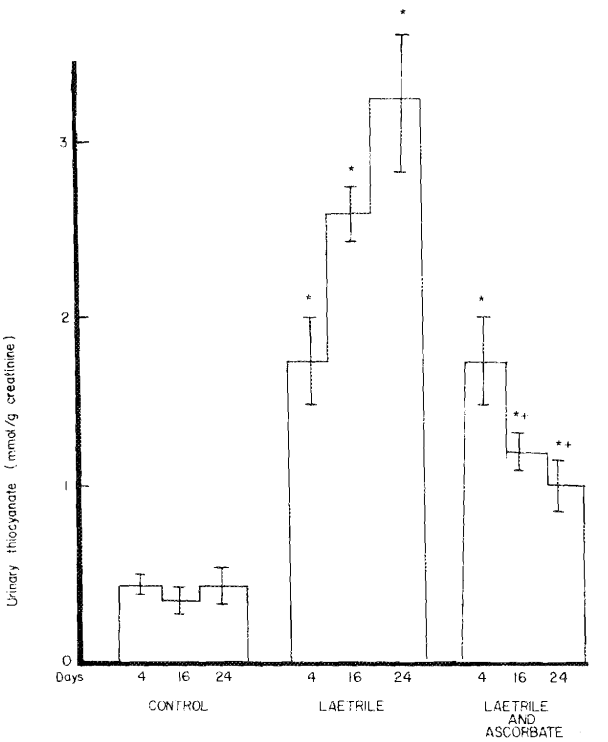

FIG. 1. Effect of megadoses of ascorbic acid on the urinary levels of thiocyanate in guinea pigs treated with laetrile. Each bar represents SEM ± with at least 10 animals. Controls versus laetrile or laetrile + ascorbate (*, $P < 0.001$), laetrile versus laetrile + ascorbate (+, $P < 0.001$).

Results

Daily oral administration of laetrile (10 mg) without or with ascorbic acid (100 mg) to guinea pigs over a period of 24 days had no significant effect on body weight and liver weight. However, treatment with laetrile alone for 4, 16, and 24 days, respectively, resulted in a significant increase in urinary excretory levels of thiocyanate, the detoxicated product of cyanide (Fig. 1). The increase was found to be progressive over the period of study. In animals treated with laetrile in combination with ascorbic acid, the urinary thiocyanate level was also found to be significantly increased at all periods when compared with the respective control groups, but the increase was less marked following 16 days than found in the laetrile group, indicating the diminishing effect of ascorbate on the detoxification of cyanide.

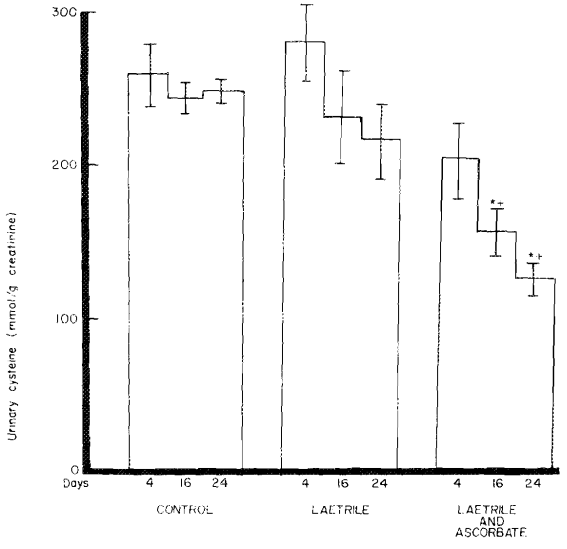

FIG. 2. Effect of megadoses of ascorbic acid on the urinary levels of cysteine in guinea pigs treated with laetrile. Each bar represents SEM ± with at least 10 animals. Control versus laetrile and ascorbate (*, $P < 0.001$), laetrile versus laetrile and ascorbate (+, $P < 0.01$).

The treatment with laetrile alone did not appear to have any effect on the cysteine excretion, but in the presence of ascorbate the amino acid excretion level was significantly decreased at 16 and 24 days (Fig. 2). The hepatic concentration of rhodanese, the principle enzyme responsible for the detoxification of cyanide, was measured in animals following administrations of laetrile either alone or in combination with ascorbate daily for 24 days. Table 1 shows that laetrile both with and without ascorbate resulted in a significantly increased rhodanese activity when compared with that of the control animals. There, however, appeared to be no difference in rhodanese activity between the treated groups.

Table 2 lists the toxic effects of KCN at 8 mg/kg in guinea pigs. Only three out of eight control animals exhibited signs of mild tremor in response to KCN; the effect disappeared within 15 min. Pretreatment with ascorbate (300 mg/day) for 3 successive days, however, exacerbated the CN toxicity. Thus all eight animals showed severe signs which included tremor, ataxia, and paralysis followed by convulsions. These effects were somewhat modified when the animals were treated with ascorbate (300 mg) concomitantly with cysteine (10 mg) daily for 3 successive days. Three out of nine of these animals remained unaffected, one showed a mild tremor followed by recovery within 15 min but the remaining five showed symptoms similar to the "ascorbate" group.

All the symptoms described in Table 2 were noted within 30 min of KCN administration. At the onset of convulsions the animals were sacrificed using chloroform anesthesia.

TABLE 1. Effect of conjoint use of laetrile and megadoses of ascorbate on the hepatic concentrations of rhodanese and total protein in guinea pigs

Groups	Total protein (mg/g liver)	Rhodanese (mg/g liver)
Control	131±9	3.16±0.37
Laetrile	140±4	4.74±0.26*
Laetrile and ascorbate	141±6	4.58±0.21*

NOTE: Guinea pigs were administered laetrile (10 mg) and laetrile plus ascorbate (100 mg) orally daily for 24 days. Each value is the mean for at least 10 animals with ± SE.

*Difference between control and treated groups, statistically significant ($P < 0.05$).

Discussion

Despite the claims that cyanide may be useful in cancer chemotherapy (Brown et al. 1960; Krebs 1970; Zeitlin 1979), others have failed to observe any significant effect of laetrile on experimental tumors (Stock et al. 1978). Thus, many do no support the use of laetrile and are calling for extensive Food and Drug Administration trials to disprove all claims (Holden 1976; Newell 1978; Greenberg 1980). However, despite the lack of objective evidence to support the use of laetrile in cancer therapy, many patients are taking laetrile either alone or in combination with other forms of treatment. Several cases of both fatal and nonfatal laetrile poisoning in humans have been reported (Smith et al. 1977; Humbert et al. 1977).

Cyanide derived from laetrile is detoxified to thiocyanate, requiring cysteine as the sulphate donor (Osuntokun 1968; Basu 1977). Sulphate formation is also an important pathway in the metabolism of ascorbic acid to ascorbic acid-sulphate, the sulphate being derived from cysteine (Baker et al. 1971). The daily oral administration of 3 g of ascorbic acid for five weeks to healthy subjects has been found to decrease the urinary levels of both cysteine and thiocyanate to 50% of preascorbate levels (Basu 1977).

The basis of the present study was the concern that the conjoint use of megadoses of ascorbic acid and laetrile, may increase the amount of cyanide released *in vivo*, thereby posing the risk of cyanide poisoning in humans. The present study has indicated that doses of laetrile and ascorbic acid in guinea pigs equivalent to those taken by man, would affect the detoxification of cyanide derived from laetrile. The synergistic diminishing effect of the agents on the urinary levels of cysteine and thiocyanate, appears to be due to monopolization of cysteine and thiocyanate by ascorbate and laetrile, rather than the deficiency of rhodanese activity

TABLE 2. Effect of ascorbate with or without cysteine on the cyanide (8 mg/kg) toxicity in guinea pigs

Groups	Total no.	Observed toxic effects of KCN
Control (10% sucrose, 1 mL)	8	5/8: no apparent symptoms 3/8: slight tremor, complete recovery within 15 min
Ascorbate (300 mg in 10% sucrose, 1 mL)	8	8/8: severe tremor, motor ataxia, bizarre neuromuscular manifestations, rhythmic head movements, paralysis, convulsions
Ascorbate (300 mg) + cysteine (10 mg) (in 10% sucrose, 1 mL)	9	3/9: no apparent symptoms 1/9: slight tremors, complete recovery within 15 min 5/9: severe symptoms as described for the "ascorbate" group

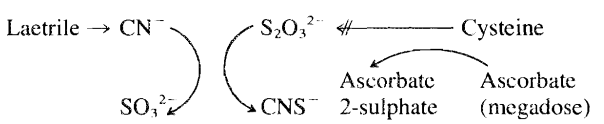

FIG. 3. Possible interaction between ascorbate and laetrile.

in the liver, which is the principal enzyme for the detoxification of cyanide to thiocyanate (Fig. 3). These results are further substantiated by the clinical manifestations of sublethal doses of KCN. Unlike controls, all ascorbate-treated animals manifested severe symptoms of CN poisoning, while the presence of cysteine counteracted such toxic effects in four out of nine animals. Although the remaining animals showed symptoms as severe as the "ascorbate only" group, it must be realized that the animals were preexposed to cysteine with ascorbate only for 3 days. It is possible that a longer exposure to cysteine may result in further alleviating the symptoms of CN poisoning.

It appears, therefore, that the use of ascorbic acid in large doses may exacerbate the laetrile-mediated cyanide burden in the body. Subjects taking megadoses of ascorbic acid to prevent or cure cancer are expected to be at increased risk of experiencing the adverse side effects from laetrile.

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Cardiovascular effects of cadmium on intravenous and intracerebroventricular administration in rats[1]

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Cardiovascular responses to the intravenous (i.v.) and the intracerebroventricular (i.c.v.) administration of cadmium acetate were evaluated in rats anaesthetized with urethane. Cadmium acetate (1 mg/kg i.v.) caused an initial fall followed by a persistent rise in blood pressure. Cadmium acetate (1 μg i.c.v.) produced a more marked hypertensive effect. In the spinal-transected rat, the effect of intravenous cadmium was reduced but the effect of intraventricularly administered cadmium was completely abolished. It is, therefore, suggested that both central and peripheral mechanisms are involved in the pressor response to cadmium exposure.

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On a évalué, chez des rats anesthésiés à l'uréthane, les réponses cardiovasculaires à l'administration intraveineuse (i.v.) et intracérébroventriculaire (i.c.v.) d'acétate de cadmium. L'administration i.v. d'acétate de cadmium (1 mg/kg) provoqua une chute initiale suivie d'une augmentation continue de la pression sanguine. L'administration i.c.v. d'acétate de cadmium (1 μg) induisit un effet hypertensif plus marqué. Chez le rat ayant subi une section spino-transversale, l'effet du cadmium intraveineux fut réduit alors que l'effet du cadmium intracérébroventriculaire fut totalement aboli. Par conséquent, on suggère que des mécanismes tant centraux que périphiques sont impliqués dans la réponse pressive à l'exposition aux cadmium.
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Introduction

The effect of cadmium on the blood pressure of the rat has been described by Schroeder and Venton (1962). The mechanism of this hypertensive effect has not been elucidated, except for the explanation put forward by Puri and Kapoor (1981), that chronic administration of cadmium acetate might cause an increase in plasma catecholamine levels. It is still not clear whether the action of cadmium acetate is peripheral or central. This has prompted us to undertake the present investigation to delineate the mechanism of action of cadmium ions.

Method

Experiments were conducted on male Charles Foster strain rats weighing 200–250 g which were bred at the Central Drug Research Institute and maintained on a standard diet. Rats were anaesthetized by urethane (0.6 mL/100 g body weight of 25% solution) given intraperitoneally. Systemic blood pressure was recorded on a Grass Polygraph (model 7) by connecting the carotid artery to a pressure transducer with a polyethylene tube. The jugular vein was cannulated for injection of various solutions. Heart rate was counted from the blood pressure pulses by increasing the paper speed. The i.c.v. injection was given as described by Noble et al. (1967). High spinal transection was done at the atlantooccipital level according to the method of Burn (1952). Cadmium acetate solution was prepared in triple distilled water. The pH of the normal saline, cadmium acetate, and sodium acetate were

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