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Laboratory Report

Hydroxocobalamin Therapy of Cyanide Intoxication in Guinea Pigs

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The efficacy of hydroxocobalamin (vitamin B12a) as a specific, nontoxic antidote in acute cyanide poisoning was tested. Guinea pigs receiving lethal intravenous NaCN injections were treated with either vitamin B12a or saline solution. There was a statistically significant antidotal effect of the vitamin. No toxic effect was observed with large doses of the vitamin. (Key words: Toxicity, cyanide; Biotransformation, nitroprusside; Anesthetic techniques, hypotensive, nitroprusside; Pharmacology, hydroxocobalamin.)

THE INCREASING CLINICAL USE of the valuable hypotensive agent sodium nitroprusside raises the possibility of patients' receiving toxic doses of cyanide. Nitroprusside is metabolized to free cyanide and, if used in large amounts, may have lethal effects.[1–4]

The mechanism of cyanide poisoning is through the binding of the ferric (Fe^{+3}) iron in the cytochrome oxidase system, blocking the electron transport chain. Death results through the rapid inhibition of cellular respiration throughout the body unless the cyanide is promptly released from the cytochrome oxidase.

Currently recommended treatment involves the *in-vivo* formation of methemoglobin.[5] This is not a benign intervention, however, since neither it nor cyanmethemoglobin is capable of reversibly combining with oxygen. Overzealous treatment may merely convert a cytotoxic hypoxia to an anemic hypoxia.

Previous investigations[6,7] into the use of hydroxocobalamin (vitamin B_{12a}) as a specific nontoxic antidote for cyanide poisoning have been suggestive of antidotal activity but were not definitive. For this reason, we have given uniformly lethal intravenous cyanide injections to guinea pigs, treated with either vitamin B_{12a} or saline solution, and determined a significant antidotal activity of the vitamin. We also administered very large doses of vitamin B_{12a}, which appear to be nontoxic even in the absence of cyanide.

Methods

EXPERIMENT I (ANESTHETIZED ANIMALS, POST-CYANIDE TREATMENT)

Twelve guinea pigs weighing between 368 and 704 g were anesthetized with intraperitoneal dialurethane§ (0.6 ml/kg). A jugular vein was cannulated with polyethylene tubing (P.E. 10). Freshly prepared 0.0408 M sodium cyanide (2.0 mg/ml) in isotonic saline solution was injected intravenously on the basis

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§ Dial-urethane: diallylbarbituric acid crystals, 100 mg; monoethyl urea, 400 mg; urethane, 400 mg; disodium calcium EDTA, 0.5 mg; purified water, q.s. to 1,000 ml.

of 4 mg/kg. Immediately following the injection of sodium cyanide (NaCN), and in a randomized order, six of the animals were given 0.0408 M vitamin B_{12a} (54.9 mg/ml) in isotonic saline solution in a ratio of 2 ml B_{12a} to 1 ml NaCN; the remaining six animals received isotonic saline solution in a ratio of 2 ml saline solution to 1 ml NaCN. Total volume injected in all animals was 6 ml/kg. The polyethylene tubing was removed, the skin incision closed, and the animals returned to their cages for observation. Death was confirmed by an isoelectric ECG recording.

Experiment II (Unanesthetized Animals, Pre-cyanide Treatment)

Twelve guinea pigs weighing between 479 and 696 g were anesthetized with diethyl ether, and a cannula filled with heparinized saline solution was placed in a jugular vein. The cannula was brought out the posterior aspect of the neck through a subcutaneous tunnel, sealed, and placed in a protective sleeve sewn to the animal's back. The animals were allowed to recover from anesthesia and, when awake and active, were divided randomly into two groups. Six of the animals received vitamin B_{12a} followed immediately by NaCN in the same dose schedule as in Experiment I; the second group of six animals received saline solution followed immediately by NaCN, also in the same dose schedule as in Experiment I. The intravenous tubing was resealed, replaced in its protective sleeve, and the animals returned to their cages for observation. Death again was confirmed by ECG recording.

Statistical analysis of the results of Experiments I and II was by the fourfold table test of the hypergeometric distribution.[8]

Experiment III (Vitamin B12a Toxicity)

Eight randomly selected guinea pigs weighing between 382 and 625 g were prepared as in Experiment II and allowed to recover from anesthesia. Four guinea pigs received intravenous injections of vitamin B_{12a} only, in the same concentration and dose as in Experiments I and II. The remaining animals were given 1 g/kg intravenous injections of 100 mg/ml vitamin B_{12a} in isotonic saline solution. All animals were observed for 72 hours.

Results

Experiment I

All guinea pigs were anesthetized at the time of injection and had normal, regular respirations. The administration of NaCN promptly produced irregular and gasping respirations in all 12 animals. The six control animals receiving saline solution rapidly became apneic, and all had electrocardiographic confirmation of death within 30 minutes. The six animals receiving vitamin B_{12a} all resumed regular respirations within 3 minutes, recovered from anesthesia within 36 hours, and appeared healthy and normal at that time. Observation for an additional 36 hours revealed apparently normal and active guinea pigs. Analysis of these data revealed a statistically significant difference between the two groups, $P \leq .005$.

Experiment II

All guinea pigs were awake and active at the time of injection. There was no noticeable alteration in any animal's behavior during the injection of either saline solution or vitamin B_{12a}. During the NaCN injection, all 12 animals had rapid onset of tonic–clonic seizure activity, with gasping irregular respirations. In six control animals, pretreated with saline solution, and one of the vitamin B_{12a}-treated animals, regular respiration never resumed; apnea occurred and was promptly followed by death. In five of the six B_{12a}-pretreated animals, regular respiration resumed within 3 minutes, and the animals were walking about within 10 minutes. These "recovery" animals appeared normal and healthy that day and during a further 48-hour observation period. Analysis of these data revealed a statistically significant difference between the two groups, $P \leq 0.01$.

Experiment III

The eight animals receiving only vitamin B_{12a} showed no reaction to the injection of the drug and appeared normal and healthy that day and during a further 48-hour observation period.

Discussion

Classic methods of treatment for cyanide intoxication center about the formation of methemoglobin by the inhalation of amyl nitrite, followed by the intravenous injection of sodium nitrite. The methemoglobin thus produced combines with cyanide and reverses the inhibition of the cytochrome oxidase system. Thiosulfate is then administered to facilitate conversion of cyanide to nontoxic thiocyanate.[9] While this regime is effective, it is not of itself nontoxic. Berlin[10] reported the case of a child who, having ingested a sublethal dose of cyanide, was treated with adult doses of sodium nitrite. This child died after having received sufficient nitrite to convert more than 90 per cent of his hemoglobin to methemoglobin.

Hydroxocobalamin (vitamin B_{12a}) is a water-soluble molecule containing a cobalt-coordinated hydroxyl group. Kaczka *et al.*[11] have demonstrated the *in-vitro* reaction of hydroxocobalamin with cyanide, resulting in the replacement of the hydroxyl group by a cyano group, with the subsequent formation of cyanocobalamin. Mushett *et al.*[6] suggested a protective effect of vitamin B_{12a} given intravenously and tested it as an antidote to intraperitoneal potassium cyanide injected into mice. Uniformly lethal doses of cyanide were not used, however, and the longest period of observation reported was only two and a half hours after injection. Intravenous administration of sodium cyanide was tested by Friedburg, Grutzmacher and Lendel,[7] who demonstrated 1) an increase in the lethal dose of sodium cyanide in guinea pigs pretreated with vitamin B_{12a}; 2) a temporary return of normal respiration and ECG activity in guinea pigs given vitamin B_{12a} during a NaCN infusion that had produced gasping and signs of myocardial ischemia; 3) the return of phrenic nerve potentials when vitamin B_{12a} was injected during apnea produced by cyanide. Besides these reports, which suggest the efficacy of vitamin B_{12a} in the treatment of cyanide poisoning, the question of toxicity of cyanocobalamin (vitamin B_{12}) and its tightly bound cyanide was tested by giving as much as 1,600 mg/kg to mice as a single intravenous injection.[12] This dose contains several times the lethal dose of cyanide, yet when given as vitamin B_{12}, was completely nontoxic.

Three successful adult resuscitations from cyanide intoxication using vitamin B_{12a} have been reported.[13] These patients received 5-g intravenous injections of the vitamin, in conjunction with cobalt EDTA (Kelocyanor), and other general supportive measures. We are unaware of any case in which a larger dose of vitamin B_{12a} was used as the sole antidote in human cyanide poisoning.

It is interesting that despite pretreatment with vitamin B_{12a} in Experiment II, five of the animals required as long as 3 minutes before regular respirations resumed after injection of the cyanide. Also, one of the six died very rapidly. Thus, it would appear that, although a double molar quantity of vitamin B_{12a} was administered, the blood level was not high enough to prevent some signs and symptoms from cyanide poisoning. This may indicate a rapid redistribution, biotransformation, and/or excretion of the drug; higher doses may be required as a pretreatment for this type of acute intoxication. Further investigations are necessary to determine antidotal activity during more gradual cyanide infusions.

Although cyanide intoxication is relatively rare and usually is associated with suicide or accidental exposures, the number of cases may increase because of mounting interest in the use of sodium nitroprusside as a treatment drug for acute hypertensive crises,[14,15] acute myocardial infarction,[16] congestive heart failure,[17–19] and the production of elective hypotension during anesthesia.[20–22] The nitroprusside molecule has five CN^- groups, and free cyanide production has been demonstrated[4] *in vitro* and *in vivo*. Statistically significant increases in plasma cyanide levels have been shown following nitroprusside infusions, and a decrease in erythrocytic cyanide in one patient followed the intravenous injection of only 5 mg of vitamin B_{12a}.[23] McDowall *et al.*[24] reported a 50 per cent incidence of tachyphylaxis and progressive metabolic acidosis in baboons infused with nitroprusside. None of the animals requiring considerably higher infusion rates of nitroprusside to maintain a given level of hypotension recovered when the nitroprusside was discontinued. While blood cyanide levels were not determined by these investigators, the pattern of progressive deterioration and

death of the animals is quite compatible with cyanide intoxication. Two recent reports[25,26] of deaths following the infusion of sodium nitroprusside in healthy adult surgical patients undergoing hypotensive anesthesia are also highly suggestive of cyanide intoxication.

If further studies reveal a lack of toxicity for vitamin B_{12a}, it could be administered prophylactically to patients receiving nitroprusside. In cases where cyanide poisoning is suspect but not confirmed, hydroxocobalamin could be used intravenously in large doses without fear of serious complication.

Conclusions

Our results clearly confirm the ability of hydroxocobalamin to reverse lethal cyanide poisoning in guinea pigs dramatically, and suggest that antidotal doses of the vitamin are nontoxic.

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