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# RAPID COMMUNICATIONS

## PRODRUGS OF CYANAMIDE AS (LONG-ACTING) ALCOHOL DETERRENT AGENTS

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Cyanamide ($H_2NC{\equiv}N$), a potent aldehyde dehydrogenase (AlDH) inhibitor, is used therapeutically in Europe, Canada and Japan as an alcohol deterrent agent. It is available commercially (but not in the United States) as a citrated form of its calcium salt (Temposil, Dipsan, Abstem) (1), or as formulated aqueous solutions (2). The insoluble calcium salt is hydrolyzed in the stomach by the acidic pH aided by the calcium ion sequestering citrate, and free cyanamide is rapidly absorbed via the gastrointestinal tract (3). Cyanamide itself does not inhibit AlDH, but must be enzymatically activated by catalase to an as yet unidentified active metabolite (4).

Unlike disulfiram (DS), cyanamide has a short duration of pharmacologic activity lasting approximately 24 hr (5). This is due to its facile conversion *in vivo* to an acetylated derivative, viz. acetylcyanamide (AC), which is rapidly excreted in the urine (6). At least 94% of the administered cyanamide is eliminated within 6 hr via this route by the rat (7). Like cyanamide, AC is devoid of AlDH inhibitory activity *in vitro* (7,8).

We have now found that AC shows some DS-like activity when administered to rats due to a small, but finite, amount of deacetylation that takes place *in vivo*. Based on this result, we prepared cyanamide derivatives that had been acylated with other fatty acyl, α-aminoacyl (amino acid) or peptidyl groups as prodrug forms of the parent drug. We wish to report that these acyl and peptidyl prodrugs of cyanamide are even more effective than the parent cyanamide in elevating ethanol-derived blood acetaldehyde when administered to rats, and that this DS-like effect can be prolonged up to 72 hr with the more lipophilic derivatives.

## MATERIALS AND METHODS

Cyanamide prodrugs. The synthetic procedures for the preparation of benzoylcyanamide, palmitoylcyanamide, stearoylcyanamide, and N-benzoyl-L-leucylcyanamide will be described in detail elsewhere. The sodium salt of [1-$^{14}$C]AC was prepared by the acetylation of sodium cyanamide (192 mg, 3.0 mmoles) with [1-$^{14}$C]acetyl chloride (1.4 mg, 1.4 mmoles, New England Nuclear Corp., Boston, MA) diluted with unlabeled acetyl chloride (77.1 mg, 0.080 mmole) in 10 ml of acetonitrile at an ice bath temperature over 18 hr. Workup followed the previously described procedure for the unlabeled compound (6). The product was crystallized from absolute ethanol/diethyl ether to give 38 mg (36% yield) of a white solid which was slightly contaminated with sodium cyanamide as shown by TLC. A 10 mg sample was therefore purified by preparative TLC on Silica gel GF (1000 μm, Analtech, Inc., Newark, DE) and developed with chloroform:methanol:2.5 N $NH_3$ (200:100:15). The band which corresponded to the product was scraped off and the scrapings were extracted with methanol. Carrier AC (sodium salt, 55.3 mg, 0.50 mmole) was added and the methanol evaporated to dryness. Recrystallization of the residue from absolute ethanol/diethyl ether gave 51.4 mg of the pure [$^{14}$C]-labeled product, sp. radioactivity = 0.159 mCi/mmole. TLC: $R_f$ = 0.50 in the solvent system described above, detected by a characteristic orange color with a nitroprusside/ferricyanide spray reagent (6,9).

Drug administration protocols. The sodium salt of [1-$^{14}$C]AC (1.0 mg in 0.5 ml of water) was diluted with unlabeled carrier (1.81 mg in 1.8 ml of physiological saline) and administered i.p. to a male rat of Sprague-Dawley descent (Biolab, Inc., St. Paul, MN) weighing 160 g (dose: 1.1 mmoles/kg; 3.22 x $10^6$ dpm). Urine, feces and expired $CO_2$ were

collected and monitored for radioactivity as previously described (10). A similar procedure was followed with a second rat.

The synthetic prodrugs of cyanamide dissolved or suspended in 2% aqueous carboxymethylcellulose were administered (0.5 mmole/kg, i.p., or 1.0 mmole/kg, p.o.) to fasted male rats weighing 176-200 g at zero time. Ethanol (2.0 g/kg, i.p.) was given at 1, 4, 11, 23, 35, 47 and 71 hr, and the animals were killed 1 hr subsequent to each ethanol dose for measurement of blood acetaldehyde. Cyanamide treated animals served as positive controls.

Blood acetaldehyde. This was measured by head space GLC as described previously (4).

Mitochondrial AlDH activity. Liver mitochondria from rats were isolated and assayed for the low $K_m$ AlDH isozyme by measuring the rate of disappearance of acetaldehyde from a closed incubation system as previously described (11).

## RESULTS

As shown in Table 1, AC, the major urinary metabolite of cyanamide, as well as a synthetic analog, benzoylcyanamide, when administered to rats at a dose of 1.0 mmole/kg, elevated ethanol-derived blood acetaldehyde, 25-fold over control values for AC and 56-fold for benzoylcyanamide. These acyl derivatives of cyanamide did not inhibit the low $K_m$ AlDH of rat liver mitochondria when tested *in vitro*, except for the slight inhibitory activity displayed by benzoylcyanamide at the highest concentration tested (1.0 mM) (Table 1).

Table 1. Effect of acyl derivatives of cyanamide on ethanol-derived blood acetaldehyde *in vivo* and on AlDH *in vitro*

| Drug | In Vivo | | In Vitro | | |
|---|---|---|---|---|---|
| | Dose (mmole/kg) | Blood acetaldehyde | Concn (mM) | AlDH activity (nmoles acetaldehyde oxid/min/mg protein) | % Inhibition |
| Acetylcyanamide | 1.0 | 256.0 ± 31.2 | 0.01 | 10.33 ± 0.12 | 3.9 |
| | | | 0.1 | 10.74 ± 0.09 | 0.1 |
| | | | 1.0 | 10.04 ± 0.06 | 6.6 |
| Benzoylcyanamide | 1.0 | 549.7 ± 75.5 | 0.01 | 11.32 ± 0.27 | 0.0 |
| | | | 0.1 | 10.32 ± 0.43 | 4.1 |
| | | | 1.0 | 7.61 ± 0.43 | 29.3* |
| Control | - | 9.8 ± 0.4 | - | 10.75 ± 0.33 | - |

* $P < 0.005$.

When [1-$^{14}$C]-acetyl-labeled AC was administered to rats, 94-99% of the dose was excreted in the urine within 8 hr as unchanged AC, based on the specific color reaction produced with a ferricyanide spray reagent and the identity of its $R_f$ values on TLC in two solvent systems with authentic AC (Table 2). Approximately 2% of the administered radioactivity appeared in the expired air as $^{14}CO_2$. The bulk of this $^{14}CO_2$ was excreted within 4 hr, suggesting that a small but finite fraction of AC was hydrolyzed *in vivo* to liberate cyanamide and [1-$^{14}$C]acetate, the latter being then rapidly metabolized to $^{14}CO_2$.

These results encouraged us to synthesize other fatty acyl, α-aminoacyl and peptidyl derivatives as prodrug forms of cyanamide, in the expectation that the acyl, α-aminoacyl and peptidyl groups would be hydrolyzed by non-specific tissue amidases, esterases and/or aminopeptidases to release the cyanamide moiety *in vivo*. Palmitoylcyanamide and stearoylcyanamide represent long chain fatty acyl derivatives of cyanamide that were designed to increase lipophilicity and to prevent premature renal excretion, whereas N-benzoyl-L-leucylcyanamide represents an N-protected amino acid derivative of cyanamide.

Blood acetaldehyde levels measured from 2 to 72 hr after these cyanamide prodrugs were given to rats are shown in Fig. 1. It can be seen that these prodrugs of cyanamide were uniformly superior to the parent cyanamide in elevating ethanol-derived blood acetaldehyde,

a direct consequence of the inhibition of the hepatic mitochondrial low $K_m$ AlDH (8). Indeed, these cyanamide derivatives appeared to be generally effective over the entire 72 hr time course studied.

Table 2. Excretion of radioactivity in the urine and expired carbon dioxide by the rat after a single dose of [1-$^{14}$C]AC, sodium salt

| Rat No. (Dose) | Time interval (hr) | % of Radioactive dose excreted in* Urine | $CO_2$ | Total |
|---|---|---|---|---|
| 1 (1 mmole/kg) | 0-8 | 93.6† | 1.69 | |
| | 8-24 | 0.39 | 0.03 | 95.7 |
| 2 (1 mmole/kg) | 0-8 | 98.9† | 1.88 | |
| | 8-24 | 0.24 | 0.06 | 101. |

*Feces were collected, but radioactivity was not determined.
†TLC showed only a single radioactive spot corresponding to AC.

To evaluate the effectiveness of oral doses, palmitoylcyanamide and N-benzoyl-L-leucyl-cyanamide were given orally to rats at twice the i.p. dose. The pharmacologic responses to ethanol by these cyanamide derivatives given orally, as measured by increased blood acetaldehyde over 72 hr, were somewhat less effective than the i.p. route of administration (data not shown).

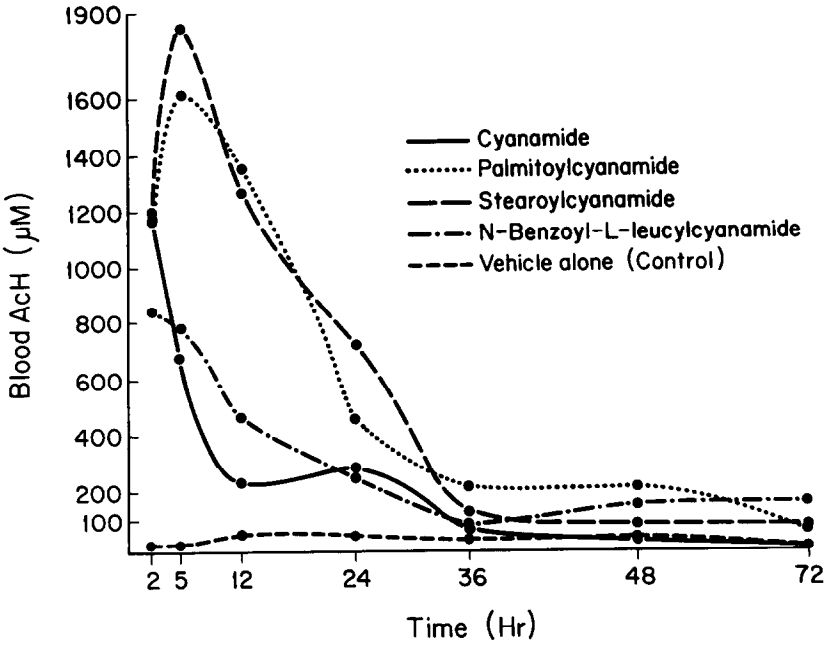


Fig. 1. Effect of a single i.p. dose of palmitoylcyanamide, stearoylcyanamide or N-benzoyl-L-leucylcyanamide on blood acetaldehyde in rats after ethanol administration. The experimental details are described under Materials and Methods. Each point represents the average of duplicate determinations from two animals. A statistical evaluation was not possible, being limited by the prohibitive number of animals that would be involved.

## DISCUSSION

A prodrug is defined as a substance which is converted after administration to the actual substance which combines with receptors (12). Accordingly, the citrated calcium

salt of cyanamide--the usual dosage form of this alcohol deterrent agent--is a prodrug, since calcium cyanamide must be solvolyzed in the stomach before cyanamide can be absorbed (3). In fact, cyanamide itself can be considered to be a prodrug requiring metabolic activation before inhibitory activity toward AlDH is manifest.

We chose to prepare prodrugs of cyanamide by acylation on the amide nitrogen with various lipophilic acyl and N-protected α-aminoacyl groups to allow for the slow release of cyanamide in vivo by enzymatic cleavage of these acyl groups. We were successful in obtaining latentiated (13) derivatives of cyanamide that appear to be superior to the parent compound with respect to (a) duration of action and (b) the ultimate and desired pharmacologic response, viz. elevation of blood acetaldehyde after ethanol administration. The AlDH inhibition in vivo by these acylcyanamides is mechanistically similar to that elicited by cyanamide itself, since treatment of rats with ethanol 1 hr prior to benzoylcyanamide (resulting in blood ethanol levels of 44.8 ± 1.9 mM) protected against the rise in blood acetaldehyde by 86%. Such protection is also shown for cyanamide (14) and would therefore be expected of the acylcyanamides if in vivo liberation of cyanamide were involved in their overall mechanisms of action.

Other acyl-, α-aminoacyl-, and peptidylcyanamides synthesized and evaluated along these lines will be reported elsewhere as a separate full paper.

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