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Metabolic Effects and Pharmacokinetics of Intravenously Administered Dichloroacetate in Humans

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Summary. Dichloroacetate decreases plasma glucose, lactate, and alanine concentrations in normal and diabetic subjects, and lowers lactate concentrations and increases survival in animals with experimentally induced lactic acidosis. The relationship between these effects and plasma dichloroacetate concentrations have not been previously studied in man. Dichloroacetate (1–50 mg/kg) was infused over 30 min to 16 healthy subjects and plasma drug concentrations were followed by gas chromatography over the next 8 h. Peak plasma concentrations were linearly related to the dose ($r = 0.98$, $p<0.001$) up to 30 mg/kg, above which 4 of 7 subjects had disproportionately high plasma drug concentrations. Nonlinear disposition was also indicated by the convex decreasing plasma elimination curves; levels declining less rapidly initially than later. At plasma concentrations below 10 μg/ml, elimination was monoexponential with a half-life of 32 ± 11 min (mean ± SD). Plasma drug clearance also decreased with doses greater than 20 mg/kg. Within 2 h of administration of the maximally effective dichloroacetate dose of 35 mg/kg, plasma lactate concentrations fell 75% below baseline and alanine fell 50% below baseline, while blood glucose was unaffected.

Key words: Dichloroacetate, pharmacokinetics, lactate, lactic acidosis, alanine, glucose.

Over the past several years, the effects of dichloroacetate (DCA) on intermediary metabolism have been studied extensively in several experimental models. DCA reduces blood glucose concentrations in both diabetic and fasted animals but not in healthy, fed animals [1–4]. The probable mechanism is through activation of pyruvate dehydrogenase via direct inhibition of pyruvate dehydrogenase kinase [5, 6]. Because lactate and alanine exist in equilibrium with pyruvate, their metabolic fates are also influenced by pyruvate oxidation. By stimulating pyruvate dehydrogenase activity, DCA accelerates pyruvate, lactate and alanine oxidation. Consequently, release of lactate and alanine from peripheral tissues into the circulation is reduced [7], and fewer three-carbon precursors are available for hepatic glucose synthesis.

We recently reported that orally administered sodium DCA, at a daily dose of approximately 50 mg/kg, rapidly and significantly decreased plasma lactate and alanine concentrations in maturity-onset diabetic patients who had normal or slightly elevated basal plasma lactate concentrations [8]. Other workers have shown that intravenously administered DCA can prevent or even reverse hyperlactatemia and lactic acidosis induced in animals by phenformin administration [9–13], acute hepatitis [14], functional hepatectomy [15, 16], epinephrine infusion [12, 17], exercise [18, 19] or hypoxia [12].

These findings suggested that DCA may be useful in the treatment of lactic acidosis in humans, where the mortality with current therapy exceeds 50% [20]. Accordingly, the present study was designed to evaluate in healthy volunteers the effects of intravenously administered DCA on plasma concentration of lactate, alanine and glucose and their relationship to the pharmacokinetics of DCA.

Materials and Methods

Sixteen healthy subjects (15 male, 1 female), 25 to 45 years old (mean 30 years), and within 10% of ideal body weight, were studied. They were taking no drugs, and their health was normal on physical examination, complete blood count and routine biochemical tests of hepatic and renal function. The protocol was

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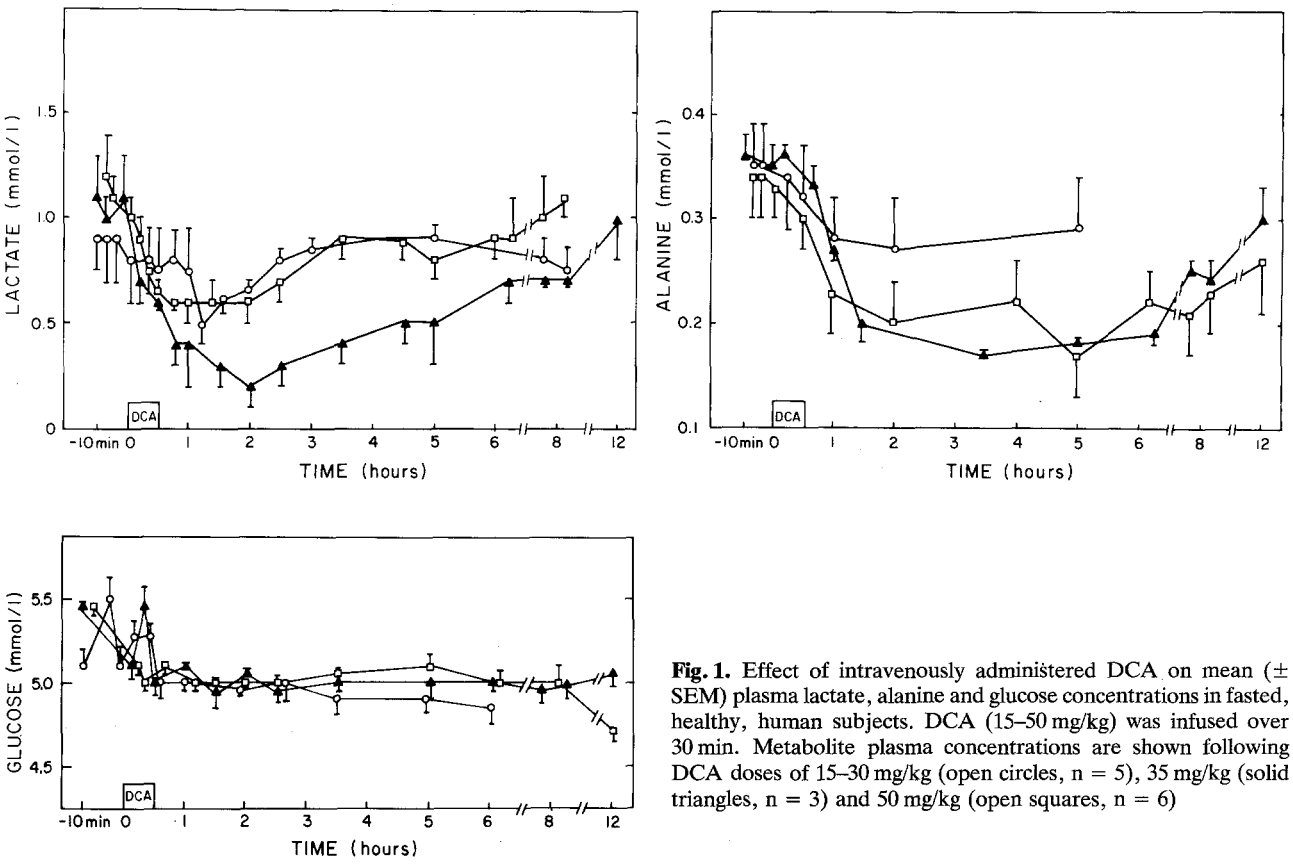

Fig. 1. Effect of intravenously administered DCA on mean (± SEM) plasma lactate, alanine and glucose concentrations in fasted, healthy, human subjects. DCA (15–50 mg/kg) was infused over 30 min. Metabolite plasma concentrations are shown following DCA doses of 15–30 mg/kg (open circles, n = 5), 35 mg/kg (solid triangles, n = 3) and 50 mg/kg (open squares, n = 6)

approved and monitored by the Vanderbilt University Committee for the Protection of Human Subjects. Informed written consent was obtained from each person before the study.

DCA was supplied in 10 ml ampules as the sodium salt, 100 mg/ml, in phosphate buffer, pH 7 (Ciba-Geigy Corp., Summit, NJ). On the morning following an overnight fast, DCA was diluted in 130 to 200 ml normal saline and infused at a constant rate by a Harvard infusion pump into a superficial forearm vein for 30 min. Doses of 1, 5, 10, 15, 20, 25, 30, 35 and 50 mg/kg body weight were administered in increasing strength, each subject being studied once at a single dose. Blood samples were drawn from a superficial vein in the other arm at 30 or 60 min intervals over a 12 h period. Blood pressure, pulse and electrocardiogram (ECG) were monitored intermittently, and subjects remained fasting and supine throughout the study.

Blood samples were centrifuged at 4 °C, and plasma was separated for analysis of pH and glucose [21], lactate [22], alanine [23], bicarbonate, and DCA [24] concentrations. For determination of plasma DCA 50 µl of internal standard in aqueous solution (trichloroacetic acid, 100 µg/ml; J. T. Baker Chemical Company, Phillipsburg, NJ) was added to 1 ml plasma followed by 2 ml 14% (w/v) boron trifluoride-butanol (Analabs, North Haven, CT) in Viton®-sealed 6 ml vials (Pierce Chemical Company, Rockford, IL). The vial was placed in a water bath (100 °C) for 10 min, cooled to room temperature and then distilled water (1 ml) and benzene (2 ml) were added. After shaking for 5 min, the benzene layer was separated by centrifugation and a 1 µl aliquot injected into a 2100 series gas chromatograph (Varian, Walnut Creek, CA) equipped with a tritiated titanium electron capture detector. The glass column was 1.83 m × 2 mm, packed with 100/120 mesh Chromosorb 101 (Analabs), and the nitrogen flow rate was 30 ml/min. Operating temperatures were: injector, 250 °C; oven, 180 °C; detector 260 °C. The concentration of DCA was obtained from daily linear calibration curves of the DCA/internal standard peak height ratios obtained after the addition of known amounts of DCA. Urinary recovery of DCA over 12 h was measured in three subjects.

In vitro plasma binding of [1,2-^{14}C] DCA (> 99% pure, New England Nuclear, Boston, MA) was estimated for one subject by equilibrium dialysis against phosphate buffer pH 7.4, using semimicrocells at 37 °C as previously described [25].

The half-life was calculated by linear regression from the log-linear portion of the plasma disappearance curve, and plasma clearance was estimated by dividing the dose of DCA administered intravenously by the total area under the plasma concentration-time curve using the trapezoidal rule with extrapolation to infinity.

Termination of the study because of emergent toxicologic information precluded full statistical analysis of the data as insufficient subjects had been studied at each dose level.

Results

Metabolic Effects

Figure 1 shows the time course of changes in lactate, alanine and glucose following administration of DCA 15–50 mg/kg. At concentrations of 1 and 10 mg/kg

(data not shown), DCA had no effect on these indices. The results obtained from infusions of 15–30 mg/kg were combined and expressed as the mean ± SEM because of the close similarity in metabolic responses achieved throughout this dose range. While there was intersubject variability of four- and two-fold among basal plasma lactate and alanine concentrations, respectively, this was not reflected in any consistent trend with respect to the response to DCA.

Maximal lactate depression for DCA doses between 15 and 50 mg/kg occurred 1–2 h after beginning drug infusion. Lactate concentrations remained below control values for 8–10 h, but returned to baseline within 12 h. At a maximally effective DCA dose of 35 mg/kg in 3 subjects, plasma lactate concentrations fell 75% below basal concentrations within 2 h. This effect was greater than that achieved with the 50 mg/kg dose in 6 subjects. Plasma alanine concentrations decreased 50% by 1 h, remained maximally depressed for 6 h, and were still below baseline at 12 h with both 35 mg/kg (n = 3) and 50 mg/kg (n = 6) of DCA. Decreases in plasma lactate and alanine correlated linearly ($r = 0.93$, $p < 0.01$) with DCA dose and peak plasma concentration from a DCA dose of 20 mg/kg up to the maximally effective 35 mg/kg dose, despite the small number of subjects (n = 8). Plasma glucose concentration fell only slightly, as shown by a maximal 13% decrease by 12 h in 6 subjects at the 50 mg/kg DCA dose.

Plasma bicarbonate concentration and venous pH did not change at any DCA dose. Similarly, blood pressure, pulse and ECG remained stable throughout the study. Subjective responses were confined to one subject who complained of drowsiness persisting 24 h following a dose of 50 mg/kg.

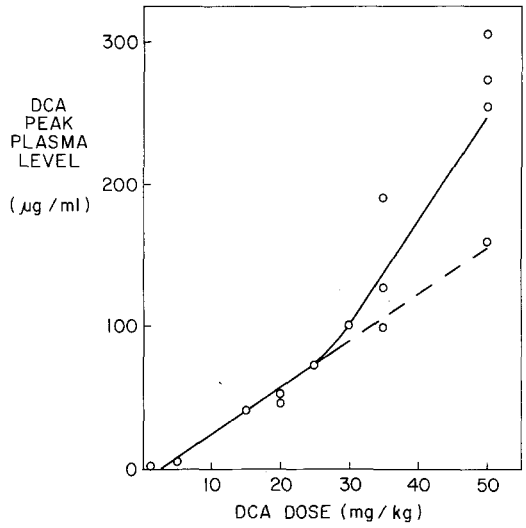

Fig. 2. Relationship between peak DCA plasma concentration (μg/ml) and DCA dose (mg/kg) in 14 fasted, healthy, human subjects. DCA (1–50 mg/kg) was infused over 30 min

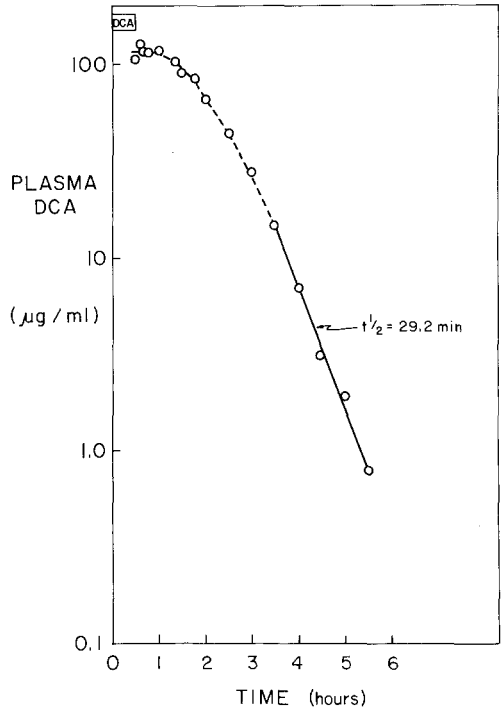

Fig. 3. Nonlinear time-course of DCA plasma elimination exemplified by data from one subject receiving 35 mg/kg of DCA over 30 min

Pharmacokinetics

Peak plasma DCA concentrations always occurred at the end of the 30 min infusion. Figure 2 shows that the peak plasma DCA concentration was apparently linearly related to the DCA dose, up to a dose of 30 mg/kg ($r = 0.98$, $p < 0.001$). At doses of 35 and 50 mg/kg, however, 4 of 7 subjects exhibited peak plasma drug concentrations disproportionately higher than predicted by the linear relation seen at lower doses. Nonlinear disposition was also indicated by the time course of plasma DCA concentrations following drug administration (Fig. 3). The plasma DCA concentration fell in a convex decreasing fashion with respect to time, elimination becoming more rapid as plasma DCA concentrations fell to about 10 μg/ml, below which elimination became monoexponential. This biphasic elimination was seen in all but one subject at all doses. The plasma clearance of DCA ranged from 0.57 to 0.08 l/kg/h, with an overall trend to decrease as the dose was increased above 20 mg/kg (Table 1). The mean (± SD) half-life of the monoexponential phase was 32 (± 11) min with a

Table 1. Half-life and clearance of DCA following a 30 min infusion of 15–50 mg/kg

Subject	Dose of DCA (mg/kg)	Half-life[a] (min)	Clearance (l/kg/h)
1	15	16.4	0.33
2	20	22.4	0.57
3	20	22.7	0.54
4	25	38.1	0.20
5	30	29.9	0.14
6	35	29.2	0.12
7	35	41.7	0.15
8	35	50.3	0.08
9	50	23.2	0.10
10	50	29.7	0.20
11	50	46.4	0.11
Mean (± SD)		31.8 (± 10.9)	

[a] Calculated from plasma concentrations below 10 μg/ml, as described in the methods section

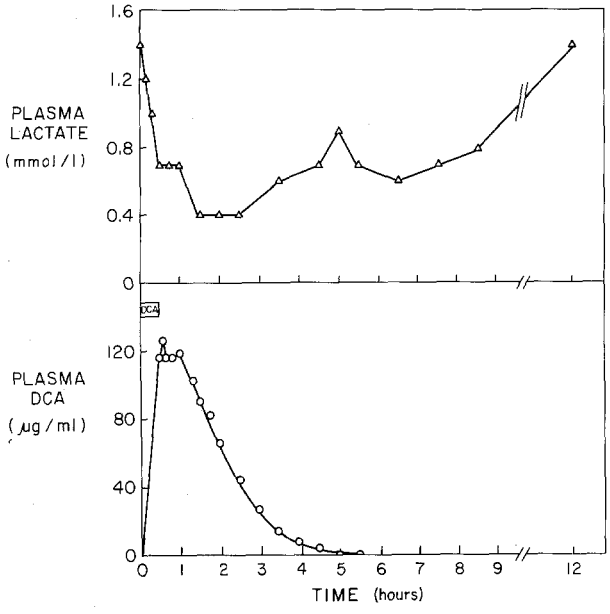

Fig. 4. Time-course relationship between plasma lactate concentration (upper panel) and DCA concentration (lower panel) exemplified by data from one subject receiving 35 mg/kg of DCA over 30 min

range of 16 to 50 min (Table 1). Urinary recovery of DCA over 12 h was less than 1% of the administered dose in 3 patients. The percent of DCA bound to plasma proteins in vitro for one subject decreased from 51% to 29% over the clinical plasma concentration range of 12.5 to 300 μg/ml, respectively.

The time course of plasma lactate depression in relation to the plasma DCA concentration is shown in Figure 4. DCA elimination from plasma was complete in 5 h, while lactate was depressed at least 9 h.

Discussion

These studies demonstrate that intravenously administered DCA rapidly reduces plasma lactate and alanine concentrations in humans (Fig. 1). The maximal depression of lactate and alanine occured at a DCA dose of 35 mg/kg, equivalent to a plasma drug concentration of 130 μg/ml. Despite a marked decrease in alanine and lactate concentrations, glucose concentrations remained fairly stable over the 12 h study period. This stability may have been due to glycogen mobilization which compensated for the effects of fasting and the blood glucose-lowering action of DCA.

There are differences in the effects of orally versus intravenously administered DCA on plasma lactate and alanine. In diabetic patients receiving 50 mg/kg of DCA orally for seven days, depression of plasma lactate and alanine persisted several days beyond cessation of DCA treatment (8). In contrast, the lactate and alanine concentrations after intravenously administered DCA had returned to or toward basal levels within 12 h of drug administration. The reason for this discrepancy in response between chronic oral dosing and single dose intravenous drug administration is unknown, although even in the intravenous studies, lactate and alanine concentrations remained depressed up to 8 h after elimination of DCA from the plasma.

Peak plasma DCA concentrations increased disproportionately when DCA was infused for 30 min at doses above 30 mg/kg (Fig. 2), and plasma clearance of DCA decreased with doses greater than 20 mg/kg (Table 1). In addition, a slower rate of DCA elimination from the plasma compartment was observed when plasma drug concentrations exceeded 10 μg/ml. A number of potential mechanisms, including saturable metabolism and plasma binding may be invoked to explain this nonlinear elimination, but the presently available data are insufficient to identify any specific process involved.

In light of the nonlinear pharmacokinetics, increasing doses or multiple dosing conditions would be expected to produce disproportionately high plasma DCA levels, as occurs with other drugs, such as phenytoin [26] and salicylate [27]. This phenomenon of DCA metabolism is particularly important in view of recent evidence of drug-related chronic toxicity in animals [28, 29], and a report of polyneuropathy developing after 4 months of daily DCA therapy in one human subject [30]. Knowledge of these toxicologic data, led to the premature termination of this study. Quantitative or qualitative interspecies variability in metabolic pathways of DCA and similar intersubject variability in humans may have

considerable toxicologic significance, and deserve further investigation. One subject in this study complained of drowsiness up to 24 h after DCA administration. He was found to have a high peak DCA plasma concentration (273 μg/ml), which had only begun to decline by the end of the sampling period. Thus, the prolonged, high plasma concentrations, together with an increased free fraction at higher drug levels, may have been responsible for the drowsiness observed in this and other [8] individuals. The relevance of the toxicologic data from chronic, oral DCA administration with respect to the acute intravenous use of DCA in life-threatening lactic acidosis awaits further study. It is possible that less toxic analogues of DCA may provide a better therapeutic index.

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