Dichloroacetate

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Dichloroacetate (DCA) represents a potentially novel class of oral antidiabetic agents that reduce blood glucose and lipids without stimulating insulin secretion. DCA reduces blood glucose by inhibiting hepatic glucose synthesis and stimulating glucose clearance and use by peripheral tissues. A major site of action of the drug is pyruvate dehydrogenase (PDH), the rate-limiting enzyme of aerobic glucose oxidation. Stimulation of PDH by DCA increases peripheral oxidation of alanine and lactate, thereby interrupting the Cori and alanine cycles and reducing the availability of three-carbon precursors for gluconeogenesis. In experimental models of ketosis, DCA reduces ketonemia and ketonuria while significantly lowering blood glucose.

DCA inhibits hepatic triglyceride and cholesterol biosynthesis. Short-term studies in patients with non-insulin-dependent diabetes have demonstrated a capacity of the drug to markedly reduce circulating a very-low-density lipoprotein cholesterol and triglyceride concentrations. In genetic models of insulin-dependent diabetes, oral administration of DCA significantly reduces insulin requirements and blood levels of glucose and triglycerides.

Several derivatives of DCA have been synthesized and found to have biological activity in animals. Further work is required to determine whether DCA and its analogues may be safe and effective agents for chronic treatment of the carbohydrate and lipid abnormalities of human diabetes.

Diabetes mellitus is a disease of metabolic integration in which the coordinate control of diverse pathways of intermediary metabolism becomes impaired. When attempts to regain metabolic control by nutritional interventions alone fail, the traditional allopathic recourse has been to administer either insulin or oral agents, such as sulfonylureas, that stimulate insulin secretion and/or act directly on cells to modify glucose synthesis or uptake. The fact that insulin facilitates glucose translocation from blood into cells, however, does not necessarily ensure a normal intracellular disposition of glucose and, therefore, glucose fuel homeostasis. There is increasing evidence that insulin itself may be incapable of restoring in diabetic patients the precise balance of glycogenesis, glycolysis, and glucose oxidation present in healthy individuals (1,2). Moreover, improving glycemic control with insulin may not correct the abnormalities in lipid and lipoprotein metabolism that frequently occur in diabetes and predispose to atherosclerosis (3).

Drugs that act independently of insulin on key processes of intracellular carbohydrate and fat metabolism might have important therapeutic roles as single agents or as adjuncts to insulin administration. Several such innovative pharmacological approaches have been summarized in other articles in this issue. Herein, we focus on the pharmacological and therapeutic effects of a novel drug class epitomized by dichloroacetate (DCA), a drug whose simple chemical structure belies a complexity of metabolic effects that offer insight into the pathology of diabetes and its treatment. The complete pharmacological spectrum of DCA has been reviewed elsewhere (4).

GLUCOSE-LOWERING EFFECT

— DCA significantly decreases hyperglycemia in insulin-dependent, ketosis-prone animals with chemically or surgically induced diabetes (5–11) and in patients with non-insulin dependent (type II) diabetes (12). DCA also modestly reduces glucose levels in fasted animals (13), but does not effect glycemia in healthy fed animals or humans (5,6,14,15). In diabetic patients treated with daily oral dosages of 50 mg/kg for 1 wk, blood glucose declined on average 24%, did not fall below normal fasting levels, and did not return to basal levels for up to 2 wk after treatment was stopped (12). Concomitant reductions of up to 70% from basal were recorded in plasma lactate and alanine concentrations that also remained suppressed for up to 2 wk after cessation of DCA dosing. Similar decrements in circulating glucose, lactate, and alanine occur in diabetic animals (8,13) and hyperglycemic patients with lactic acidosis (16; unpublished observations) treated with the drug. DCA administration has not been extended beyond 1 wk in diabetic humans or beyond 2 wk in diabetic animals. It has not been administered to subjects with insulin-dependent (type I) diabetes mellitus.

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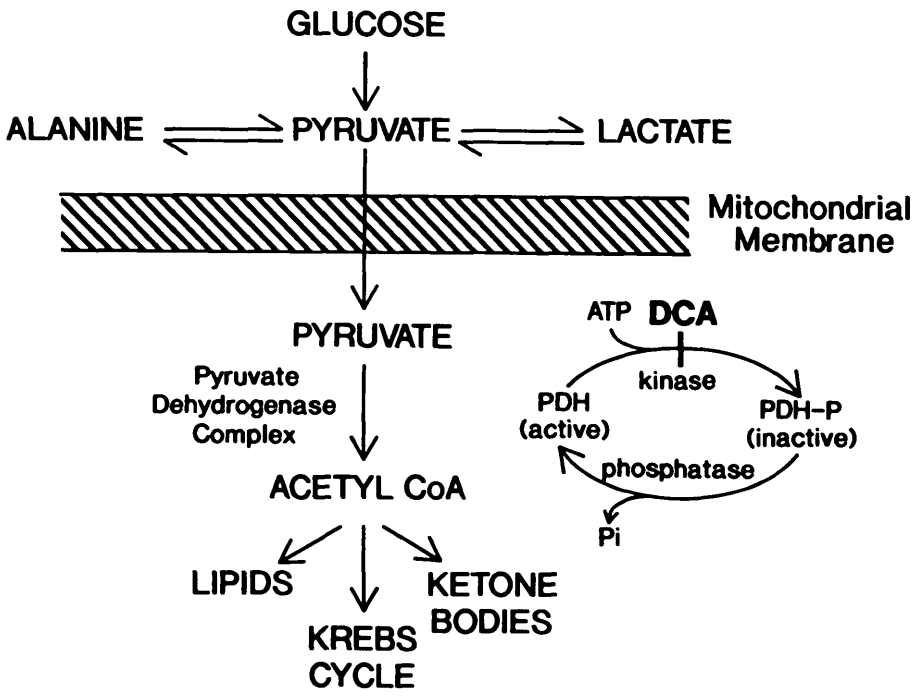

Figure 1—*The mitochondrial pyruvate dehydrogenase complex and site of action of DCA.*

Mechanisms of action

DCA does not stimulate endogenous insulin secretion (4). That its glucose-lowering action is independent of insulin has been dramatically illustrated in ketoacidotic rats, in whom DCA decreases both circulating glucose and ketone bodies and significantly improves short-term mortality (7,8). Likewise, its glucose-lowering effect cannot be attributed to suppression of glucagon secretion, intestinal glucose transport, or stimulation of renal glycosuria (4). DCA may stimulate glycogen synthesis in skeletal muscle and liver under fed conditions, but does not impair glycogenolysis during fasting (4).

The primary mechanism by which DCA selectively reduces blood glucose in diabetic animals or humans relates to its ability to shift cellular metabolism in diabetes or starvation from predominantly fat to predominantly carbohydrate use. This shift occurs because of three interdependent insulinlike effects of the drug: *1*) stimulation of glycolysis, 2) stimulation of glucose oxidation, and 3) inhibition of fatty acid oxidation. These properties are more fully detailed elsewhere (4) and will be only briefly summarized herein.

DCA inhibits fatty acid and ketone body oxidation in skeletal and cardiac muscle derived from diabetic or fasted rats and in cardiac tissue perfused with high concentrations of ketones (17). The precise site at which DCA inhibits fat oxidation in muscle is unknown. However, apparently as a result of this inhibition, tissue citrate levels fall. Citrate is an allosteric inhibitor of phosphofructo-1-kinase that catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate, a step that can be rate determining for glycolysis. Intracellular citrate levels are elevated in experimental models of diabetes consequent to enhanced fatty acid oxidation, and provide one plausible mechanism for the inhibition of glycolysis in cardiac muscle and probably most types of skeletal muscle in diabetes (18). Though some evidence suggests DCA may actually inhibit glycolysis in some types of skeletal muscle (19), this may not be the case in most actively glycolysing tissues.

However, perhaps the most important mechanism by which DCA reduces hyperglycemia in diabetes is by stimulating the activity of the pyruvate dehydrogenase (PDH) complex. PDH is a multienzyme complex located in the inner mitochondrial membrane that catalyzes the stepwise conversion of pyruvate to acetyl-CoA (Fig. 1). The physiological relevance of the PDH complex, particularly as it relates to fuel homeostasis in starvation or diabetes, has been reviewed (20,21). Extensive experimental data indicate that, under aerobic conditions, PDH is the rate-controlling enzyme of glucose and pyruvate oxidation. Because lactate and alanine are in equilibrium with pyruvate, their rates of oxidation are dependent on the activity of the PDH complex.

PDH is regulated by substrate activation (pyruvate, ADP), end product inhibition (acetyl-CoA, NADH), and reversible phosphorylation in which the phosphorylated enzyme is inactive. Magnesium and calcium ions activate PDH phosphatase and thus shift PDH into its catalytically active form. PDH kinase is activated by acetyl-CoA, NADH, and a recently described kinase activator protein (20) and is inhibited by CoA, NAD (reversal of the effects of acetyl-CoA and NADH, respectively), pyruvate, ADP and DCA. Therefore, PDH is inhibited in states such as diabetes and starvation when intracellular acetyl-CoA and NADH levels are high because of accelerated fatty acid oxidation. Insulin rapidly stimulates adipocyte PDH in vitro, but its effect on the enzyme in other tissues is either less pronounced or absent (19).

In contrast, DCA is a rapid and potent stimulator of PDH activity in virtually all tissues (4). The kinetics of inhibition of PDH kinase by DCA are complex, but may involve synergism with ADP, a known competitive inhibitor of

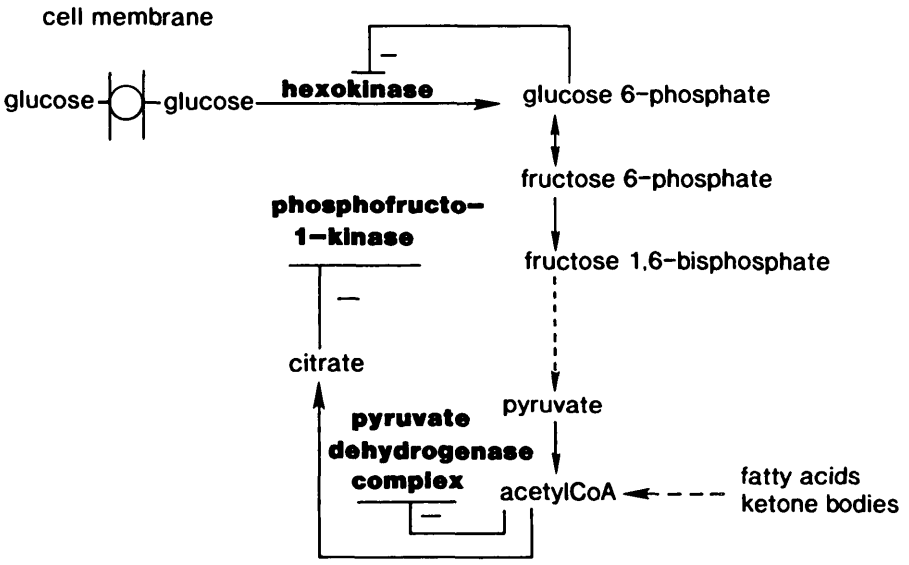

Figure 2—*Sites of inhibition of glucose metabolism in diabetes. Modified from Randle et al. (20).*

the enzyme (23). However, DCA may also stimulate PDH activity by mechanisms independent of direct kinase inhibition. Some evidence exists that DCA may facilitate calcium entry into mitochondria (24), thereby providing a mechanism for stimulating PDH phosphatase. In addition, by inhibiting fatty acid and ketone oxidation, DCA may reduce acetyl-CoA/CoA and NADH/NAD ratios intracellularly, leading to further inhibition of PDH kinase. These additional mechanisms for stimulating the PDH complex by DCA, while plausible, remain to be verified.

Locking PDH into its unphosphorylated, catalytically active form provides the mechanism for the dramatic lactate- and alanine-lowering effects of DCA observed in vivo. By shunting these glucose precursors toward Krebs cycle oxidation, DCA decreases their release from peripheral tissues and thereby limits their availability for gluconeogenesis. DCA also has direct intrahepatic inhibitory effects on gluconeogenesis that may be independent of hepatic PDH activation (25,26).

Repeated administration of DCA to rats causes a dose-dependent apparent increase in the half-life of PDH activation that may be independent of protein synthesis (13). This probably accounts for the sustained falls in circulating glucose, lactate, and alanine that persist beyond the period of drug administration (12,16). Because DCA or a metabolite may inhibit its own metabolism (4), repeated dosing may lead to sustained high concentrations of DCA at active sites, such as PDH kinase, and may also involve irreversible (covalent) inactivation of the enzyme by drug metabolites. These hypotheses remain to be tested.

It should be apparent from the above that the sites and mechanisms of action of DCA in controlling blood glucose are best understood within the context of the so-called glucose-fatty acid cycle developed by Randle et al. (18). In brief, this theory proposes the existence of a reciprocal relationship between cellular carbohydrate and fat metabolism, such that conditions leading to excessive fatty acid oxidation restrain glucose metabolism by inhibiting the activity of regulatory enzymes involved in glycolysis and glucose oxidation and may also accelerate hepatic gluconeogenesis by generating reducing equivalents (NADH) through fatty acid oxidation (Fig. 2). Inhibition of glucose metabolism at the levels of phosphofructo-1-kinase by citrate and the PDH complex by oxidative products of fatty acids lead to secondary inhibition of glucose phosphorylation and uptake by cells. These changes, demonstrated most readily in skeletal or cardiac muscle obtained from starved or diabetic animals, can be demonstrated in human diabetic patients under certain circumstances (20,27,28). Thus, the two primary causes of hyperglycemia in diabetes, increased hepatic glucose output and decreased peripheral glucose clearance, are ameliorated by DCA's effects on rate-limiting enzymes of intracellular carbohydrate metabolism.

The pathological consequences of the glucose-fatty acid cycle are reported to be operative in patients with type II diabetes who manifest elevated resting levels of blood lactate (1,12) and diminished capacity for cellular glucose oxidation. Limited data suggest that DCA may correct these abnormalities in humans, but its quantitative impact on human hepatic glucose production and peripheral glucose use remains unknown.

Lipid-lowering effect

DCA reduces circulating triglycerides in diabetic animals (10,29) and patients with type II diabetes and types IIb, IV, or V hyperlipoproteinemia (12). Again, the duration of the triglyceride-lowering effect in human subjects extends days to weeks beyond cessation of drug administration. Some patients with triglyceride levels >1000 mg/dl achieve normal or near-normal concentrations within 1 wk of daily administration of 50 mg/kg (12; G. Moore, unpublished observations).

In two patients with the low-density lipoprotein receptor-negative form of homozygous familial hypercholesterolemia, DCA reduced circulating total and low-density lipoprotein cholesterol concentrations ~35% within 2 wk of daily administration of 50 mg/kg (30).

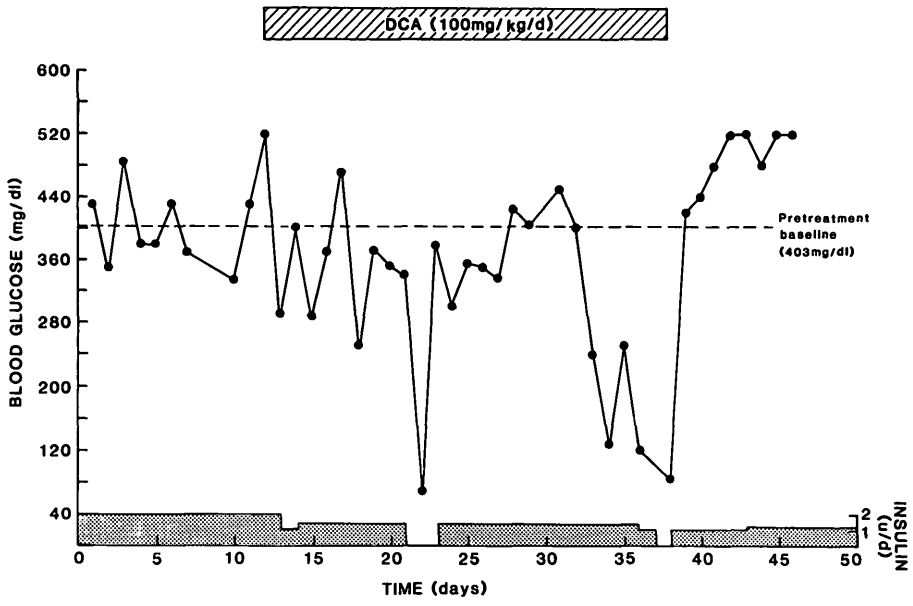

Figure 3—*Effect of DCA (100 mg · kg^{-1} · day^{-1}) in drinking water on daily blood glucose levels and insulin dose in a diabetic BB Wistar rat.*

Other lipoprotein fractions remained unchanged. Restoration of basal lipid and lipoprotein levels occurred within ~4 wk after stopping treatment.

Mechanisms of action

In healthy fed rats, DCA inhibits hepatic synthesis of triglycerides and cholesterol from tritiated water (31). If this effect also occurs in intestinal epithelial cells, it may account for the profound triglyceride-lowering property of the drug under conditions, like diabetes, in which synthesis and secretion of triglyceride-rich lipoproteins by liver and intestine may be accelerated. The inhibition site of triglyceride synthesis is unknown. The effect of DCA on apoprotein synthesis has not been investigated.

DCA is a noncompetitive inhibitor of HMG-CoA reductase, the rate-controlling enzyme of cholesterol synthesis (31). It is unlikely, however, that this mechanism accounts for the reduction in low-density lipoprotein cholesterol observed in homozygous familial hypercholesterolemia, because more potent competitive inhibitors of reductase are ineffective in this disease.

Although speculative, it is conceivable that a common site of action explains the inhibitory effect of DCA on hepatic triglyceride and cholesterol formation. It has been postulated (4) that DCA may inhibit an enzyme, such as ATP citrate lyase, proximal to the reductase-catalyzed step and common to the pathways of lipogenesis and cholesterolgenesis.

Studies in genetic models of diabetes

Experiments conducted in surgically or chemically induced diabetes have been criticized as not accurately reflecting the pathobiology of type I diabetes in humans and of introducing hepatic and other toxic drug effects. Nevertheless, the use of such experimental models has indicated DCA may have therapeutic potential as sole or at least adjunctive therapy in humans or animals ordinarily dependent on exogenous insulin for diabetes control.

Accordingly, we recently tested the hypothesis that DCA might ameliorate the metabolic abnormalities present in the BB Wistar rat and the NOD mouse, two genetic models of type I human diabetes that standard oral hypoglycemic drugs would not be expected to benefit.

Nine acutely diabetic BB Wistar male rats and 11 female diabetic NOD mice were fed ad libitum and treated each morning with subcutaneous injections of park protamine-zinc insulin. Animals were stabilized for at least 7 days at an insulin dose sufficient to maintain fasting blood glucose levels between 19.4 (350) and 22.2 mM (400 mg/dl) and mild ketonemia. From the onset of this period of stabilization until death, rats and mice received thiamine (600 μg/day) in their drinking water, because DCA may deplete tissue levels of this vitamin (32). Sodium DCA was then added to the water and the volume adjusted for each animal to provide a daily drug dose of ~100 mg/kg. Tail vein blood was sampled daily for blood glucose and weekly for lactate, alanine, B-hydroxybutyrate, cholesterol, and triglycerides.

RESULTS— Usually within 1 day of beginning DCA treatment, severely diabetic BB Wistar rats developed marked falls in blood glucose levels (Fig. 3). Animals frequently became hypoglycemic (blood glucose <5.6 M [<100 mg/dl]), despite repeated decrements in insulin dose. Mean values before and during 7–14 days of treatment are given in Table 1. DCA significantly decreased glucose, alanine, B-hydroxybutyrate, and triglyceride levels while also reducing mean insulin requirements 25%. Body weight did not change during this period. Figure 4 summarizes the effect of DCA administered to one BB Wistar animal before onset of type I diabetes. The drug maintained near normoglycemia during 16 days of treatment. After discontinuation of DCA, unequivocal hyperglycemia ensued. Figure 5 illustrates the effect of periodic administration of DCA in a previously poorly controlled BB Wistar rat. DCA induced a rapid and profound fall in blood glucose. When drug adminis-

Table 1—*Metabolic effects of DCA in poorly controlled diabetic BB Wistar rats*

Treatment Conditions	Blood Glucose (mg/dl)	Insulin Dose (U/day)	Blood Lactate (mEq/L)	Blood Alanine (mEq/L)	Blood B-OHB (mEq/L)	Serum Triglycerides (mg/dl)	Serum Cholesterol (mg/dl)	Body Weight (g)
Insulin	404	2.0	3.1	0.34	0.73	377	110	328
Insulin + DCA	269	1.5	2.6	0.17	0.60	216	107	324
% Change	34	25	16	56	18	43	1	1
P	0.0005	0.05	0.20	0.0005	0.005	0.005	0.325	0.375

tration was stopped, circulating glucose levels rose promptly and declined again on reinstituting drug therapy.

We also conducted pilot studies of the effect of DCA in NOD mice, stabilized with 0.5 U insulin/day for 1 wk at a mean ± SE blood glucose concentration of 19.1 ± 1.0 mM (358 ± 19 mg/dl). Oral administration of DCA resulted in profound lowering of blood glucose levels (Table 2) and death in three animals from hypoglycemia within 3 days of treatment. The mean ± SE glucose level over the 3 days of combined DCA and insulin therapy was 7.8 ± 1.6 mM (141 ± 28 mg/dl). Blood glucose levels in the remaining five animals decreased progressively during this brief period and averaged only 4.8 ± 0.0 mM (87 ± 1 mg/dl) by the third treatment day, a 76% fall from basal. After 3 days of DCA administration, the daily insulin dose was reduced 50% to 0.25 μ/day while DCA was continued at 100 $mg \cdot kg^{-1} \cdot day^{-1}$. Twenty-four-hours after decreasing the insulin dose, blood glucose had increased to 6.9 ± 1.6 mM (124 ± 28 mg/dl). After 1 wk, the mean ± SE blood glucose level was 15.1 ± 1.7 mM (271 ± 31 mg/dl), or 25% below basal.

This study is the first, to our knowledge, to demonstrate oral efficacy of any drug in genetic models of type I diabetes. Only parenteral administration of insulin or large dosages of nicotinamide (33) have previously been effective in treating hyperglycemia in autoimmune experimental diabetes. Our data show that daily oral administration of DCA markedly reduces both hyperglycemia and insulin requirements in severely diabetic BB Wistar rats and NOD mice. Circulating levels of lactate, alanine, B-hydroxybutyrate, and triglycerides were also decreased by this therapy.

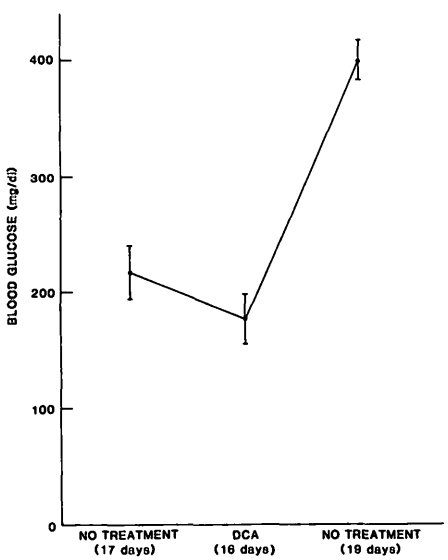

Figure 4—*Effect of DCA (100 mg $kg^{-1} \cdot day^{-1}$) in drinking water on blood glucose levels in a diabetes-prone BB Wistar rat.*

SHOULD DCA BE USED IN HUMAN DIABETES?— Early clinical studies with DCA in the treatment of diabetes and hyperlipoproteinemia were suspended when a mild reversible neuropathy appeared in one adult patient, and other toxicity was induced in animals chronically receiving DCA (30). Since then, it has become evident that the potential chronic toxicity of DCA may be related to certain features of its metabolism in vivo and its effects on enzymes requiring thiamine as a cofactor.

The plasma half-life of DCA is substantially longer in adult rats and dogs than in humans, and repeat dosing in adult humans markedly delays its plasma clearance (15,16). In addition, DCA stimulates the activity of at least some thiamine-dependent enzymes (including PDH) and may deplete thiamine stores with long-term treatment. Coadministration of DCA and thiamine signif-

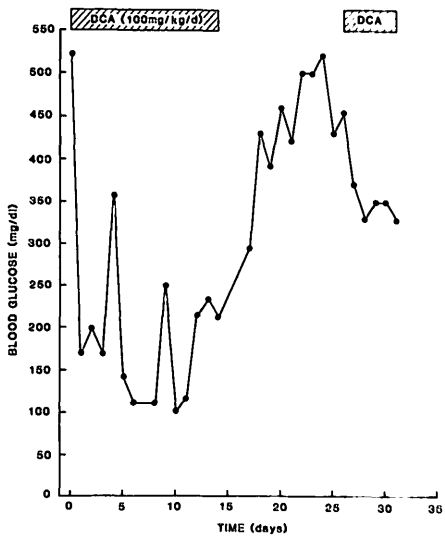

Figure 5—*Effect of intermittent treatment with DCA (100 $mg \cdot kg^{-1} \cdot day^{-1}$) in drinking water on blood glucose levels in a diabetic BB Wistar rat.*

Table 2—*Metabolic effects of DCA in poorly controlled diabetic NOD mice*

Treatment Conditions	Duration (day)	Blood Glucose (mg/dl)	Insulin Dose (U/day)	Body Weight (g)
Insulin	7	358 ± 19	0.5	30 ± 2
Insulin + DCA	3	141 ± 28	0.5	. . .
Insulin + DCA	7	271 ± 31	0.25	29 ± 2

icantly decreases the incidence and severity of toxicity, including neuropathy, due to DCA alone (32). Finally, DCA metabolism may be age dependent. This postulate is based on data accumulated in >20 infants and children with congenital forms of lactic acidosis treated daily with DCA for several months or years without apparent toxicity (4). DCA is now designated as an orphan drug for the chronic treatment of congenital lactic acidosis and homozygous familial hypercholesterolemia.

Minimally effective dosages of DCA in treating diabetic animals or humans remain to be established, but the dose and dose interval may be considerably less than those used previously, and thiamine should probably accompany its chronic administration. It is possible that DCA, or drugs with similar actions, may reduce insulin requirements and "smooth out" daily glucose and lipid excursions in insulin-requiring patients.

Several halogenated derivatives of acetic and other short-chain fatty acids have been reported to possess glucose-lowering properties and/or to stimulate PDH complex activity (34,35). Some are clearly too toxic for human administration and none have undergone as extensive a pharmacological evaluation as DCA.

Derivatives of DCA, in which DCA exists either in prodrug form esterified to polyols or as an ionic complex to provide sustained release of the parent drug, have been synthesized (36; Fig. 6). These derivatives are orally effective in decreasing blood glucose and lactate levels in fasted rats and in inhibiting hepatic triglyceride and cholesterol synthesis.

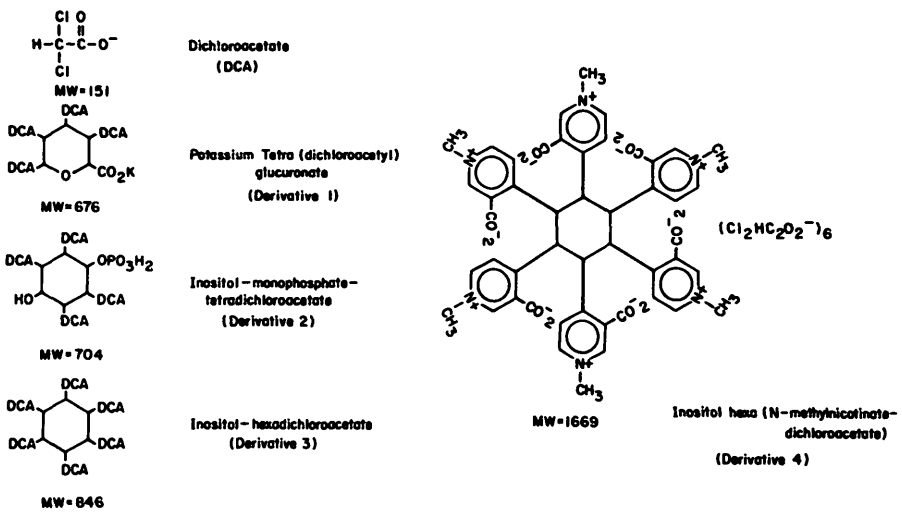

Figure 6—*Biologically active derivatives of DCA.*

In summary, it is premature to consider chronic treatment of diabetic humans with DCA. However, carefully controlled short-term investigations of DCA pharmacokinetics and pharmacodynamics appear warranted to determine safe and effective drug dosages in healthy subjects and, subsequently, in patients. Apart from its possible clinical usefulness, DCA and its derivatives can be valuable probes with which to investigate intermediary metabolism and fuel homeostasis under diverse nutritional and pathological conditions.

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