DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 20(15), 2425-2437 (1994)

DEVELOPMENT OF AN ORAL DRUG FORMULATION FOR DICHLOROACETATE AND THIAMINE

George N. Henderson[*,a], Patrick O. Whalen[a], Rebecca A. Darr[a], Stephen H. Curry[b,1], Hartmut Derendorf[b], Thomas G. Baumgartner[c] and Peter W. Stacpoole[a]

[a]Department of Medicine (Division of Endocrinology and Metabolism), Box 100226, College of Medicine, [b]Department of Pharmaceutics, College of Pharmacy, and [c]Shands Hospital Pharmacy, University of Florida, Gainesville, FL 32610.
[1] Present Address: Fisons Pharmaceuticals, 755 Jefferson Road, Rochester, NY 14603. [*]Author to whom correspondence should be addressed.

ABSTRACT

Dichloroacetate (DCA) is an investigational drug for the treatment of several metabolic and cardiovascular disorders. Hitherto, it has been used mostly in intravenous, short term treatment regimens. Chronic administration of the drug may be toxic, due in part to the depletion of tissue thiamine stores. We therefore developed a stable liquid preparation of the sodium salt of DCA and thiamine·HCl suitable for chronic oral administration. The DCA-thiamine mixture is formulated as a palatable solution containing glycerol, sodium benzoate and aspartame in phosphate buffer at pH 3.5. A thermally accelerated decomposition study of the oral DCA formulation revealed a shelf-life of about 5 years at 4°C and about 156 days at 25°C. The stability was also followed for 1 year at its storage condition of 4°C and it was found to be stable at least for 1 year, which is its current recommended storage time. A new high performance liquid chromatographic method was developed to quantitate DCA, thiamine, aspartame and sodium benzoate in the formulation. The DCA-thiamine formulation is palatable and well tolerated by children, some of whom have received it continuously for over three years. Under chronic treatment, at a dose of 12.5 mg/Kg or 25 mg/Kg, DCA has a mean plasma half life of 7.9 hrs in children with congenital lactic acidosis.

INTRODUCTION

Dichloroacetate (DCA) is an investigational drug with clinical potential in treating several metabolic and cardiovascular disorders, such as lactic acidosis, diabetes mellitus, hyperlipidemia, ischemic heart disease and heart failure (1). The drug acts independently of insulin to reduce hyperglycemia in diabetes and decreases circulating cholesterol or triglycerides or both in various forms of hyperlipidemia. DCA stimulates myocardial glucose and lactate oxidation and improves myocardial left ventricular function in experimental or clinical states of cardiac ischemia or failure (2). It also decreases morbidity in adult patients with lactic acidosis (3). Recently, chronic oral treatment with DCA has been reported for children with congenital forms of lactic acidosis (1,4), in whom the drug may reduce hyperlactatemia and improve neurological status.

Chronic oral administration of DCA, however, may be toxic (5,6). Rats, dogs and two human subjects receiving $\geq$50 $mg.kg^{-1}.d^{-1}$ sodium DCA by mouth for several weeks or months exhibited a reversible peripheral neuropathy. Other evidence of chronic drug toxicity observed in animals has not been reported in humans. Oral administration of large (approx. 1 $g.kg^{-1}.d^{-1}$) doses of DCA for several weeks to rats decreases tissue thiamine (vitamin B_1) stores and induces a peripheral neuropathy and other clinical signs of thiamine deficiency that can be prevented or ameliorated when the drug is co-administered with thiamine (6). Thus, it is reasonable to postulate that the safety of chronic DCA administration might be enhanced if thiamine supplementation were made.

Chronic treatment of diseases such as congenital lactic acidosis with DCA will include infants and children. Moreover, the drug may have to be administered at home for extended periods. Therefore, it is desirable to have a liquid formulation of the drug that includes an appropriate dose of thiamine. DCA/thiamine mixtures have a bitter-salty taste and are not palatable. In addition, thiamine is a labile vitamin.

We now report the development of a stable, liquid formulation containing DCA and thiamine suitable for chronic, oral administration to human subjects. Temperature and pH optima for the preparation are described, as is a new high performance liquid chromatographic (HPLC) technique for the detection of both DCA and thiamine in this formulation.

EXPERIMENTAL SECTION

Reagents and Materials:

Sodium dichloroacetate (DCA) was purchased from American Tokyo Kasei (Portland, Oregon). Its purity and homogeneity were established as described

previously (6). Other chemicals were used as obtained from the supplier as follows: thiamine hydrochloride (USP grade, Lyphomed, Inc., Chicago, Illinois); aspartame (NutraSweet Company, Deerfield, Illinois); glycerol (USP grade, Humco Laboratory, Texarcana, Texas); disodium hydrogen phosphate and phosphoric acid (ACS grade, Fisher Scientific, Pittsburgh, Pennsylvania); acetonitrile (HPLC grade, Fisher Scientific); red #40 food color and imitation strawberry extract (C.F. Sauer Company, Richmond, Virginia); Hartle's neutral slush base and Hartle's cherry syrup (H&R products, Orlando, Florida).

High Performance Liquid Chromatography:

A Perkin-Elmer Series 410 Bio Liquid Chromatograph was used for HPLC analyses. Samples were detected by a Perkin-Elmer model LC-95 UV-VIS detector at a wavelength of 210 nm and aniline sulfate was used as an internal standard. The flow rate was 1 mL . min^{-1}. Analysis of DCA, thiamine and sodium benzoate (without added aspartame) employed a Zorbax phenyl column (4.6 mm x 25 cm) and the following eluents: water (35%), acetonitrile (30%) and 0.1 M sodium dihydrogen phosphate (35%). For quantitation of the final oral drug solutions and stability studies involving aspartame, we used a Zorbax phenyl cartridge column (4 mm x 8 cm) with a 5 μm packing in conjunction with the corresponding guard column and an eluent composition of water (46.5%), acetonitrile (7%) and 0.1 M sodium dihydrogen phosphate (46.5%).

Stability Studies in Phosphate Buffers (without Aspartame):

Three sets of experiments were performed to examine the stability of DCA formulations: 1) thiamine in phosphate buffers, 2) DCA in phosphate buffers and 3) DCA and thiamine in phosphate buffers, For the first set of experiments, 100 mg of thiamine was dissolved in 5 mL 1 M sodium dihydrogen phosphate and deionized water was added to make 40 mL of solution. The pH of the solution was adjusted to 2.5, 5, 5.5, 6, 7.4 or 9 with either concentrated phosphoric acid or sodium hydroxide. Water was added to bring the final volume to 50 mL and the solutions were refrigerated at 4°C until use. A 10 mL aliquot of each sample was heated to 110°C in tightly capped (Teflon lined caps) Pyrex test tubes, protected from light. Aliquots (500 μL) were withdrawn periodically from 0 to 360 minutes of heating and frozen at -20°C for later analysis by HPLC.

Samples used in the second set of experiments contained 5 g DCA in 5 mL of sodium dihydrogen phosphate buffer and water to make 50 mL. The pH was adjusted as described above. The third set of experiments employed pH-adjusted mixtures of 100 mg thiamine and 5 g DCA in 5 mL sodium dihydrogen phosphate buffer and water to a volume of 50 mL.

An internal standard solution was prepared by diluting 5 mL 1 M aniline sulfate stock solution to 200 mL. For the HPLC analysis, 50 μL of sample was diluted with 2 mL internal standard solution and 20 μL of this diluted mixture was injected.

Stability Studies in Phosphate Buffers (with Aspartame):

The following stock solutions were prepared: 1 M monobasic phosphate solution (1 L), 1% sodium benzoate solution (100 mL), 10% thiamine solution (10 mL), 1% aspartame solution (100 mL) and 10% red color solution (10 mL). Five different test solutions (A-E) were prepared by adding various constituents (A-E: 2.5 mL phosphate buffer, 5 mL sod. benzoate, 10 mL glycerine, 5 mL aspartame; C-E: 1 mL thiamine; B,D and E: 2.5 g DCA; E: 100 μL flavoring, 50 μL coloring) and adjusting the pH to 3.5 using phosphoric acid and the final volume to 50 mL. Thus, solution E represents the final oral formulation and A-D represent formulations with one or more components absent from the complete formulation.

Four 10 mL samples from each solution (A-E) were heated at 100, 95, 80 or 60°C and 500μL aliquots were withdrawn at various time intervals (0 to 48 hrs) and stored in a freezer at -20°C for later analysis by HPLC. Separate studies at 25°C and 4°C did not reveal any change in the concentrations of DCA, thiamine or aspartame when followed for 30 days.

Long Term Stability of Oral DCA Formulation:

For clinical use, the final oral drug formulation (5% DCA syrup) was prepared in 4 L lots. Sodium DCA (200 g), 8 g thiamine.HCl, 4 g sodium benzoate, 4 g aspartame, 800 mL glycerine, 200 mL 1M monobasic sodium phosphate and 35 mL of 1:10 diluted phosphoric acid were added to a 6 L Erlenmeyer flask, equipped with a large magnetic stirrer. Sterile water was added with stirring to make up most of the 4 L volume and stirring was continued until all solids dissolved. The pH of the solution was adjusted to 3.5 using about 5 mL of 1:1 diluted phosphoric acid. After adjustment of the pH to 3.5 was complete, 0.4 mL Sauer brand red food coloring, 8.0 mL Sauer brand strawberry extract and water to make up 4.0 L were added. The resulting syrup was mixed well and the final pH was measured. Samples were withdrawn for analysis and bacterial testing. The syrup was transferred to dispensing bottles (500 mL brown glass) and stored at 4°C.

The stability of the final drug formulation was followed for 1 year under its recommended storage condition of 4°C by assaying stored samples. Periodic testing for bacteria and fungi were also conducted for 1 year, using thioglycolate (7) and blood agar (8) assays.

RESULTS AND DISCUSSION

HPLC Method Development:

Previous analyses of DCA concentrations in biological fluids have utilized a gas chromatography (GC) method that employs derivatization of the drug to its methyl ester, followed by GC analysis using electron capture detection (9,10). Thiamine levels have been determined by enzymatic (11) or HPLC techniques (12), and aspartame (13) and sodium benzoate (14) have also been analyzed by HPLC. No method currently exists for the analysis of these components together in a mixture. To facilitate quality control and analysis of the oral DCA formulation, we developed a sensitive and reliable HPLC method to quantitate all the components of oral formulation, including DCA, from a single injection of the sample.

Two HPLC methods were developed, the first to analyze drug mixtures with thiamine and second to analyze drug samples with thiamine and aspartame. In the first method, optimal HPLC peak separation was achieved with a mixture (by volume) of water (35%), acetonitrile (30%), and 0.1 M sodium dihydrogen phosphate (35%) on a Zorbax C18 column (25cm x 4.6 mm). Each peak was resolved to the baseline. Retention times were 2.42 min for DCA, 3.52 min for thiamine, 4.81 min for sodium benzoate and 6.55 min for aniline sulfate. Calibration graphs of thiamine (0.094 to 0.016 mg/mL) and DCA (0.375 to 1.563 mg/mL) were linear, with the correlation coefficients of 0.996 and 0.997, respectively. The intra-assay C.V. were below 1.5% and inter-assay C.V. were below 2% for both thiamine and DCA. The method was stability indicating because none of thiamine's decomposition products (0.2% aqueous thiamine solution was heated at 100°C for 16 hrs) interfered with the analysis and DCA was stable under all the conditions studied.

The first method developed for the analysis of thiamine and DCA was found not to be suitable for the analysis of mixtures containing aspartame, thiamine and DCA. Therefore, a second HPLC method was developed and it utilized a Zorbax phenyl cartridge column (8 cm x 4 mm) with a 5μm packing in conjunction with the corresponding guard column and an eluent composition of water (46.5%), acetonitrile (7%), and 0.1 M sodium dihydrogen phosphate (46.5%). The following retention times were observed (Figure 1): DCA (1.08 min), thiamine (2.22 min), aniline sulfate (3.68 min), sodium benzoate (5.45 min) and aspartame (9.30 min). Calibration curves for DCA (concentration range 0.125 - 1.25 mg/mL), thiamine (0.005 - 0.05 mg/mL), sodium benzoate (0.0025 - 0.025 mg/mL) and aspartame (0.0025 - 0.025 mg/mL) were linear, with the correlation coefficients of 0.999, 0.985, 0.991 and 0.999, respectively. The intra-assay C.V. were below 2% and inter-assay C.V. were below 2.6% for all the compounds assayed. Periodic replacement of the guard column (2-3 months) was necessary to maintain good separation of all the components. The

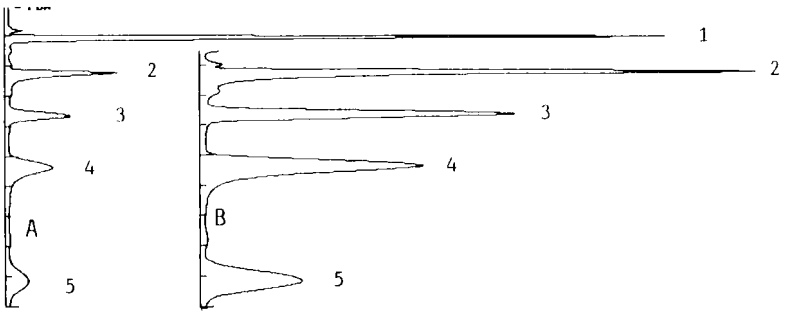

FIGURE 1.

Chromatogram of oral DCA formulation (A) and its expansion (B). Column: Zorbax phenyl cartridge (4 mm x 8 cm). Eluent: water (46.5 %), acetonitrile (7 %) and 0.1M sodium dihydrogen phosphate (46.5 %). Key: 1. DCA (1.07 min); 2. thiamine.HCl (2.25 min); 3. aniline sulfate (3.67 min); 4. sodium benzoate (5.38 min) and 5. aspartame (9.10 min).

method was stability indicating because none of thiamine and aspartame decomposition products (aqueous aspartame solution (0.1%) was heated at 110°C for 24hrs when it had completely decomposed to a colorless solution (decomposition followed by HPLC)) interfered with the analysis and DCA was stable under all the conditions studied.

Effect of pH on the Stability of DCA and Thiamine:

Parenteral solutions of DCA are stable at near-to-neutral pH (15), while thiamine is most stable under acidic conditions (16). Both DCA and thiamine are bitter and a potent sweetener is required to render oral solutions palatable. As discussed in a later section, aspartame was chosen as the sweetener and it is reported (17) to be stable in acidic pH.

Preliminary experiments were conducted to test the stability of thiamine, DCA and DCA plus thiamine in phosphate buffers over the pH range 2.5-9. A temperature of 110°C was chosen for the study because of the measurable rate at which thiamine decomposed at this temperature in preliminary experiments.

Figure 2A relates thiamine concentration vs reaction time as a function of pH. At 7.4 or 9, thiamine was rapidly degraded and was unmeasurable at 360 min. At pH 5-6, however, thiamine decomposition was slower and at least 50% of the original concentration remained after 360 min. No significant change in the concentration of thiamine was observed in solutions of pH 2.5 - 5.

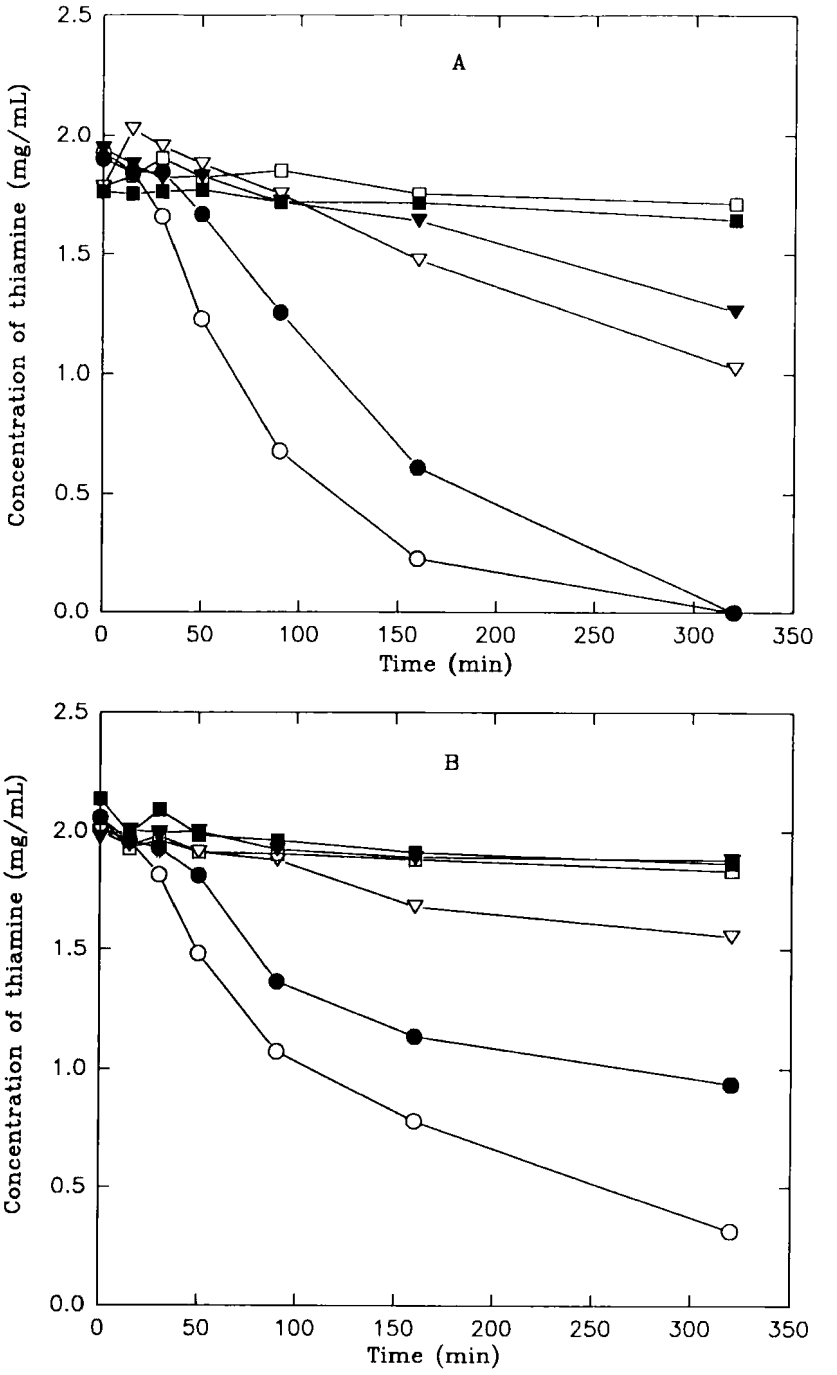

FIGURE 2.

Effect of pH on the degradation of thiamine at 110°C in the absence (A) and in the presence (B) of 10% DCA in 0.1M phosphate buffer. Key: pH 9.0 (open circle), 7.4 (closed circle), 6.0 (open triangle), 5.5 (closed triangle), 5.0 (open square) and 2.5 (closed square).

DCA was stable within the pH range tested and added thiamine had no effect on the stability of DCA. In addition, it was interesting to note that, at every pH, the rate of decomposition of thiamine was reduced by the presence of DCA (Figure 2B), with measurable decomposition of the vitamin observed only at pH 6 and 9. From the shape of the lines obtained from a plot of log concentration vs time (not shown), it can be inferred that the decomposition of thiamine followed apparent first order kinetics.

Oral DCA Preparations:

Initial oral DCA/thiamine preparations made were from the commercial cherry and neutral slush base syrups (10% DCA). Cursory taste tests suggested incomplete masking of the bitter-salty taste of the DCA/thiamine formulations. Stability studies at elevated temperatures showed that DCA was stable in these formulations. However, the components of syrups interfered with the HPLC analysis of thiamine. At this point it was decided to use aspartame because of its safety and sweetness (180-200 times that of sucrose (18)). Aspartame is the methyl ester of the dipeptide of aspartic acid and phenylalanine and it is extensively used as an additive in foods, beverages, multivitamins and pharmaceuticals, including those used by children and infants (19). It is most stable in the pH range of 2.5 to 5.5 in aqueous solutions. Thus, the choice of pH 3.5 for the oral DCA preparation was considered optimum for the stability of both thiamine and aspartame.

After preliminary trials, an oral DCA solution containing 10% DCA, 0.4% thiamine hydrochloride and 0.1% sodium benzoate in 0.1 M phosphate buffer at pH 3.5 was formulated. The concentration of the preservative sodium benzoate is the same as that present in commercial syrups and oral formulations and the thiamine concentration chosen was based on amounts previously determined to protect against depletion of this vitamin by DCA (6). Both aspartame and glycerol were found to mask the taste of DCA/thiamine formulations (Table 1). Several solutions were made with and without 20% glycerol (chosen to give sufficient viscosity to the formulation) at pH 3.5 and were taste-tested and graded subjectively by a 5 person panel to derive a consensus opinion.

From the foregoing data a solution containing 0.12% or 0.16% aspartame and 20% glycerol appeared to be the most suitable compositions, although a residual salty taste persisted with these formulations. In order to further mask the taste of the oral formulation, the following food flavors were tried in formulations with aspartame and glycerol at pH 3.5: orange extract, lemon extract, strawberry extract, pineapple flavor, peppermint extract, maple syrup extract and vanilla extract. The panel chose the formulation with the strawberry extract to be most palatable. Even though the current formulation substantially masked the taste of DCA, there was still a slight bitter after-

TABLE 1.
Taste Test of Oral DCA with and without 20% Glycerol.

#	% Aspartame	No glycerol	20 % Glycerol
1	0.04	bitter-salty	bitter-salty
2	0.06	bitter-salty	bitter-salty
3	0.08	bitter-salty	bitter-salty
4	0.10	bitter-salty	salty
5	0.12	bitter-salty	palatable
6	0.16	bitter-salty	palatable
7	0.20	salty	too sweet
8	0.22	palatable	extremely sweet

taste and at this point it was decided to reduce the concentration of DCA, thiamine and buffer to half the original amounts. The new formulation with 5% DCA was taste-tested using concentrations of aspartame from 0.01% - 0.12%. The 0.1% solution was the most palatable, with 0.06 and 0.08% in the acceptable range. Red food coloring was also added to the formulation to enhance its appearance.

Stability of Oral DCA Formulation and Aspartame:

The stability of the oral drug formulation for long term storage was evaluated by the method of thermally accelerated testing of the drugs (20). The five test solutions A-E (see Experimental Section) at pH 3.5 were heated to 110, 95, 80 and 60°C and samples withdrawn at various intervals up 48 hrs were analyzed by HPLC. Although DCA and thiamine were stable at all temperatures, degradation of aspartame increased progressively with increasing temperature. We estimated a 10% loss of aspartame would still maintain palatability of the formulation and thus calculated shelf-lives based on this assumption. k values were obtained from the degradation studies of the solution of aspartame alone and as part of the final oral drug formulation (Table 2). Table 3 lists the predicted shelf-lives of aspartame in the drug solution and aspartame alone. As with the case of thiamine, DCA appeared to enhance the stability of aspartame. Studies conducted at 25°C and 4°C showed no degradation and hence did not contribute to the calculations. Since aspartame degradation was small and since higher temperatures were used to elicit decomposition, the predicted shelf-lives are approximate.

TABLE 2.

k values of aspartame alone and aspartame in oral DCA.

Temp °C	Aspartame in phosphate buffer	Aspartame in oral DCA
110	9.6×10^{-4} min^{-1}	6.7×10^{-4} min^{-1}
95	3.3×10^{-4} min^{-1}	3.9×10^{-4} min^{-1}
80	1.8×10^{-4} min^{-1}	1.4×10^{-4} min^{-1}
60	2.4×10^{-5} min^{-1}	1.6×10^{-5} min^{-1}

TABLE 3.

Predicted Shelf-life of Oral DCA

Item (units)	Aspartame in phosphate buffer	Aspartame in oral DCA
-Ea (cal . $mole^{-1}$)	-18132	-9646
log A	7.37	7.88
A (min^{-1})	2.352×10^{6}	7.667×10^{6}
k at 4°C (min^{-1})	1.129×10^{-7}	5.787×10^{-8}
Shelf life at 4°C (days)	925	1806
Shelf life at 4°C (years)	2.53	4.95
k at 25°C (min^{-1})	1.181×10^{-6}	6.706×10^{-7}
Shelf life at 25°C (days)	88	156
Shelf life at 25°C (years)	0.24	0.43

Figures 3A and B show the plot of log k vs 1/T of aspartame in phosphate buffer and the DCA solution, respectively. There is sufficient linearity to the plot to justify estimation of the shelf-lives that are presented in Table 3.

Further studies conducted at the recommended storage condition of 4°C for 1 year did not show any decomposition of the components of oral drug formulation and no fungal or bacterial growth was present. Therefore, the formulation can be stored refrigerated for at least one year.

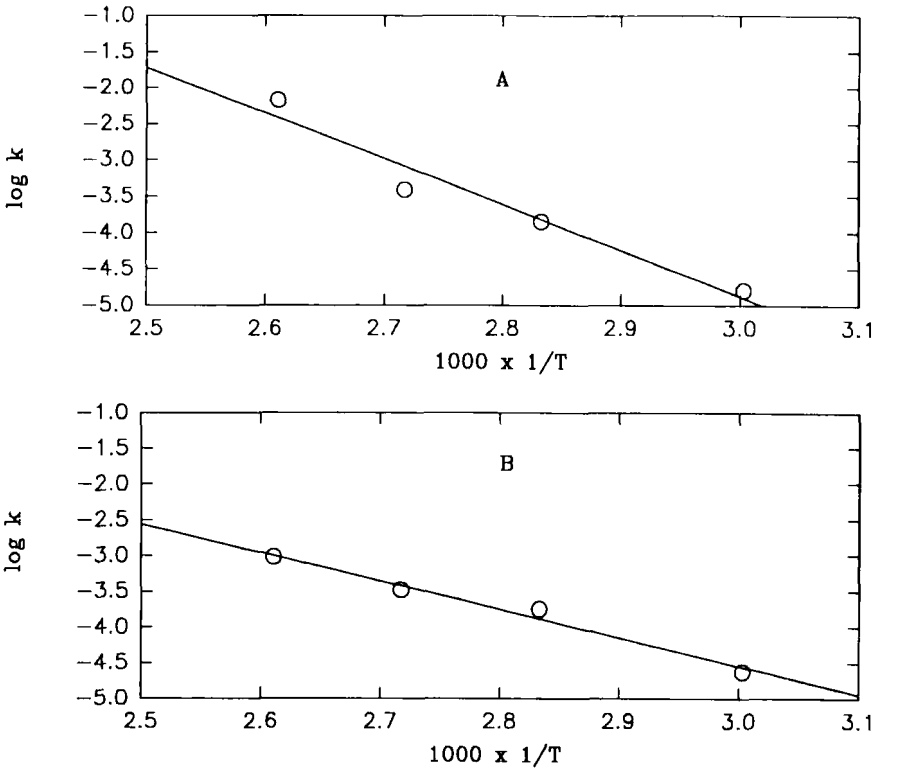

FIGURE 3.

Plot of log k against 1/T for aspartame in phosphate buffer (A) and in oral DCA formulation (B).

Pharmacokinetic Studies:

The DCA-thiamine formulation has so far been administered in oral doses of 12.5 or 25 mg/kg twice daily for up to 3 years to six children, aged 18 months to 14 years, with congenital lactic acidosis due to mitochondrial enzyme deficiencies. The preparation has been well tolerated and no unpalatability or adverse side effects have been reported.

Periodic pharmacokinetic studies were carried out on the children as part of their treatment. DCA plasma half-lives varied widely from 0.70 to 21.5 hrs with the mean being 7.9 hrs (n=19). Cmax varied from 23 to 167μg/mL (mean 72.7) and Cmin were from 0 to 67.5μg/mL (mean 23.9). Figure 4 shows a typical plasma concentration of DCA after an oral dose for a patient under chronic treatment.

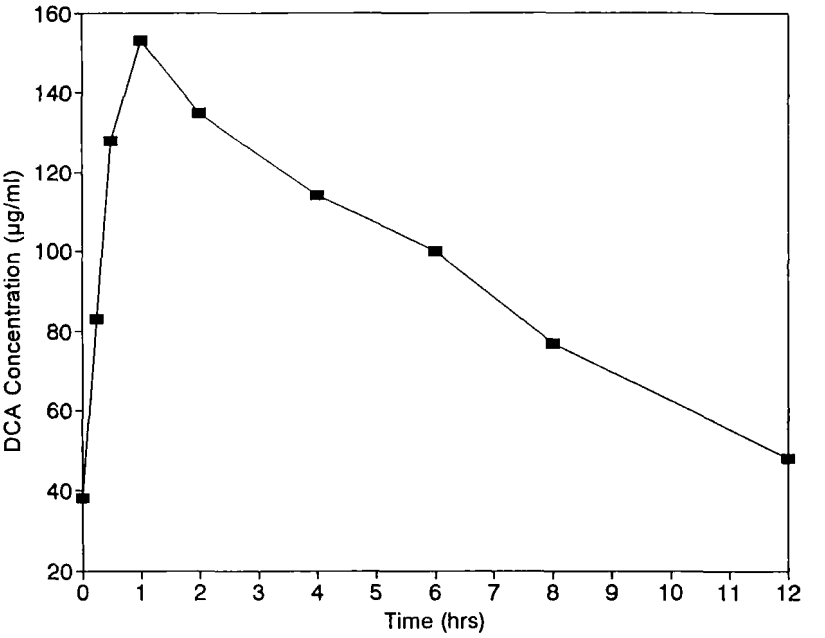

FIGURE 4.

Plasma DCA concentration in a single patient after an oral DCA dose at t=0 (dose = 25 mg/Kg every 12 hrs).

CONCLUSIONS

The study of the stability of thiamine and DCA illustrates that drug preparations of thiamine and DCA in the pH range of 5.5 to 2.5 will be the most stable. An HPLC method was developed for single injection analysis of all the components of an oral DCA formulation containing 5 % DCA, 0.2% thiamine.HCl, 0.1% sodium benzoate and 0.1% aspartame in 0.05 M phosphate buffer at pH 3.5. The formulation has an estimated shelf-life of approximately 5 years at 4°C from an accelerated degradation study. It was found to be stable for at least for one year under refrigeration at 4°C. Preliminary data indicate this formulation is palatable, safe and effective in reducing blood lactate concentrations in children with congenital lactic acidosis.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the secretarial assistance of Melody Riedy and the support of NIH grant DK35448.

REFERENCES

1. P.W. Stacpoole, Metabolism, 38, 1124 (1989).
2. T.J. Wargovich, R.G. MacDonald, J.A. Hill, R.L. Feldman, P.W. Stacpoole and C.J. Pepine, Am. J. Cardiol., 61, 65 (1988).
3. P.W. Stacpoole, E.M. Harman, S.H. Curry, T.G. Baumgartner and R.I. Misbin, N. Engl. J. Med., 309, 390 (1983).
4. D.C. DeVivo, A.H. Jackson, C. Wade, K. Altman and P.W. Stacpoole, Ann. Neurol., 28, 437 (1990).
5. P.W. Stacpoole, G.W. Moore and D.M. Kornhauser, N. Engl. J. Med., 298, 526 (1978).
6. P.W. Stacpoole, H.J. Harwood Jr., D. Cameron, S.H. Curry, M. Schneider, I. Alvarez and H.E. Sauberlich, Fund. Appl. Toxicol., 14, 327 (1990).
7. J.W. Levhuk, R.J. Nolly, and N. Lander, Am. J. Hosp. Pharm., 45, 1311 (1988).
8. M.H. Bronson, D.J. Stennett and P.K. Egging, J. Parenter. Enteral Nutr., 12, 25 (1988).
9. S.H. Curry, P.I. Chu, T.G. Baumgartner and P.W. Stacpoole, Clin. Pharmacol. Ther., 37, 89 (1985).
10. P.G. Wells, G.W. Moore, D. Rabin, G.R. Wilkinson, A.J. Oates and P.W. Stacpoole, Diabetologia, 19, 109 (1980) .
11. J.C. Somogyi, J. Nutr. Sci. Vitaminol., 22 (Suppl.), 29 (1976).
12. D.L. Hilker and A.J. Clifford, J. Chromatogr., 231, 433 (1982).
13. S.M. Gaines and J.L. Bada, J. Chromatogr., 389, 219 (1987).
14. Y. Maeda, M. Yamamoto, K. Owada, S. Sato, T. Masui and H. Nakaswa, J. Assoc. Off. Anal. Chem., 72, 244 (1989).
15. P.I. Chu, "Pharmacokinetics of sodium dichloroacetate", Ph.D. Dissertation, University of Florida, Gainesville, FL. (1987).
16. K.I.H. Farrer, Biochem. J., 41, 162 (1947).
17. B.E. Holmer, in "Aspartame Physiology and Biochemistry", L.D. Stegink and L.J. Filer, Jr., eds., Marcel Dekker, Inc., New York, 1984, p 247.
18. Council Report on Aspartame Safety Issues, Council on Scientific Affairs, Aspartame - Review of safety issues, J. Am. Med. Assn., 254, 400 (1985).
19. NutraSweet Co Bulletin, The NutrSweet Center, 1751 Lake Cook Road, Deerfield, IL 60015. (2/1989).
20. E.R. Garrett and R.F. Carper, J. Am. Phar. Assn., XLIV, 515 (1955).