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Solubilization and stabilization of an investigational antineoplastic drug (NSC no. 278214) in an intravenous formulation using an emulsion vehicle

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Summary

The use of parenteral fat emulsions for development of an extemporaneous preparation of an intravenous formulation of a poorly water-soluble and unstable investigational anticancer agent (NSC no. 278214) is presented. The incorporation into a commercial fat emulsion of the drug, dissolved in dimethylacetamide-cremophor solution, results in a suitable parenteral formulation in which the drug is approximately 100-fold more stable than in simple aqueous solutions.

Introduction

Due to the exciting and interesting results obtained in the antineoplastic testing in suitable screening systems (Anderson et al., 1980), carbamic acid (1-methylethyl)-, [5-(3,4-dichlorophenyl)-2,3-dihydro-1H-pyrrolizine-6,7-diyl] bis(methylene) ester (NSC no. 278214; I) was selected for investigation for further antitumor evaluation and to that end the development of a suitable intravenous formulation was undertaken.

The initial concern in the formulation of I was its insolubility. Since compounds of this type are extremely weak bases (Perrin et al., 1965), $pK_b - 14$, salt formation was not a viable approach for enhancing the solubility of I. An alternative approach to solubilization might normally be considered to be the use of organic co-solvent

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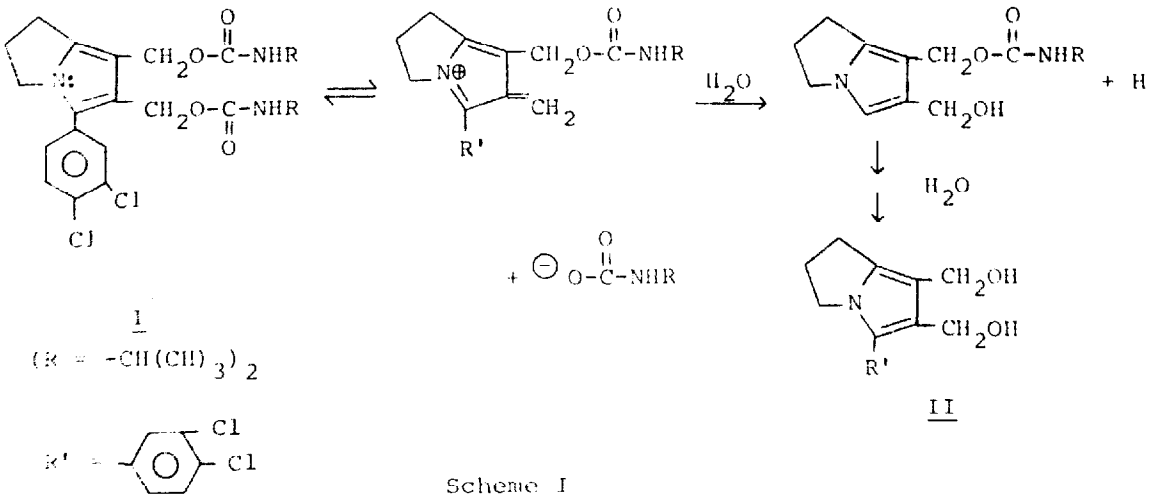
mixtures. However, during the course of preliminary studies involving water–organic co-solvent mixtures, it became apparent that the chemical stability of I was also a very serious problem, with extensive degradation occurring in less than an hour in mixed organic–aqueous solutions. While carbamates as a chemical class are not highly stable (Kuhr and Dorough, 1976; Dittert and Higuchi, 1963), the rate of hydrolysis in the present case was exceptionally fast. On the basis of stability studies and chemical data for related compounds (Anderson and Corey, 1977), this is probably due to conjugation of the electron pair of the tertiary amine with allylic methylene groups involved in the carbamate ester functionality as shown in Scheme I.



Scheme I

One possible approach to enhancing both solubility and stability of I (and related compounds) is through the prepartion of a suitable pro-drug. From the work of Dittert and Higuchi (1963), it appears that substantial enhancement of stability of carbamate linkages may be achieved by replacing the hydrogen atom of the carbamate nitrogen with an alkyl substituent. Such derivitization of the carbamate might be accomplished by the formation of either a N-hydroxymethyl derivative (Johansen and Bundgaard, 1979; Soignet et al., 1972) or an N-Mannich base (Bundgaard and Johansen, 1980). Both derivatives would be expected to revert to the parent compound (I) under appropriate conditions in aqueous media (Bundgaard and Johansen, 1979; Johansen and Bundgaard, 1980), although the reversion rate might be rather slow. However, in view of the chemistry depicted in Scheme I, it seemed unlikely such derivatives of the carbamate nitrogen would be likely to significantly affect carbamate hydrolysis in the present case since cleavage appeared to involve the O-alkyl bond (Anderson and Corey, 1977) rather than the O-acyl bond as occurs in those carbamates shown to be stabilized by a second N-substituent (Dittert and Higuchi, 1963).

While a species such as a 'soft' quaternary derivative (Bodor et al., 1980) of the tertiary N-atom in the pyrrolizine portion of NSC no. 278214 might be expected to decrease the rate of hydrolysis of the carbamate linkage through localizing the electron pair on the N-atom, the synthesis of such a species directly from I

was unsuccessful, presumably, because of the poor nucleophilic reactivity of the tertiary nitrogen.

Another alternative pro-drug approach involved the formation of a N-oxide of the pyrrolizine tertiary nitrogen atom. Such a derivative would again be expected to exhibit enhanced stability through a decreased availability of the electron pair of the N-atom. However, the N-oxide would require enzymatic reduction to the parent compound. A literature report on indicine N-oxide (Powis et al., 1979) suggests that in vivo N-oxide reduction does not occur to any appreciable extent.

In addition to the stability considerations observed above, the co-existent poor water solubility of NSC no. 278214 was also a concern and did not appear likely to be greatly enhanced by any of the pro-drug approaches considered.

Based on the above findings and considerations, the formulation of compound I in o/w emulsion was considered. Fortner et al. (1975) described the formulation of a parenteral preparation of a water-insoluble antineoplastic agent, methyl CCNU [1-(2-chloroethyl)-3-(4-methyl cyclohexyl)-1-nitrosourea] in an intravenous emulsion vehicle. Although no details were provided, it was alleged that the drug was stable for 8 h at room temperature. Emulsions have also been used as parenteral vehicles for other lipid-soluble drugs including diazepam (Jeppsson and Ljunberg, 1975), cyclandelate (Jeppsson and Ljunberg, 1973), barbiturates (Jeppsson, 1972), and valinomycin (Repta, 1981). The tolerance for the emulsion vehicle itself has been shown to be high in animal experiments (Jeppsson and Ljunberg, 1979). Also, the incidence of thrombophlebitis in patients injected with barbiturate-containing emulsions was lower than patients injected with an aqueous solution (Van Dardel et al., 1973) of the corresponding barbiturates.

Valinomycin, an antitumor agent, was formulated in emulsion form utilizing the commercially available Intralipid-10% (Repta, 1981). Animal testing of the system (Repta, 1981; Johnson, 1981) showed that the emulsion formulation produced similarly shaped dose–response curves to that of an aqueous suspension, but the emulsion formulation required a 30-fold lower dose than the suspension to produce similar effects.

The enhanced stability afforded a substance, which is unstable in aqueous media, by dissolving in the oil phase of an emulsion has been recently discussed (Repta, 1981). For a compound which undergoes first-order loss in aqueous media and is essentially stable in the oil phase, the observed rate constant may be expressed as

$$k_{obs} = \frac{k_a}{1 + K_p\phi} \tag{1}$$

where k_a is the rate constant for the loss of drug in the aqueous media; K_p is the partition coefficient for the substance between the oily and aqueous phases; and ϕ is the phase volume ratio of oil to water. From Eqn. 1 it is clear that as the value of K_p and ϕ are increased, the observed stability would be increased in the emulsion system.

Since preliminary data obtained in this work indicated I was very poorly soluble in water and quite soluble in less polar solvents, it appeared that I was a suitable

candidate for stabilization as well as solubilization in an emulsion vehicle. The purpose of this study was to determine whether or not I (and possibly related compounds) might be formulated as an emulsion and the effects of such a formulation on the mutual stability of the drug and the vehicle. The studies and findings described in the report represent rational development of the approach and data necessary for identifying a suitably acceptable intravenous dosage form of a difficult-to-formulate investigational drug substance.

Experimental

Materials

Compound I was used as supplied by the drug development branch of the National Cancer Institute. Liposyn [1] (Abbott Laboratories), Intralipid-10% and -20% [2] (Cutter-Vitrum Laboratories), and macrogol ricinoleate (Cremophor, Sandoz) was provided (Lot no. BV-RM-80-1088) by Ben Venue Laboratories, Cleveland, OH. All other chemicals were commercially available analytical or chromatographic grades.

Method of analysis of I

Analysis of I was done at ambient temperature by normal phase HPLC on a 30 cm × 3.9 mm μ-Bondapak-CN column (Waters Ass., Milford, MA) using a mobile phase of 80% (v/v) isopropanol in hexane at a flow rate of 1 ml/min. Detection was at 280 nm and sensitivity of 0.1 AUFS. Injection volumes of 20 μl were used. Quantitation was done by electronic integration of peak areas of sample and compared with values obtained from calibration curves prepared by dissolving I in dichloromethane. Using this system, the capacity factor of I was $k' = 2.48$. The major degradation product (presumably completely hydrolyzed product II) exhibited $k' = 1.87$.

Extraction of compound I from emulsion

Compound I was extracted from the emulsion formulations using dichloromethane. An aliquot (1 ml) of the formulation was added to 10 ml of dichloromethane contained in a screw-top centrifuge tube. The mixture was agitated on a vortex-Genie mixer (Fisher Scientific) for 5 min. The mixture was centrifuged at 650 × g for 5 min. The upper layer was removed by aspiration and discarded. The remaining (predominantly dichloromethane) layer was then centrifuged for another 5 min and any additional upper layer formed was removed. An aliquot (20 μl) of resulting clear solution was injected and analyzed by high performance liquid chromatography.

The efficiency of the extraction procedure was assessed by shaking 10 ml aliquots

[1] Liposyn contains 10% safflower oil as the internal phase.

[2] Intralipid-10% contains 10% soybean oil as the internal phase of the emulsion, while Intralipid-20% contains 20% of the same oil.

of dichloromethane (containing a range of concentrations of I) with 1 ml of emulsion in a manner analogous to that described above, then separating and assaying the chloroform layer of I, and comparing the concentration of I with that of the unextracted dichloromethane solution. From such studies it was determined that the efficiency of extraction was 95 ± 3% for all of the emulsion systems.

Results and discussion

Initially, a series of studies were conducted for assessing the solution stability and solubility of NSC no. 278214 in order to define potentially promising formulation approaches. On the basis of the data obtained, alternative or modified strategies had to be developed in order to expeditiously achieve a suitable formulation.

Solubility and stability in selected solvents

In the initial studies carried out with I, its solubility and stability in a variety of solvents was evaluated. While dissolution was fast and the apparant solubility was > 4 mg I per ml in methanol, 95% ethanol and formamide, analysis by HPLC showed extensive degradation at room temperature in 1 h in all these solvents. In acetone, ethylacetate, dimethylacetamide (DMA) and dimethylsulfoxide (DMSO), respective solubility values of ~ 12, 5, 110 and 60 mg of I per ml were observed and no significant degradation appeared to occur in 24 h. These results suggested that solvents which are relatively non-polar and which did not contain functional groups with a replaceable hydrogen atom or a strong nucleophilic centes might yield relatively stable solutions of I.

The degradation rate of I in pure water could not be studied due to its low solubility and associated slow rate of dissolution. However, it was possible to prepare stable acetone solutions of I which were then conveniently diluted with water to yield 33% (v/v) aqueous solutions of the drug. Total loss of I (as determined by HPLC) occurred in such systems (at ambient temperature within 15 min of mixing, indicating the very poor stability of I in solutions containing substantial concentrations of water.

The solubility properties of I in commercial soybean and safflower oils were examined, but the rate of dissolution was so slow in these viscous oils that meaningful values could not be directly obtained. An estimate of the solubility of I in both oils was obtained by an indirect approach which involved preparation of concentrated DMA solutions of I. Aliquots of such DMA solutions were added with mixing to each oil at system compositions of 0.8, 1, 2, 3 and 4 mg I/ml in either 5% or 10% (v/v) DMA in each of the oils. While precipitation of I occurred (in ≤ 24 h) from solutions prepared at ≥ 4 mg I/ml in the oils containing both 5% and 10% DMA, no such precipitation was obtained in either oils at ≤ 3 mg I/ml in the presence of 5% and 10% DMA. These results indicate that the intrinsic solubility of I in these oils might be sufficiently great to allow I to be incorporated into the oil phase of o/w emulsion containing either of these oils. Additionally, no drug loss appeared to occur periods of ≤ 24 h in the DMA-oil solutions.

Rather than totally develop an emulsion formulation, the choice was made to evaluate, as vehicles, the commercially available i.v. fat emulsions, Liposyn and Intralipid, which contain safflower and soybean oils as their respective internal phases. In order to utilize these products as vehicles, it was necessary to devise a procedure for rapidly and reproducibly dissolving I in the internal phase of these emulsions. The selected approach involved the use of concentrated solutions of I in the suitable water-miscible organic solvent DMA [3]. In order to assess its suitability for use in this present case, its compatibility with the emulsion vehicles was studied.

The effects of DMA on the physical stability of the emulsion were examined both macroscopically and microscopically over periods of 24 h. At concentrations of 1 and 3% added DMA, no observable change in emulsion stability was observed, while at 5% DMA concentration, the physical characteristics of the emulsion evidenced deterioration at 24 h. This was reflected microscopically by increased oil droplet size. Macroscopic examinations showed that the emulsion appeared to stick to the glass surface of the vials in contrast to the emulsion containing lesser concentrations of DMA.

Thus it appeared that DMA concentrations of $\leq 3\%$ were well tolerated by all 3 emulsion vehicles and the utility of incorporating I into emulsion vehicles as a solution of DMA was subsequently evaluated. Initial attempts involved the addition to 10 ml of the well-stirred emulsions of 100 μl of a DMA solution of I in concentrations sufficient to yield a final product containing 0.3, 0.5, 0.75, 0.90 or 1.0 mg I/ml of emulsion formulation.

From the results of such studies, it became clear that at concentrations of 0.9 or 1 mg I/ml, precpitation of I was frequently encountered even with the most careful addition of the DMA solution to the well-stirred emulsion. This problem may not have reflected the saturation of the oil phase of the emulsion with respect to I, but rather the development of highly supersaturated solution of I due to dilution of the concentrated DMA solution at the point of its contact with the aqueous continuous phase of the emulsion, resulting in precipitate formation before redistribution of I into the oil phase could occur.

In order to minimize the problems of such precipitate formation, all subsequent stability studies were carried out in systems containing 0.7 mg I/ml of emulsion formulation (with ~ 1% DMA).

Stability in various emulsion vehicles

Solutions of I at a concentration of 0.7 mg/ml were prepared in the commercial fat emulsions, Intralipid-10%, Intralipid-20% and Liposyn by careful addition (with agitation) of 95 μl of a DMA solution of I (75 mg I/ml of DMA solution) to 10 ml samples of each of the emulsions. After incorporation of I, aliquots were removed, extracted with dichloromethane and analyzed by HPLC. The chemical degradation of I from such systems was markedly slower than from the aqueous acetone solution mentioned above. The kinetics of degradation in such a system appeared to be a

[3] DMA was the solvent chosen because of the good solubility of I in this solvent, and because of its acceptable status for use in i.v. solutions (NCI Investigational Drugs, a–c, 1981).

TABLE I

VALUES OF k_{obs} FOR THE FIRST-ORDER LOSS OF I FROM EMULSION VEHICLES AT EITHER 20 OR 25°C

Vehicle	$k_{obs}(\times 10^2\ h^{-1})$	T(°C)
Intralipid-10%, 1% DMA	6.8 [a] (7.1) [b]	25
Intralipid-20%, 1% DMA	6.4 [a] (5.7) [b]	25
Liposyn, 1% DMA	9.5 [a] (9.5) [b]	25
Intralipid-10%, 1% DMA, 1% Cremophor	6.8	25
Intralipid-10%, 1% DMA, 3% Cremophor	6.6	25
Intralipid-10%, 1% DMA, 5% Cremophor	5.0	25
Intralipid-10%, 1% DMA, 3% Cremophor	5.5	20
Intralipid-10%, 1% DMA, 5% Cremophor	4.7	20

[a] Unfiltered.

[b] Values in parentheses are for filtered solution.

first-order process and rate constants for degradation of I at 25°C in each of the emulsions is given in Table I. Essentially, identical kinetic results were obtained and drug concentrations were very similar when the emulsion, containing the incorporated drug, was filtered through a 0.45 μm membrane prior to extraction and analysis, indicating that a precipitation of the drug had not occurred during the incorporation process.

From the data obtained in these studies, it appeared clear that Liposyn was not as effective as the Intralipid emulsions in stabilizing I. Although the stability of I in Intralipid-20% appears slightly greater than that found with Intralipid-10%, the difference is far smaller than predicted on the basis of the oil : water phase volume ratios and Eqn. 1 which would suggest a ~ 2-fold greater stability for I in Intralipid-20%. The reasons for this apparant discrepancy were not determined. While it would have been desirable to carefully determine o/w partition values for these systems, this was not feasible because of the rapid degradation of I in the aqueous media. However, it was found that upon centrifugation of the drug–emulsion system that virtually all of the drug added was found associated with the oily phase, suggesting a high oil-to-water partition coefficient.

It was also observed in an independent study that the concentration of I, which could be obtained without precipitate formation (upon addition of 5% (v/v) DMA solution of I), in Intralipid-10% and Intralipid-20% were about 0.95 mg/ml and 1.1 mg/ml, respectively. Since the solubility and stability values of I in the Intralipid-10% were so similar to those for Intralipid-20% and the former can more rapidly be intravenously administered (Facts and comparisons, 1981), subsequent efforts were confined to use of Intralipid-10%.

During the course of the above study, which was encouraging from the standpoint of enhancing stability, it became apparent that there was considerable difficulty in the incorporation of the DMA-drug solution into the emulsions. Although the problem of drug precipitation could be avoided by extremely careful addition of the

drug solution to the emulsion, considerable skill and practice was required, thereby rendering such a system unsuitable for routine use in a clinical situation. Consequently, we examined the utility of the surfactant, Cremophor (polyethoxylated castor oil), as an adjuvant which might enhance the ease of incorporation of I into the emulsions. The intravenous use of the non-ionic surfactant, Cremophor, has been reported previously and has been used to advantage in the formulation of some investigational drugs (Davignon et al., 1972; NCI Investigational Drugs, 1980).

Because Cremophor is a very viscous oil, direct dissolution of I in Cremophor was not practical. This problem was overcome by again preparing concentrated DMA solutions of I and mixing these with Cremophor to yield clear solutions.

When various compositions of DMA–Cremophor solutions containing I were added to the fat emulsion, it was found that incorporation of I without precipitation was much more easily accomplished. The procedure involved dropwise addition (using a syringe equipped with a 22-gauge needle) of solutions of I in the DMA–Cremophor mixtures to a 20 ml serum vial containing 10 ml of emulsion. The vial was swirled either by hand or by manually holding-in-place on a vortex mixer. Cremophor concentrations tested were either 1, 3 or 5% (v/v) in the final preparation while the DMA concentration remained constant at ~ 1%. The final concentration of I was 0.7 mg/ml in all cases.

When the stability of I in the emulsion containing DMA–Cremophor was monitored by extraction of I and its subsequent analysis by HPLC, it was observed that loss of I appeared to be a first-order process. However, slightly enhanced stability (over that observed in the systems containing no Cremophor) was observed at 25°C in all systems as shown in Table I. When the temperature was lowered to 20°C, which better approximates ambient temperature in clinical settings, the stability was further enhanced. Under such conditions, 10% loss of drug was observed in ~ 1.9 h in the 3% Cremophor preparation and in ~ 2.2 h in the formulation containing 5% Cremophor.

In order to establish that the enhanced stability of I in the Cremophor-containing emulsion was not due to micellar solubilization by Cremophor alone, the stability of I in aqueous solutions containing 5% Cremophor, 5% DMA and 0.7 mg/1 ml were studied [4]. In such systems, first-order loss of drug at 25°C occurred with a rate constant of 0.259 h^{-1} ($t_{1/2}$ ~ 2.7 h). The enhanced stability and solubility of the drug in the system suggests that the drug is solubilized in a micellar phase which also affords some protection from hydrolysis. However, the stability of I in the surfactant solution is much less than in the emulsion.

Assessment of stability of I in DMA–Cremophor

On the basis of the results of the above studies, it appeared that an emulsion-based formulation of I, suitably stable for animal testing and subsequent clinical use, could be developed. However, the limited drug stability in such a formulation would require preparation just prior to use. The strategy developed to extemporaneously

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[4] These were prepared by adding 100 μl of a 1 : 1 DMA-Cremophor solution of I to 9 ml of water.

prepare a sterile product involved the preparation of a sterile DMA–Cremophor solution of I which could be aseptically incorporated into a commercially available sterile Intralipid-10% emulsion.

In order for such an approach to be useful, the stability of I in DMA-Cremophor solution needed to be assessed. Solutions containing 375 mg I dissolved in a mixture of 5 ml of DMA and either 27 or 16 ml of Cremophor [5] were prepared and stored at either 4, 25 or 50°C, and the stability of I under these conditions was monitored by HPLC over a 24-week period. At both 4 and 25°C no loss of drug could be detected over the 24-week period. At 50°C the solutions were less stable with 10% loss of I occurring at about 8 weeks and 25–27% loss at 24 weeks in both solutions.

These results indicate that the Cremophor–DMA solutions of I might be adequately stable for more than a year at refrigerated (and perhaps even at ambient) temperatures, thereby permitting the production and distribution of a sterile solution of I which could be used in the extemporaneous preparation of an emulsion-based formulation of I. It is anticipated that the in vivo evaluation of this formulation will be undertaken by the National Cancer Institute in the near future.

Conclusions

The studies and results described here demonstrate that I, a water-insoluble and unstable experimental anticancer agent, may be satisfactorily formulated for intravenous administration by incorporating it into Intralipid, a commercially available parenteral o/w emulsion. On the basis of these findings and previous work done in these laboratories (Repta, 1981) and others (Fortner et al., 1979; Jeppsson, 1972; Jeppsson and Ljunberg, 1973 and 1975), it appears that the use of parenteral emulsions may be generally useful in overcoming the solubility and/or stability problems of selected drug substances for which intravenous formulations are desired and difficult to develop.

Moreover, it seems reasonable and desirable that more extensive use be made of oil-in-water emulsions as vehicles in the screening and evaluation of investigational drug substances which are poorly water-soluble and/or which in their early stages of evaluation/development do not merit extensive, costly and time-consuming formulation studies. The merit of the suggested use of parenteral emulsions stems from the expectation that the limiting factor in the availability of such drugs for absorption, whether given orally or parenterally (as suspensions) may be the dissolution rate while in those cases where the drug would be dissolved in the oil phase of the emulsion the in vivo dissolution step is obviously bypassed.

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[5] Solutions of this composition when incorporated into 500 ml of Intralipid-10% would yield products containing ~ 0.7 mg I/ml, 0.1% (v/v) DMA and either ~ 3% or 5% (v/v) Cremophor.

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