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The effect of solvent composition upon the blood and lymph levels of phenytoin in rats after gastric administration

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Summary

The effects of six different solvents on the blood and lymph levels of [^{14}C]phenytoin after intragastric administration were studied in rats. The solvents studied were a diverse group which consisted of water, olive oil, 10% Liposyn, 10% ethanol, and aqueous solutions of 1% and 2.5% Na oleate. Chronic collection of lymphatic fluid from the unanesthetized rat was accomplished by cannulation of the major lymphatic structure in the abdominal cavity, the cisterna chyli, which is the terminal sac of the thoracic lymph duct. Blood and lymph were sampled for 24 h. The solvents which resulted in the greatest bioavailability in blood and lymph were the 10% ethanol and 1% Na oleate solutions. The 10% ethanol, olive oil and 10% Liposyn treatments resulted in total lymph volumes which were statistically larger ($P < 0.05$) than the other groups. Thus, the solvent may not only affect the bioavailability of a drug in the blood and lymph, but may also affect the lymphatic flow. This may be of significance in the design of dosage forms which are concerned with both the concentration of the drug in the blood and lymph, and with the total amount of drug delivered into the lymph.

Introduction

The use of the lymphatic system as an alternative route of drug absorption and distribution after oral administration has a least two significant advantages over the vascular route: (1) circumvention of the hepatic first-pass effect; and (2) higher concentrations or amounts of drug in the lymphatic system. The latter characteristic may be preferable in some disease states. The former advantage could result in an increase in bioavailability if the drug undergoes an extensive hepatic first-pass effect. Orally absorbed compounds which exhibit a significant first-pass effect may produce active hepatic metabolites in sufficient concentrations to effect an unwanted pharmacological response. If an increased amount of the drug could be absorbed through the lymphatic route, rather than the portal route, these metabolites may no longer be produced in sufficient concentrations to exert the undesired side-effect. Information regarding the effects of solvents upon bioavailability in the blood and lymph may aid in dosage form development.

The use of a lipophilic vehicle to enhance the adsorption of a second compound has been noted with sesame oil (Giannina et al., 1966), corn or olive oil (Williams, 1959), arachis oil (Palin et al., 1982) and long-chain fatty acids with bile salts

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(Inui et al., 1976; Muranishi et al., 1980). Some of these studies used micellar solutions (Inui et al., 1976; Muranishi et al., 1980), emulsions (Carrigan and Bates, 1973), or liposomes (Ueda et al., 1981). Several lipophilic compounds have been noted to appear in the lymph after absorption from the gastrointestinal tract (Sieber et al., 1974). An examination of the numerous lymph sampling studies in the literature indicates that the rate and extent of drug appearance in the lymph was as varied as the vehicles and constituents. Data is presented in this study to demonstrate the effect of six diverse solvents (water, olive oil, 10% Liposyn, 10% ethanol, and aqueous solutions of 1% and 2.5% Na oleate) upon the blood and lymph levels of the lipophilic drug phenytoin.

Materials and Methods

Male Sprague–Dawley rats (210–225 g) were surgically prepared for the chronic collection of lymphatic fluid (Bollman et al., 1948). Anesthesia was induced and maintained by diethyl ether inhalation. A left, lateral, subcostal incision was made. The major lymphatic structure in the abdomen (cisterna chyli) is located lateral to the left kidney and dorsal to the aorta and inferior vena cava. The cisterna chyli was exposed and ligated near the diaphragm. A polyethylene cannula (0.58×0.965 mm, Clay Adams, Parisppany, NJ) was inserted and secured with cyanoacrylate adhesive (Permabond 910, Permabond International, Englewood, NJ). The cannula was externalized between the left thigh and the anus. The subcostal incision was closed. The rat was placed in a restraining cage which prevented him from rolling and displacing the cannula (Bollman, 1948). Water was allowed ad libitum. Food was available two hours after completion of the surgery. The rats were observed over 12 h to verify successful implantation of the cannula and a lymph flow of approximately 0.5 ml/h was observed.

Carbon-14-labeled phenytoin with a specific activity of 200 μCi/mg (New England Nuclear, Boston, MA) was tested for purity by thin-layer chromatography on silica gel with a fluorescent indicator at 254 nm (Polygram SilG/UV Instruments, Westbury, NY). Solvent systems of chloroform : acetone (7 : 3) and diethyl ether : dioxane (75 : 30) indicated a radiochemical purity of at least 96%.

A non-cannulated, or control, group ($n = 6$) and a surgically cannulated group ($n = 6$) were administered the test solvents. The solvents were deionized water, 10% ethanol (ethanol U.S.P., Aaper Alcohol and Chemicals, Shelbyville, KY), olive oil (Aldrich Chemicals, Milwaukee, WI), a commercial 10% safflower oil microemulsion (10% Liposyn, Abbott Laboratories, North Chicago, IL), 1% and 2.5% Na oleate (J.T. Baker Chemicals, Phillipsburg, NJ) as aqueous solutions. A small amount of food was allowed the evening prior to the study. Water was allowed ad libitum. On the day of the study the rats were lightly anesthetized with diethyl ether inhalation. Five micrograms of [^{14}C]phenytoin in 50 μl of ethanol was loaded into a pediatric feeding tube (Pharmaseal, Toa Alta, Puerto Rico) with 1 ml of the test vehicle and injected intragastrically. The rats were weighed and placed in the restraining cages. Recovery from the light anesthesia occurred during placement of the rat in the restraining cage. 100 μl blood samples were collected from the tail tip at 30 min intervals for the first 6 h, then every hour for the next 6 h, and a final sample at 24 h. Lymph fluid was collected in vials over 30 min intervals for the first 6 h, every 2 h for the next 6 h, and a final sample at 24 h. Total lymph volumes during the collection intervals were determined gravimetrically.

The protocol for preparing the blood samples for liquid scintillation counting was a modified New England Nuclear procedure (Liquid Scintillation Counting, 1981). 100 μl of unclotted blood was added to a 22 ml glass scintillation vial containing 0.5 ml of a 1 : 2 Protosol : ethanol solution (New England Nuclear, Boston, MA). The mixture was incubated at 60°C for 1 h. Then 0.5 ml of 30% H_2O_2 (Fisher Scientific, Fair Lawn, NJ) was added. The vial was allowed to incubate at room temperature for 2 h. Observation of the vial was necessary to prevent a possible loss of contents due to foaming. To the vial 0.5 ml of 0.5 N HCl (MCB Chemicals, Norwood, OH) was then added and gently swirled. 15 ml of scintillation cocktail

TABLE 1

AVERAGED DATA FOR TIME-TO-PEAK DPM, MAXIMUM DPM OBSERVED AND THE AREA UNDER THE CURVE FOR THE ACTIVITY FOUND IN THE BLOOD OF THE CANNULATED RATS (n = 6 FOR EACH TREATMENT)

Parameter	Averaged data (S.D.)					
	Water	Olive oil	10% Liposyn	10% Ethanol	1% Na oleate	2.5% Na oleate
AUC_{0-24h}[a] (dpm · h/ml × 10^4)	19.11 (2.80)	14.43 (2.86)	15.45 (2.75)	23.03 (8.02)	25.72 (7.86)	11.64 (1.05)
Time-to-peak dpm (h)	1.33 (0.52)	1.17 (0.75)	1.42 (0.58)	1.25 (0.76)	1.42 (0.38)	1.83 (0.68)
Maximum dpm observed[b] (dpm/100 μl)	1908 (510)	1681 (360)	1275 (208)	2401 (959)	2017 (405)	1526 (191)

Results of the Newman-Keuls Analysis (0.05 level of significance).
[a] 1% Na oleate > 10% Liposyn, olive oil, 2.5% Na oleate, 10% ethanol > 2.5% Na oleate.
[b] 10% ethanol > 10% Liposyn.

(Ready-Solv MP, Beckman Instruments, Fullerton, CA) was added. The samples were allowed to stand for 24 h to decrease the background counts. Each sample was then counted twice during a 24 h period in a Beckman LS 7500 Liquid Scintillation Counter equipped with automatic quench control. The 100 μl lymph samples were mixed with 15 ml of the scintillation cocktail, allowed to stand for 24 h, and counted twice over 24 h.

An analysis of variance (ANOVA) was performed on the time-to-peak-dpm, maximum dpm observed, and the area under the curve (AUC_{0-24h}) for the blood and lymph data (Tables 1 and 2). If a significant difference existed, the Newman-Keuls method (Snedecor and Cochran, 1980) was used to determine which means were statistically different. Each treatment group consisted of six rats. All analyses were performed at the 0.05 level of significance. In addition, a within treatment comparison of the time to peak dpm, maximum dpm observed, and AUC for the blood of the cannulated group and the blood of the non-can-

TABLE 2

AVERAGED DATA FOR TIME TO PEAK DPM, MAXIMUM DPM OBSERVED, AND THE AREA UNDER THE CURVE FOR THE ACTIVITY FOUND IN THE LYMPH OF THE CANNULATED RATS (n = 6 FOR EACH TREATMENT)

Parameter	Averaged data (S.D.)					
	Water	Olive oil	10% Liposyn	10% Ethanol	1% Na oleate	2.5% Na oleate
AUC_{0-18h}[a] (dpm · h/ml × 10^4)	15.26 (1.11)	13.48 (2.93)	14.20 (3.02)	22.70 (5.69)	22.10 (3.33)	11.11 (1.06)
Time-to-peak dpm (h)	1.67 (0.61)	1.08 (0.20)	1.58 (0.49)	1.50 (0.55)	1.58 (0.59)	1.25 (0.99)
Maximum dpm observed[b] (dpm/100 μl)	1319 (186)	1231 (196)	1372 (558)	1700 (381)	2075 (502)	1837 (242)

Results of the Newman-Keuls Analysis (0.05 level of significance).
[a] 10% ethanol, 1% Na oleate > water, 10% Liposyn, 2.5% Na oleate.
[b] 1% Na oleate > olive oil.

nulated group was performed to determine if the surgery had affected the rate or extent of absorption.

Results

The effects of the different solvents on the blood and lymph radioactivity–time curves are presented in Figs. 1–3. The 1% Na oleate solution exhibited a high radioactivity in the blood and lymph for approximately 12 h. Fig. 3 demonstrates the effect of the two Na oleate solutions on the blood curves in comparison to the water treatment.

The results of the within treatment Student's *t*-test comparing the time-to-peak dpm, maximum dpm observed, and AUC_{0-24h} in the blood of the cannulated rats versus the blood of the non-cannulated rats indicated no statistical differences existed.

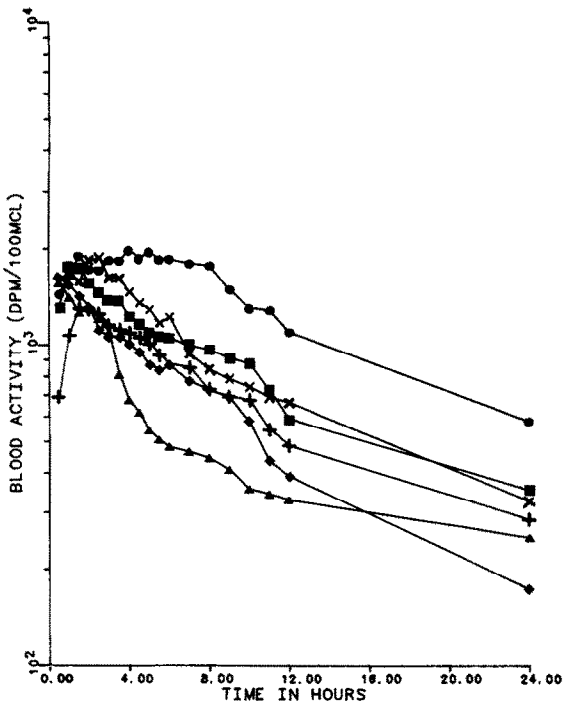

Fig. 1. Blood radioactivity–time curves for the [^{14}C]phenytoin after intragastric coadministration with one of the test solvents. Each point is the mean radioactivity found in 100 μl of blood from 6 rats. The solvents were water (■), olive oil (◆), 10% Liposyn (+), 10% ethanol (×), 1% Na oleate aqueous solution (●), and a 2.5% Na oleate aqueous solution (▲).

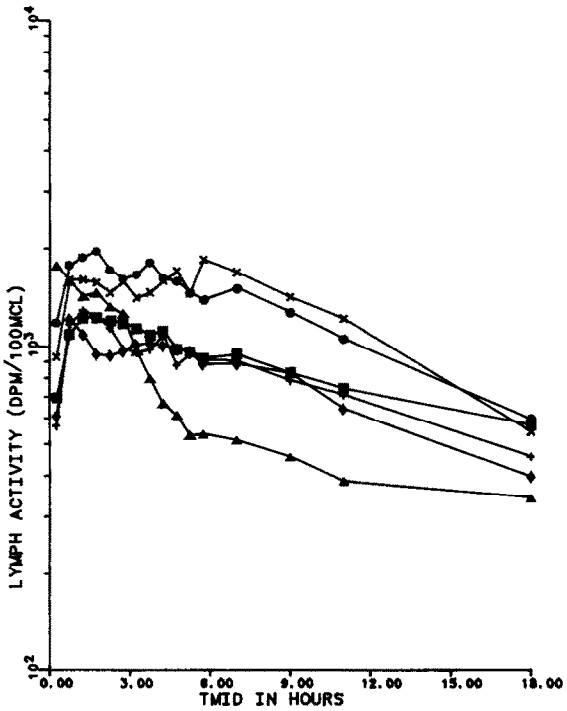

Fig. 2. Lymph radioactivity–time curves for the [^{14}C]phenytoin after intragastric coadministration with one of the test solvents. Each point is the mean radioactivity found in 100 μl of lymph from 6 rats. The solvents were water (■), olive oil (◆), 10% Liposyn (+), 10% ethanol (×), 1% Na oleate aqueous solution (●), and a 2.5% Na oleate aqueous solution (▲). Note that the data points are plotted at the midpoint of the collection interval.

The ANOVA of the blood $AUC_{0-24\ h}$ demonstrated statistically significant differences among treatments. All areas were calculated using the trapezoidal rule. The Newman-Keuls comparison between treatment means resulted in the following differences: 1% Na oleate > 10% Liposyn, olive oil, 2.5% Na oleate; and 10% ethanol > 2.5% Na oleate. Since no data point was taken between 12 and 24 h, the AUC_{0-12h} was also analyzed. The results of the ANOVA and Newman-Keuls analysis were identical to the AUC_{0-24h} data. ANOVA of the lymph AUC_{0-18h} among treatments was also statistically significant and resulted in these specific differences between treatments: 10%

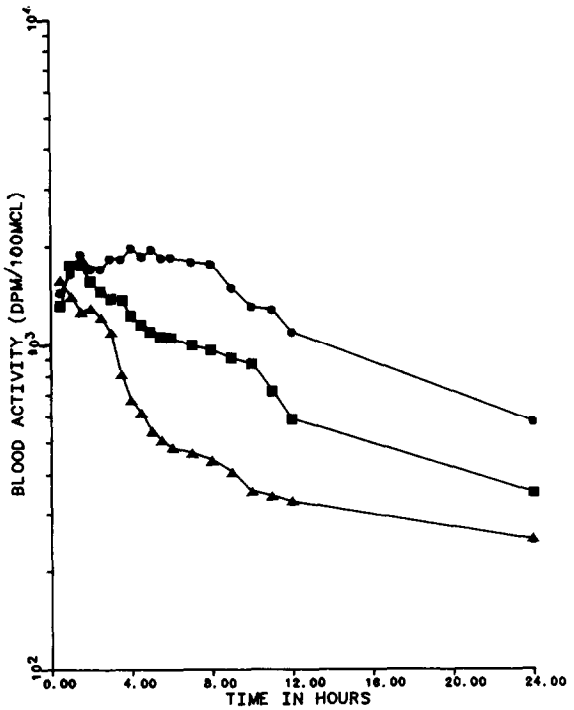

Fig. 3. Blood radioactivity--time curves illustrating the effect of the different Na oleate concentrations upon the blood curves of [^{14}C]phenytoin when compared to the water treatment. Water treatment (■), 1% Na oleate aqueous solution (●), and a 2.5% Na oleate aqueous solution (▲).

ethanol, 1% Na oleate > water, 10% Liposyn, olive oil, 2.5% Na oleate.

ANOVA of the maximum dpm observed in the blood and lymph of each treatment demonstrated significant differences among treatments. The mean of the 10% ethanol treatment was significantly greater than the 10% Liposyn in the blood, and the mean of the 1% Na oleate was greater than the olive oil treatment in the lymph. There was no statistical difference between the time-to-peak dpm in any of the treatments in either the blood or the lymph.

Table 3 presents the data for the total radioactivity collected in the lymph and the total volume of lymph collected over the 24 h study. Significant differences were found between the treatments in both categories. The 10% ethanol treatment had significantly greater total lymph radioactivity than the water, 10% Liposyn, 1% Na oleate, and 2.5% Na oleate treatments. Specific differences were also found in the total volume of lymph collected over 24 h: olive oil > water and the 1% Na oleate treatment; 10% ethanol > 1% Na oleate treatment; and the 10% Liposyn > 2.5% Na oleate treatment.

TABLE 3

AVERAGED DATA FOR TOTAL LYMPH COUNTS AND TOTAL LYMPH VOLUMES COLLECTED OVER 24 h (n = 6 FOR EACH TREATMENT)

Treatment	Averaged data (S.D.)	
	Total lymph counts [a] (dpm × 10^3)	Total lymph volume [b] (ml)
Water	43.26 (6.20)	13.71 (1.52)
Olive oil	53.29 (5.43)	18.98 (2.13)
10% Ethanol	73.00 (14.45)	17.98 (1.91)
1% Na oleate	50.20 (15.36)	12.78 (1.98)
2.5% Na oleate	40.39 (2.82)	16.37 (1.62)
10% Liposyn	42.26 (5.43)	17.62 (2.13)

Results of the Newman-Keuls Analysis (0.05 level of significance).

[a] 10% Ethanol > 1% Na oleate, water, 10% Liposyn, 2.5% Na oleate.

[b] Olive oil > water, 1% Na oleate, 10% Ethanol > 1% oleate.

Discussion

The solubility of phenytoin in water at pH 7.0 and 24°C is approximately 14 μg/ml, and approximately 100 μg/ml in intestinal fluid at pH 7.8 at 37°C (Woodbury et al., 1972). In this study 5 μg of [^{14}C]phenytoin in 50 μl of ethanol was loaded into a pediatric feeding tube with 1 μl of a test solvent for intragastric coadministration. Once injected into the stomach it can be assumed that this minute amount of labeled drug was completely dissolved either in the test solvent alone, or in combination with the fluid present in the gastric milieu. Other studies in rats have shown virtually 100% oral absorption of 100 μg/ml phenytoin after 90 min (Woodbury et al., 1972). Therefore, the differences in the blood curves observed in Fig. 1 are probably due to the coadministration of the solvent and not to factors affecting solubility.

The figures presented depict the total radioactivity collected in the blood and lymph as a function of time, thus, they include both parent compound and metabolites. Phenytoin undergoes rapid hepatic oxidation. The major metabolite is the 5-(*p*-hydroxphenyl)-5-phenylhydantoin which undergoes glucuronidation and renal excretion. After a single dose administration of 5 μg no accumulation of the metabolites is expected. Thus, the majority of the radioactivity in the blood and lymph should reflect the concentration of the parent compound.

A study of the blood and lymph curves in Figs. 1 and 2 demonstrated that the treatments which were the most bioavailable in the blood were also the most bioavailable in the lymph. The similarity between the curves may be due to the site of cannulation. The cisterna chyli is the major junction for the lymphatic ducts from the abdomen and the lower limbs. Since the origin of the lymphatic fluid is either the interstitial fluid or the fluid expressed from the capillaries (Guyton, 1976), the concentration of a compound in the lymph may be in equilibrium with the concentration of the compound in the blood. Therefore, in this study, the radioactivity collected in the lymph may not only reflect the absorptive process (via the mesenteric lymph duct) but the total systemic concentration and flow.

An examination of Table 3 demonstrates that the lipid vehicles generally resulted in larger lymph volumes than the non-lipid or low lipid solvents. The absorption of long chain fatty acids preferentially through the lymphatic system has been reported (Bloom et al., 1951). Long chain fatty acids have also been shown to stimulate the flow of lymph (Demarco and Levine, 1969). Since the olive oil (primarily oleic acid) (Cocks and van Rede, 1966), the 10% safflower oil microemulsion (primarily linoleic acid) (Cocks and van Rede, 1966), and oleic acid are all long chain fatty acids the volumes noted in Table 3 are not unexpected. The large volume of lymph generated by the 10% ethanol treatment was unexpected. This may be due to the fact that ethanol has been reported to increase the permeability of the gastric mucosa and stimulate the flow of lymphatic fluid (Majchrowicz and Noble, 1979). An ANOVA of the water, 1% and 2.5% Na oleate treatments indicated a significant difference in the volume of lymph collected from these different treatments. The 2.5% Na oleate solution resulted in a statistically greater volume than the other two treatments. This may indicate that a certain concentration of oleic acid is required to generate a significant increase in the lymph flow.

The treatments that resulted in the highest total amount of radioactivity collected in the lymph were those treatments that had the largest volumes of collected lymph and were the most bioavailable. This is best demonstrated with the 10% ethanol solution. However, high bioavailability is not synonymous with high total lymph radioactivity. The olive oil treatment resulted in a large total radioactivity due to the enhanced lymph flow and despite its low bioavailability data. If one were developing a formulation in which high lymphatic concentration of a drug was the objective then (in this study) the 10% ethanol or the 1% Na oleate solutions would be the logical choices. However, if the amount of drug being delivered to the potential site of action (i.e. the lymph nodes) was the objective, then knowledge of the solvent's effects on the lymph flow is essential to evaluate the total amount of drug delivered to the site of action. Both vehicles demonstrated high bioavailability in the blood and lymph (Tables 1 and 2) but the ethanolic vehicle delivered significantly more drug through the lymphatic system.

Another interesting point about the Na oleate solutions is how the 1% Na oleate solution generated high bioavailability in the blood and lymph and the 2.5% solution demonstrated the opposite. The magnitude of the effect on the blood curves can be seen in Fig. 3. The 2.5% solution also consistently demonstrated a prominent biexponential disposition phase in all 6 rats. It is possible that at this oleate concentration the gastric emptying or motility were inhibited (Hunt and Knox, 1968; Davenport, 1971; Keinke and Ehrlein, 1983) or the labeled drug was bound in micelles which decreased its bioavailability. It is also possible that the oleate affected the systemic transport or distribution of phenytoin through a protein binding interaction (Rudman et al., 1971; Gugler et al., 1974; Spector, 1975). Further studies are under-

way in an attempt to understand this observation.

This study demonstrates that different solvents may significantly affect the bioavailability of a lipophilic drug in the blood and lymph of a rat. The 1% Na oleate solution and the 10% ethanol treatments demonstrated larger amounts of radioactivity in the lymph than the other treatments. Bioavailability and the total amount of drug in the lymph may be dependent upon the effect of the solvent. This effect may be significant when evaluating blood and lymph concentration data without acknowledging the potential effect of the vehicle upon the total amount of drug in the lymph. This may be of importance when one is concerned with not only the drug concentration, but the amounts of drug, being delivered into the lymphatic system.

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