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Report

The Influence of Liposomal Encapsulation on Sodium Cromoglycate Pharmacokinetics in Man

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The pharmacokinetics of pulmonary-administered sodium cromoglycate (SCG) has been studied in five healthy volunteers. SCG, 20 mg, was inhaled as a solution and encapsulated in dipalmitoyl phosphatidylcholine/cholesterol (1:1) liposomes. Liposomal SCG produced detectable drug levels in plasma from four volunteers taken 24 and 25 hr after inhalation. Inhaled SCG solution, although producing peak plasma levels more than sevenfold greater than liposomal drug, was not detectable in 24-hr samples from any volunteer. The decline in plasma levels following inhalation of liposomal SCG (reflecting the absorption phase) was best described by a biexponential equation. The two absorption rate constants differed by more than an order of magnitude. The rapid absorption phase was probably due to free or surface-adsorbed SCG in the liposomal formulation, since the absorption rate constant for this phase did not differ significantly from the absorption rate constant for SCG in solution. The phase of slow drug absorption may then be attributed to absorption of drug released from vesicles. The data indicate that encapsulation of SCG prior to pulmonary administration prolonged drug retention within the lungs and altered its pharmacokinetics.

KEY WORDS: sodium cromoglycate; liposome; liposomal; drug delivery system; pulmonary drug delivery.

INTRODUCTION

Studies of the pulmonary clearance of inhaled ^{99m}Tc-labeled liposomes have shown that the short-term retention profiles for multilamellar vesicles (MLVs) and small unilamellar vesicles (SUVs) were indicative of clearance via the mucociliary transport mechanism (1). Gamma camera images taken 20 hr after dosing indicated that greater than 50% of liposomes remained in the lungs, representing the fraction of liposomes that were alveolar deposited (2). Studies in rats on the pulmonary absorption of cytarabine showed that liposomal encapsulation altered the pharmacokinetics of the drug, resulting in minimal distribution to other organs (3).

Sodium cromoglycate (SCG) is widely used in the prophylactic treatment of bronchial asthma and in other diseases having an allergic basis. SCG is highly polar and is not administered orally due to poor oral absorption (4,5). It is, however, rapidly absorbed from the lungs (4,6) and is rapidly excreted, unmetabolized in the bile and urine of man in approximately equal proportions (5). A sensitive radioimmunoassay method for determining SCG in human plasma has been described (7) and was used to establish maximum protection against exercise-induced asthma when SCG plasma concentrations were 4 ng ml^{-1} or greater (8). Administration of 20 mg SCG as a dry powder aerosol produced peak plasma concentrations of up to 50 ng ml^{-1} (7), which may be 10-fold greater than necessary.

In this paper, the effect of encapsulation of SCG in liposomes on the pharmacokinetics of pulmonary deposited drug was investigated in plasma samples up to 25 hr after inhalation by volunteers of liposomal and nonliposomal formulations.

MATERIALS AND METHODS

Preparation of Liposomes

Two hundred sixty-two milligrams of dipalmitoylphosphatidylcholine (DPPC) (Sigma Chemical Co. Ltd., U.K.) and 138 mg cholesterol (Chol) (99+%, Sigma Chemical Co. Ltd., U.K.) were weighed into a 200-ml long-necked round-bottom flask and dissolved in 60 ml of diethyl ether/chloroform (1:1) (AnalaR, BDH Chemicals Ltd., U.K.). Ten milliliters of a sterile 3.2% solution of SCG in 0.9% (w/v) saline was added. The flask was sealed under nitrogen and the mixture sonicated at 50°C for 6 min to facilitate emulsification. Slow removal of organic solvent at 45°C resulted in the production of REVs, which were extruded through polycarbonate membrane filters (pore diameter, 1.0 μm; Nucleopore Inc., U.S.A.) and maintained for 1 hr at 45°C to anneal the liposome structure (9), before being placed in dialysis sacs.

The liposome preparations were dialyzed for 120 hr at

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4°C against 100 vol of 0.9% (w/v) saline, continuously stirred, and changed four times in 24 hr.

Liposomes were removed from the sacs just prior to administration to volunteers. Free SCG in the liposome preparations was determined by centrifuging samples at 200,000*g* for 30 min and the supernatant assayed for SCG at 326 nm. Total SCG in the preparations was determined from the absorbance at 326 nm in the presence of Triton-X-100 (1% final concentration). The amount of SCG entrapped within vesicles was then calculated by difference.

Size Characterization of Liposomes and the Nebulized Product

The vesicle size of freshly dialyzed liposomes was determined by photon correlation spectroscopy (Malvern Instruments, U.K.). The nebulized product was characterized by directing aerosols generated from liposome preparations with an air-jet nebulizer (Hudson, Henleys Medical Supplies Ltd., U.K.) into a calibrated multistage liquid impinger (10). The mass median aerodynamic diameter (MMAD) and geometric standard deviation (σ_g) were derived from determinations of SCG deposited on each stage of the impinger.

Human Study

The volunteer study received ethics committee approval and volunteers participated with written informed consent.

Five healthy nonsmoking males aged 18 to 40 years took part in the study. Each inhaled, in two experiments, separated by 7 days, 20 mg SCG delivered by an air-jet nebulizer (Hudson; Henleys Medical Supplies Ltd., U.K.) as (a) an aqueous solution in 0.9% (w/v) saline or (b) a DPPC/Chol (1:1) liposome formulation.

The nebulized product was generated with compressed air at 172 kPa and inhaled through a mouthpiece. Volunteers maintained deep, slow inspirations, with periods of breath-holding prior to exhalation, giving a breathing frequency of 6 to 8 cycles/min. Nebulization was continued for the time calculated for delivery of a 20-mg dose, approximately 8 and 15 min for free and liposomal SCG, respectively.

Five-milliliter blood samples were taken at intervals up to 10 hr following commencement of drug inhalation, via an indwelling catheter (Critikon Inc., U.S.A.) inserted into a forearm vein. Further blood samples were taken at 24 and 25 hr by venipuncture. Following centrifugation, plasma was assayed for SCG by radioimmunoassay (7).

RESULTS

The liposomes inhaled by the volunteers had a mean (±SE) entrapment of 21.3 (0.08) mg/100 mg of lipid and a mean diameter of 1.2 μm. Filtered DPPC/Chol (1:1) liposomes were previously found to have a high entrapment of SCG and to be stable to nebulization (11).

Figure 1 shows the mean plasma concentration–time profile for SCG following inhalation as a solution by five volunteers. In each case the delivered dose was 20 mg. Peak levels occurred within 15 min of cessation of drug inhalation, and a maximum plasma concentration of 34.9 ± 7.8 ng ml^{-1} (mean ± SE) was observed. Brown *et al.* (12) reported peak plasma concentrations of 45 ± 10 ng ml^{-1} occurring 5 min after inhalation of 20 mg SCG as a dry powder.

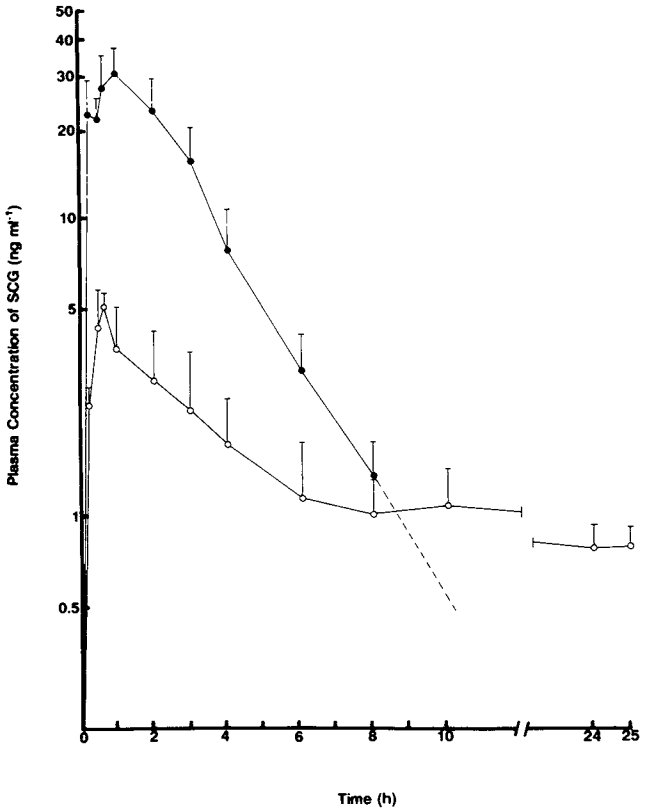

Fig. 1. Plasma levels following nebulization of 20 mg SCG to volunteers. Each point is a mean ± SE. (●) Free SCG (N = 5); (○) liposomal SCG (N = 4).

The mean plasma concentration–time profile for volunteers following inhalation of the liposomal SCG formulation is shown in Fig. 1. The peak plasma concentration produced by the liposome formulation was 4.69 ± 1.37 ng ml^{-1} for four volunteers, occurring within 20 min of cessation of drug inhalation. The liposome preparations inhaled by these volunteers had less than 5% free SCG, greater than 95% being liposomally associated. The fifth volunteer received, in addition to 20 mg entrapped SCG, 12.7 mg free drug (38% of total), due to the incomplete removal of free drug during dialysis of the liposome preparation. The peak plasma concentration for this volunteer was 31.0 ng ml^{-1}, suggesting that the initial peak plasma concentration with liposome formulations was probably the result of rapid absorption of free SCG from the preparations.

An extended least-squares nonlinear regression program (MK Model II plus) using a first-order input and first-order disposition model, adequately described the plasma concentration data for each volunteer following inhalation of the SCG solution. The log plasma concentration–time plots following peak plasma levels indicated a decline in plasma concentration with a clearly defined half-life, significantly longer than that previously reported following intravenous dosing of SCG (12). This occurs when absorption rather than elimination dictates the decline in plasma drug concentra-

tion. This type of "flip-flop" kinetics following pulmonary administration has been previously reported for SCG (13).

The absorption half-life of SCG calculated from the estimate of the absorption rate constant (k_a) following curve fitting was 1.71 ± 0.18 hr and is in close agreement with reported values of 1.61 hr (13) and 2.1 hr (12) for a dry powder SCG aerosol and 2.24 hr for nebulized drug (13). Accurate determination of the elimination rate constant was not possible because of the prolonged nature of drug absorption and the few plasma samples obtained prior to peak levels being attained.

Non-linear curve fitting of the plasma level data indicated that the fall in plasma concentrations, following inhalation of liposomal SCG, was better described by a biexponential rather than a monoexponential decline.

Tables I and II show the pharmacokinetic parameters calculated from least-squares nonlinear curve fitting of data following inhalation of SCG in solution and in the liposome formulation. AUC_∞ was calculated from the plasma concentration–time plots using the linear trapezoidal rule.

A significant proportion of the administered dose was deposited in the mouth and swallowed. The poor absorption of SCG from the gastrointestinal tract (5,12) ensured that the determined plasma concentrations were a true indicator of pulmonary drug absorption.

The lower limit of accurate determination of SCG, by the assay technique, in this work was 0.5 ng ml^{-1}, as previously reported by Brown *et al.* (7). At 24 hr the mean plasma SCG concentration after inhalation of the liposomal formulation in four volunteers was 0.77 ng ml^{-1}, as compared to the free SCG preparation, which produced 24-hr plasma levels of less than 0.5 ng ml^{-1} in all volunteers. Predicted 24-hr plasma concentrations following inhalation of free SCG were less than 0.03 ng ml^{-1}

DISCUSSION

Ethics committee approval for an intravenous study was not obtained because of the possible cardiovascular effects of intravenous SCG (5). A number of pharmacokinetic parameters for SCG following intravenous administration of 4 mg drug were determined by Fuller and Collier (13); these were used in this work for the calculation of bioavailability. A bioavailability of 13.3 ± 4.0% was similar to previous reported values of 12.4 to 23.4% for nebulized SCG solutions and of 9.7% for a dry powder aerosol (13).

Some 80 to 85% of tracheobronchial deposited particles are cleared by the mucociliary clearance process within 6 hr of inhalation, and after 20 hr all remaining particles are in the alveoli (2). Farr *et al.* (1) suggested that the regional deposition of liposomes in the respiratory tract of man is dependent upon the droplet size of the inhaled aerosol cloud rather than on vesicle size. The air-jet nebulizer employed in this study produced droplets having a MMAD of 2.6 μm and a σ_g of 1.9, which have a high probability of deposition in the alveolar lung regions (14). Alveolar clearance mechanisms are very slow; investigations of insoluble particulates in human lung have suggested alveolar clearance half-lives of 240 days to 1 year (15). This suggested that almost all the SCG entrapped in deposited liposomes would ultimately become available to the lung. Hence, if loss of drug from the liposomes is by random destruction of the liposome structure, extrapolation of the plasma concentration–time profile to infinity using the calculated rate constant for absorption of liposomal SCG was justified. The bioavailability using a model-independent AUC_∞ was calculated to be 14.0 ± 3.5%, which did not differ significantly ($P < 0.05$) from the availability of the SCG solution. However, if loss is envisaged as due to the progressive destruction of liposome bilayers, then at a finite time the final bilayer surrounding the aqueous core will be destroyed and all the entrapped drug will be released and, hence, available for absorption. Thus an availability was calculated by extrapolating the plasma concentration curve from 25 hr to infinity with the absorption rate constant for free drug. This value for bioavailability of 3.9 ± 1.5% represents a minimum calculated for complete loss of SCG from liposomes at 25 hr. The true availability of the drug from the liposome formulation will lie between the minimum and the maximum calculated values, and multiple dosing is necessary to characterize properly the terminal phase of the plasma decline before a more accurate value of bioavailability can de derived.

The two absorption rate constants for the liposomal formulation calculated from the fitted data differed by more than an order of magnitude, having mean values of 0.70 and 0.027 hr^{-1}. This suggested that the rapid absorption phase was probably due to the free SCG in the liposome formulation, since the first absorption rate constant did not differ significantly (*t* test) from the absorption rate constant for the SCG solution. The phase of slow drug absorption could then be attributed to absorption of drug released from the pulmonary deposited vesicles. A similar biphasic plasma profile was observed when 6-carboxyfluorescein-containing liposomes were instilled into the lungs of rats (16).

The mean half-life of liposomal drug absorption was cal-

Table I. Pharmacokinetic Parameters of SCG Following Inhalation of 20 mg SCG in Solution[a]

Parameter	Mean value
k_a (hr^{-1})	0.43 ± 0.05
$t_{1/2a}$(hr)	1.7 ± 0.2
C_{max} (ng ml^{-1})	34.9 ± 7.8
t_{max} (min)	21 ± 4
AUC_∞ (μg liter hr^{-1})	101.7 ± 27.0

[a] Mean ± SE; $N = 5$.

Table II. Pharmacokinetic Parameters of SCG Following Inhalation of 20 mg Liposomal SCG[a]

Parameter	Mean value
k_{a_1} (hr^{-1})	0.70 ± 0.14
k_{a_2} (hr^{-1})	0.027 ± 0.015
$t_{1/2a_1}$ (hr)	1.24 ± 0.32
$t_{1/2a_2}$ (hr)	56.9 ± 17.7
C_{max} (ng ml^{-1})	4.7 ± 1.4[b]
t_{max} (min)	30.0 ± 4.1[b]
AUC_∞ (μg liter h^{-1})	102.0 ± 24.1[b]

[a] Mean ± SE; $N = 5$.
[b] $N = 4$.

culated from the terminal portion of the plasma concentration–time plots to be 57 hr. The *in vitro* release half-life for this preparation, determined as described previously (17), was 144 hr. Thus *in vivo* release was more rapid, suggesting that diffusion of drug across liposomal bilayers was not the primary factor determining SCG plasma levels.

A number of processes may have augmented the absorption of liposomal SCG from the lung. Alveolar macrophage activity is considered the most important mechanism of alveolar clearance for deposited particles rapidly phagocytosing inhaled particulates (18). Macrophages contain a number of lipolytic enzymes (19), which are likely to degrade liposomal phospholipid, contributing to the release of encapsulated drug. Scarpelli *et al.* (20) suggested that extracellular phospholipases were involved in the clearance of intrinsic DPPC in the foetal respiratory tract. Fusion of liposomes with lung epithelial cells has been suggested as a mechanism of cellular uptake of intratracheally administered liposomes (21). DPPC cleared from the lung distributes to tissues rich in reticuloendothelial cells (22,23), suggesting that liposomes may be absorbed intact from the lung. Liposomal phospholipid may also exchange with the proteins in lung surfactant (24) in a manner similar to exchange with the surface proteins of red blood cells (25), resulting in the loss of entrapped material.

In conclusion, liposomal encapsulation markedly altered the pharmacokinetics of SCG, with prolonged residence of drug in the lung.

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