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Monensin intercalation in liposomes: effect on cytotoxicities of ricin, *Pseudomonas* exotoxin A and diphtheria toxin in CHO cells

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Monensin, a carboxylic ionophore was intercalated in liposomes (liposomal monensin) and its effect on cytotoxicities of ricin, *Pseudomonas* exotoxin A and diphtheria toxin in CHO cells was studied. Intercalation of monensin in liposomal bilayer is found to have no effect on its stability and interaction with cells. Liposomal monensin (1 nM) substantially enhance the cytotoxicities of ricin (62-fold) and *Pseudomonas* exotoxin A (11.5-foid) while it has no effect on diphtheria toxin. This observed effect is highly dependent on the liposomal lipid composition. The potentiating ability of monensin (1 nM) in neutral vesicles is significantly higher (2.2-fold) as compared to negatively charged vesicles. This ability is drastically reduced by incorporation of stearylamine in liposomes and is found to be dependent on the density of stearylamine as well as on the concentration of serum in the medium. Monensin in liposomes containing 24 mol% stearylamine has a very marginal effect on the cytotoxicity of ricin (7.5-fold) which is further reduced (1.5-fold) in the presence of 20% serum. The uptake of ^{125}I-gelonin from neutral vesicles is significantly higher (~ 2.0-fold) than that from the negative vesicles. The uptake from positive vesicles is highly dependent on the concentration of stearylamine. The reduction in the lag period (30 min) of ricin action by monensin in neutral and negative vesicle is comparable with free monensin. However, monensin in positive vesicle has no effect on it. These studies have suggested that liposomes could be used as a delivery vehicle for monensin for selective elimination of tumor cells in combination with hybrid toxins.

Introduction

Monensin, a carboxylic ionophore is known to alter the morphology of Golgi cisternae, elevate the pH of endosomes and lysosomes and inhibit the transport of certain proteins [1–3]. All these cellular changes have been considered as a plausible basis for potentiation of cytotoxicity of ricin and conjugates of ricin A-chain in cultured cells [4,5]. Similar potentiation effect has been observed with lysosomotropic agents like NH_4Cl and chloroquine [6,7]. Among these potentiators only monensin has been reported to improve the efficacy of immunotoxins marginally under in vivo condition [8]. However, its hydrophobic nature severely limits its administration in optimum doses to realize its full potential as a potentiating agent in vivo. Therefore, it is vital to develop a delivery system for monensin for exploitation of its role as an enhancing agent.

Liposomes have been extensively studied as promising intracytoplasmic delivery vehicle to selectively direct entrapped materials to specific cells or tissues by attaching defined ligands [9,10]. In this paper, we have studied the effect of intercalation of monensin in liposomes on its stability, interaction and potentiating activity on cytotoxicities of ricin, *Pseudomonas* exotoxin A and diphtheria toxin in CHO cells.

Materials

Monensin, cholesterol, lactoperoxidase, stearylamine, dicetyl phosphate and calcein were purchased from Sigma. Egg PC was from Nippons Oils and Fatsco, Japan. [^{3}H]Leucine (153 Ci/mmol) was from NEN Research Products. Na^{125}I (7.2 mCi/μg iodine) was from BARC, India. *Pseudomonas* exotoxin A and diphtheria toxin were purchased from Swiss Serum and Vaccine Institute, Switzerland. FCS and RPMI-1640 medium were from Flow laboratories. Gelonin was purified according to the method described earlier [6].

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Abbreviations: BSA, bovine serum albumin; CHO, Chinese hamster ovary; DBSS, Dulbecco's balanced salt solution; DCP, dicetyl phosphate; DMSO, dimethylsulfoxide; FCS, fetal calf serum; HSA, human serum albumin; PBS, phosphate-buffered saline; PC, phosphatidylcholine; SA, stearylamine.

Methods

Purification of ricin

Ricin was isolated from the seeds of *Ricinus communis* and purified by affinity chromatography on cross-linked Guar Gum following the published procedure described by Appukuttan et al. [11]. This was followed by gel filtration on Sephadex G-100.

Radioiodination of gelonin

Gelonin was radiolabeled with $Na^{125}I$ by the lactoperoxidase catalyzed reaction as described earlier [6]. Briefly, 0.5 mg gelonin (5 mg/ml) was mixed with 4 μl lactoperoxidase, 5 μl 8.8 mM H_2O_2 and 5 μl (0.25 mCi) $Na^{125}I$. The reaction was carried out for 1 h at 37°C with occasional stirring. The iodinated protein was separated from free iodine on Sephadex G-25 column preequilibrated with PBS (50 mM, pH = 7.2) containing 50 μg/ml BSA. The biological activity of ^{125}I-gelonin was comparable to native gelonin.

Cell culture

A CHO cell line auxotropic for proline was a generous gift from Dr. Henry C. Wu (USUHS, USA). This cell line was maintained in RPMI-1640 medium supplemented with 10% FCS, penicillin and streptomycin (100 μg/ml).

Cytotoxicity assay

The cytotoxicities of ricin, *Pseudomonas* exotoxin A and diphtheria toxin were determined from the observed inhibition of [^{3}H]leucine incorporation into protein in cell cultures exposed to varying concentrations of the toxins as described earlier [12]. Cells at a density $4 \cdot 10^5$ cells/well were plated in 24 wells plates 18 h prior to the experiment. The monolayer cultures were washed twice with DBSS and incubated with different concentrations of either free or liposomal monensin. The control experiments were performed by preincubating the cells either with DBSS or liposomes having respective charges but without monensin. After 1 h, varying concentrations of the toxins were added for different time periods. Cells were then washed and incubated with [^{3}H]leucine (0.5 μCi/ml) for 1 h at 37°C in leucine-free medium.

The monolayers after fixation with 3% (w/v) perchloric acid/0.5% (w/v) phosphotungstic acid were dissolved in 0.5 M NaOH. A 50 μl aliquot of solubilized cell extracts was transferred to a scintillation vial containing 5 ml scintillation cocktail, neutralized with 0.1 M HCl. The radioactivity then counted in a LKB 1209 Rackbeta liquid scintillation counter.

Preparation of liposomes

Liposomes with egg PC/cholesterol/monensin at a molar ratio (7:3:1) were prepared by reverse phase evaporation technique described by Szoka and Papahadjopoulas [13]. REVs after sonication for 15 min in a bath type sonicator were extruded through a series of polycarbonate membranes of pore size (0.8–0.2 μm). Charged liposomes were prepared the same way with different mol% of either (a) dicetyl phosphate (an anionic lipid) or (b) stearylamine (a cationic lipid). Free monensin was separated by gel filtration on Sepharose CL-4B column. The amount of monensin intercalated in liposomes was determined spectrophotometrically by modified vanillin method [14]. For stability and liposome–cell interaction studies, liposomes were prepared in a similar manner except that quenched concentration (175 mM) of calcein or ^{125}I-gelonin were present in PBS. Non-encapsulated calcein or ^{125}I-gelonin were separated from the vesicles by gel filtration on Sepharose CL-4B column. 85–90% monensin was present in the liposomal fraction with only 6–7% aqueous phase marker ^{125}I-gelonin suggesting that most of the monensin is intercalated in liposomal membrane.

Internalization of liposomes in CHO cells

The uptake of liposomes (with or without monensin) containing ^{125}I-gelonin was studied as a function of temperature, time and liposome concentration. Cells at a density $3 \cdot 10^6$ cells/well in 12 well plates were incubated at 37°C with liposomes containing ^{125}I-gelonin (spec. act. $1.45 \cdot 10^5$ cpm/μg) for different time intervals or with varying concentrations of liposomes for 3 h. Cells were then washed with cold DBSS four times to remove surface adsorbed liposomes. In order to define the specificity of liposomal monensin interaction with the surface of CHO cells, we have done control experiments at 4°C, a temperature which precludes any uptake of the vesicles by the cells. Specific uptake was calculated by subtracting the uptake at 4°C from that at 37°C. 25–30% of the total cell associated ^{125}I-gelonin at 37°C was subtracted as 'unspecific uptake' for negative and neutral liposomes.

Results

Monensin liposomes; stability in culture media and interaction with cells

Lipid composition, vesicle size and surface charge on the liposomes mediate its stability and interaction with cells. To evaluate whether intercalation of monensin in the liposomes affects its stability and interaction with cells, we measured changes in liposomal stability by monitoring leakage of calcein after different time of incubation and uptake of liposomes (with or without monensin) in CHO cells using ^{125}I-gelonin as the marker. It was observed that after 5 h, latency for calcein was 80–85% in liposomes without monensin and 90–95% in case of liposomes with monensin, sug-

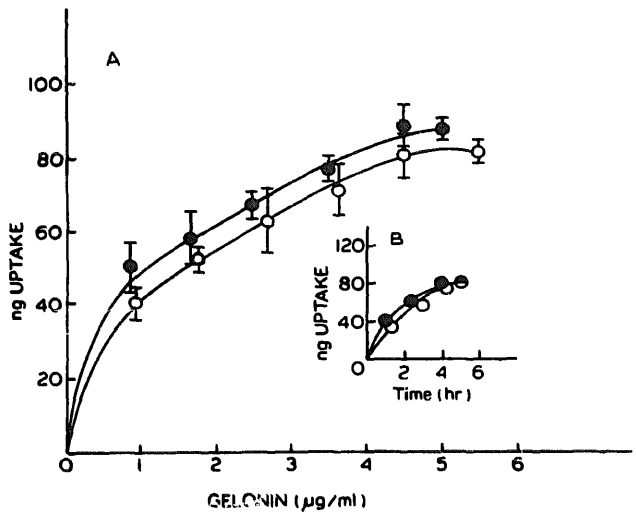

Fig. 1. Concentration- and time-dependent uptake of ^{125}I-gelonin entrapped in liposomes with or without monensin. Cells in 12 well plates ($3 \cdot 10^6$ cells/well) are incubated in RPMI-1640 medium at 37°C either with varying amounts of liposomes entrapped ^{125}I-gelonin (spec. act. $1.45 \cdot 10^5$ cpm/μg), (5 μg/ml gelonin ≡ 3.5 μmol phospholipid/well) for 3 h or at a fixed concentration of liposomes (65 nmol phospholipid/10^6 cells) for varying intervals of times. (A) Uptake as a function of concentration for 3 h. (B) (Inset) Uptake as a function of incubation time. ○, Liposomes without monensin: ●, liposomes with monensin. All the points are the means ± S.D., $n = 3$.

gesting that monensin slightly increases the stability of liposomes in serum. Chromatography of the culture media after 5 h showed a main peak corresponding to liposome peak containing 85–90% of the quantities applied suggesting the presence of intact liposomes with intercalated monensin.

We have examined the time course and concentration-dependent internalization of ^{125}I-gelonin entrapped in liposomes (with or without monensin) in CHO cells. Uptake of ^{125}I-gelonin from both types of liposomes at 37°C increased with increasing concentration of lipid with saturation at approx. 3.5 μmol phospholipid/well (Fig. 1A). A saturation type curve for liposome uptake with time plateaus around 4 h (fig. 1B).

Effect of liposomal monensin on cytotoxicities of ricin, Pseudomonas exotoxin A and diphtheria toxin

In order to ascertain whether liposomes could be used as a delivery vehicle for monensin for potentiation of cytotoxicities of hybrid toxins to selectively eliminate tumor cells, we have examined the modulatory influence exercised by monensin in liposomes on cytotoxicities of ricin, *Pseudomonas* exotoxin A and diphtheria toxin in CHO cells. The results of the studies are presented in Table I. The cytotoxicities of ricin and *Pseudomonas* exotoxin A are significantly enhanced by liposomal monensin in CHO cells and this enhancement is found to be dose-dependent. At 0.1 nM liposomal monensin, cytotoxicities of ricin and *Pseudomonas* exotoxin A are enhanced by 30- and 5.8-fold, respectively. The maximum enhancing effect (62-fold for ricin, 11.5-fold for *Pseudomonas* exotoxin A) is observed at 1 nM. Thereafter no further enhancement is observed

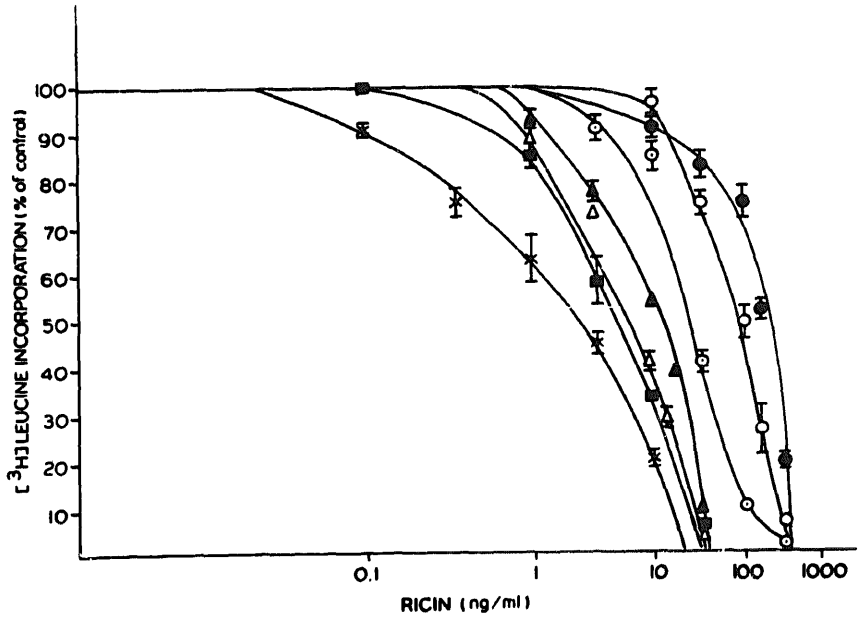

Fig. 2. Effect of charge in liposomal monensin on its ricin cytotoxicity enhancing ability. Cells ($4 \cdot 10^5$ cells/well) are plated 18 h prior to the toxin treatment. The monolayer cultures are washed twice with dBSS and incubated in RPMI-1640 medium for 1 h at 37°C with monensin in free or various liposomal formulations. The cells are then exposed to different concentrations of ricin for 2 h. Following this incubation, protein synthesis is measured as described under Materials and Methods. All points are the means ± S.D., $n = 3$. Control liposome (●); positively charged liposomal monensin: (○) 0.1 nM, (⊙) 1 nM; negatively charged liposomal monensin: (△) 0.1 nM, (▲) 1 nM; neutral liposomal monensin: (■) 0.1 nM, (×) 1 nM.

TABLE 1

Effect of liposomal monensin on cytotoxicities of ricin, Pseudomonas exotoxin A and diphtheria toxin in CHO cells

Cells ($4 \cdot 10^5$ cells/well) are incubated with either ricin, *Pseudomonas* exotoxin A or diphtheria toxin for 2, 22 and 5 h, respectively after 1 h preincubation with free or liposomal monensin. Inhibition of protein synthesis is measured by [^{3}H]leucine incorporation as described under Materials and Methods. Points are the means ± S.E. of three individual experiments.

Agent added	Toxin concentration required for 50% inhibition of protein synthesis (ng/ml)		
	Ricin	*Pseudomonas* exotoxin A	Diphtheria toxin
Control			
PBS	220 ± 16.0	80 ± 1.8	185 ± 10.8
Control liposomes	220 ± 10.6	75 ± 3.8	200 ± 5.6
Free monensin (nM)			
0.1	7.5 ± 0.5	10 ± 2.0	190 ± 15.1
1	3.5 ± 0.2	8 ± 1.5	185 ± 18.6
100	10.0 ± 0.2	> 500	650 ± 2.9
1000	-	> 500	> 1000
Liposomal monensin (nM)			
0.1	7.0 ± 0.8	8.5 ± 0.3	200 ± 12.2
1	3.5 ± 0.7	6.5 ± 0.8	200 ± 10.0
100	8.0 ± 3.5	> 500	800 ± 30.0
1000	-	> 500	> 1000

upto 10 nM. At 50 nM, the enhancement of cytotoxicity of ricin is slightly reduced whereas it has a protective influence on *Pseudomonas* exotoxin A (6-fold). In contrast, diphtheria toxin cytotoxicity is not affected by liposomal monensin upto 10 nM, while 50 nM and above confers protection. This concentration dependent enhancing and protective influence of liposomal monensin against ricin, *Pseudomonas* exotoxin A and diphtheria toxin cytotoxicities in CHO cells is highly comparable to that observed with free monensin.

Effect of lipid composition on ricin cytotoxicity enhancing potency of liposomal monensin

Lipid composition, size and charge have been reported to modulate the behaviour of liposomes under in vivo and in vitro conditions [16–18]. To evaluate whether the lipid composition affects ricin cytotoxicity enhancing potency of liposomal monensin, we have examined the influence of liposomal lipid composition on the efficacy of liposomal monensin.

As can be seen from Fig. 2, the potentiating effect of monensin in neutral vesicles is significantly higher than in charged vesicles. A concentration of 0.1 and 1 nM monensin in neutral vesicle enhances the cytotoxicity of ricin by 30- and 62-fold, respectively. However, the enhancement is reduced to 15- and 30-fold, respectively, by the same concentration of monensin in DCP (24 mol%) containing negatively charged vesicles. Furthermore, it is drastically reduced to 2.8- and 7.5-fold by monensin in stearylamine (24 mol%) containing positively charged vesicles.

The extent of modulation of the cytotoxicity of ricin by monensin either in stearylamine or DCP liposomes is highly dependent on the density of stearylamine or DCP in the liposomal formulation (Table II). The control liposomal preparations having different mol% of either positive or negative charged lipid without monensin have no effect on cytotoxicity of these toxins.

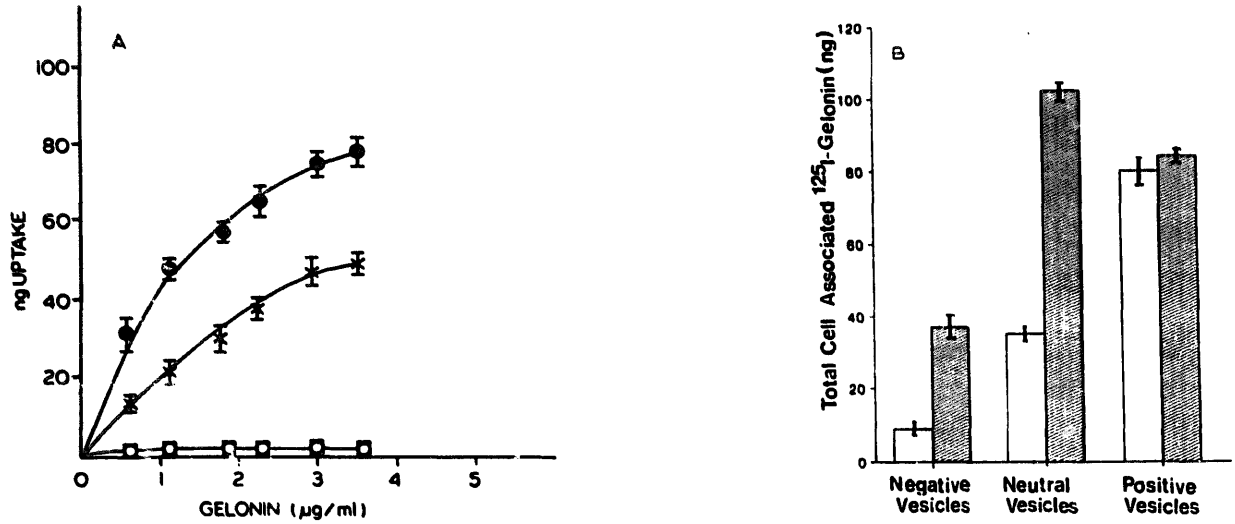

Fig. 3. Effect of liposomal surface charge on the binding and uptake of entrapped ^{125}I-gelonin. Cells ($3 \cdot 10^6$ cells/well) are plated 18 h prior to the uptake study and incubated with varying concentrations of free or liposome entrapped ^{125}I-gelonin with a specific activity $1.45 \cdot 10^5$ cpm/μg). The amount of gelonin inside the cells is measured as described under Materials and Methods. All the points are the means ± S.D., $n = 3$. (A) Specific uptake as a function of liposome concentration (0.35–3.5 μmol phospholipid/well) for 3 h. (●) Neutral liposomal monensin; (×) negatively charged liposomal monensin; (■) positively charged liposomal monensin; (○) free ^{125}I-gelonin. (B) Cell associated ^{125}I-gelonin at 4°C and 37°C after 3 h incubation with 1.7 μmol phospholipid/well (2.3 μg/ml gelonin per well). □, 4°C; ▩, 37°C.

TABLE II

Effect of stearylamine and DCP density on ricin cytotoxicity enhancing effect of liposomal monensin

CHO cells ($4 \cdot 10^5$ cells/well) are preincubated with liposomal monensin (egg PC/Chol/SA or DCP/monensin) with varying mol% of either stearylamine (SA) or DCP for 1 h in RPMI-1640 medium. Different concentrations of ricin is then added for 2 h. Inhibition of protein synthesis is measured by [^{3}H]leucine incorporation as described under Materials and Methods. Points are the means ± S.E. of triplicate determinations.

Lipid composition (molar ratio)	Monensin (nM)	ID_{50} in the presence of liposomes (ng)	
		SA	DCP
Egg PC/Chol/SA or DCP/monensin			
7:3:3:0	-	220 ±14	
7:3:0:1	0.1	7.5 ± 0.5	
	1	3.5 ± 1.2	
7:3:0.25:1	0.1	25 ±2.9	10.0 ± 2.5
	1	15 ±0.8	4.5 ± 0.8
7:3:1:1	0.1	60 ±2.0	15.0 ± 0.8
	1	20.5 ± 1.8	7.5 ± 1.8
7:3:3:1	0.1	100 ±8.2	14.5 ± 1.9
	1	30 ±2.3	7.5 ± 0.2

These results clearly indicated that alteration of one of the vesicles physical parameters, i.e., charge can have a substantial impact on the ricin cytotoxicity enhancing ability of liposomal monensin.

In order to investigate whether differential potentiating ability exhibited by monensin in various liposomal formulations is due to the difference in the uptake, we have examined uptake of various liposomes entrapped ^{125}I-gelonin. As can be seen from the data presented in Fig. 3A, the uptake of ^{125}I-gelonin from neutral vesicles at 37°C is significantly higher (approx. 2.0-fold) than that from the negatively charged vesicles. However, inclusion of increasing proportion of stearylamine (1–24 mol%) in egg PC/Chol/monensin liposomes results in a progressive decrease in the uptake by CHO cells (Fig. 4A). No uptake was observed at 16.7 mol% stearylamine although the binding at 4°C is 4- and 8-fold higher than neutral and negatively charged liposomes, respectively (Fig. 3B). Similarly, inclusion of 24 mol% DCP in the liposomes reduced the uptake by 42% (Fig. 4B). However, the uptake of free ^{125}I-gelonin in these cells was found to be negligible, suggesting liposome-mediated uptake of the former. These observations focus on the important role of density of stearylamine and DCP on liposome–cell interaction.

It has been reported that the interaction of liposomes with cells in vitro condition is markedly influenced by serum [19,20]. To examine whether serum has any influence on the enhancing potency of liposomal monensin, we studied role of serum on cytotoxicity of ricin in the presence of liposomal monensin.

The enhancement of cytotoxicity of ricin (7.5-fold) by 1 nM monensin in stearylamine (24 mol%) liposomes is reduced to 2.7- and 1.5-fold by 10 and 20% serum, respectively (Table III). However, serum has no effect on enhancing potency of monensin in neutral and negatively charged vesicles (data not shown). Binding studies revealed that only positive vesicles experience a rapid decrease in binding in the medium with increasing serum concentrations (Fig. 5).

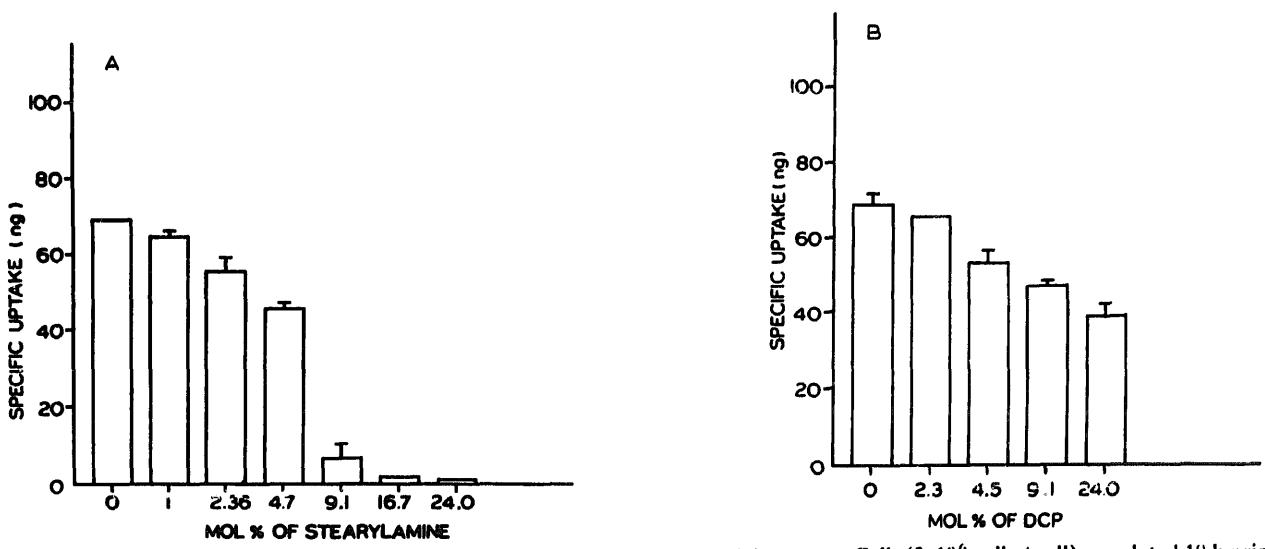

Fig. 4. Effect of increasing concentration of stearylamine or DCP on the uptake of liposomes. Cells ($3 \cdot 10^6$ cells/well) are plated 18 h prior to the experiment and are incubated in RPMI-1640 medium containing liposomes with varying mol% of either stearylamine or DCP. Liposomes at a concentration 1.75 μmol phospholipid/well and ^{125}I-gelonin with a specific activity ($1.45 \cdot 10^5$ cpm/μg) is used. Specific uptake is calculated by subtracting the binding at 4°C from the uptake at 37°C. (A) Specific uptake as a function of increasing mol% of stearylamine. (B) Specific uptake as a function of increasing mol% of DCP. All points are the means ± S.D., $n = 3$.

TABLE III

Effect of serum on ricin cytotoxicity enhancing ability of monensin in positively charged liposomes

CHO cells ($4 \cdot 10^5$ cells/well) are preincubated with liposomal monensin (egg PC/Chol/SA/monensin (7:3:3:1)) in RPMI-1640 medium with or without serum for 1 h. Different concentrations of ricin is then added for different time periods. Inhibition of protein synthesis is measured by [^{3}H]leucine incorporation as described under Materials and Methods. Points are the means ± S.E. of triplicate determinations.

Serum concentration (%)	Time of incubation (h)	Monensin (nM)	Toxin concentration required for 50% inhibition of protein synthesis (ID_{50} in ng)
0	2	0	220 ± 10.0
		0.01	250 ± 3.5
		0.1	100 ± 2.9
		1	30 ± 1.6
10	16	0	225 ± 0.8
		0.01	200 ± 1.2
		0.1	145 ± 4.2
		1	80 ± 2.2
20	16	0	300 ± 5.9
		0.01	300 ± 4.6
		0.1	275 ± 4.6
		1	200 ± 2.0

Kinetics of ricin cytotoxicity in the presence of liposomal monensin

The lag period (time interval between the addition of toxin and onset of protein synthesis inhibition) is reported to be reduced by treatment of cells with monensin, lysosomotropic amines and also by the dose of toxin [7,21]. To examine whether the mechanism of action of liposomal monensin is similar to free monensin, we have studied the kinetics of protein synthesis inhibition by ricin in the presence of liposomal monensin.

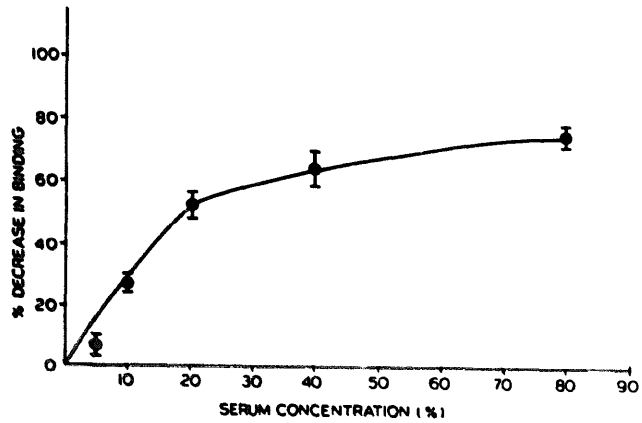

Fig. 5. Serum dependent decrease in the extent of binding (at 4°C) of positively charged liposomes. Cells ($3 \cdot 10^6$ cells/well) are incubated for 1 h in RPMI-1640 medium containing varying concentrations of serum prior to addition of stearylamine liposome (1.75 μmol phospholipid/well) entrapped ^{125}I-gelonin. % Decrease is calculated by taking the binding value in medium without serum as 100%. All points are the means ± S.D., $n = 3$.

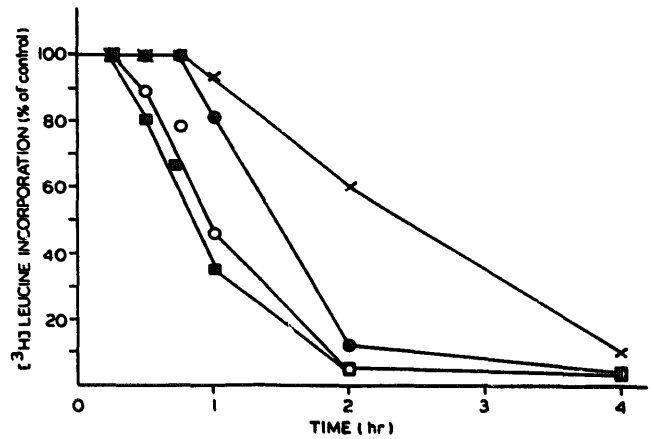

Fig. 6. Kinetics of inhibition of protein synthesis by ricin. CHO cells ($4 \cdot 10^5$ cells/well) are exposed to 10 μg/ml ricin for 10 min in the presence or absence of monensin (1 nM) in various liposomal formulations. After different time intervals, inhibition of protein synthesis is measured by [^{3}H]leucine incorporation for 30 min. (×) Control liposomes; (■) monensin in neutral liposomes; (○) monensin in DCP liposomes; (●) monensin in stearylamine liposomes.

At 10 μg/ml ricin, a lag period of 45 min is observed in control CHO cells with T_{50} (time required to achieve 50% inhibition of protein synthesis) 143 min (Fig. 6). 1 nM monensin in neutral vesicle reduces the lag period to 15 min and T_{50} to 52.0 min. Similar reduction in lag period and T_{50} is observed with free monensin. This reduction in lag period as well as T_{50} by liposomal monensin is highly dependent on liposomal lipid composition. Treatment of the cells with 1 nM monensin in negatively charged vesicle results in reduction of the lag period to a same extent as that observed with neutral vesicle and free monensin but the T_{50} is reduced to 60 min. No reduction in lag period is observed with the same concentration of monensin in positive vesicle, but T_{50} is reduced to 87 min.

Discussion

The results presented in this paper demonstrate that liposomes could be used as a delivery vehicle for monensin. This report for the first time shows that liposomal monensin is equally effective (depending on the composition of liposome) as free monensin in enhancing the cytotoxicity of ricin and *Pseudomonas* exotoxin A in cells in culture. The liposomal monensin at lower concentrations enhances cytotoxicity of ricin and *Pseudomonas* exotoxin A while higher concentrations protect the cells from *Pseudomonas* exotoxin A and diphtheria toxin. This concentration-dependent enhancing and protective effect of monensin has also been reported by other investigators. This has been attributed to differential action of monensin at subcellular level at

higher and lower concentration [22,23]. The exact mechanism by which liposomal monensin enhances cytotoxicity of ricin is not well understood. However, it has been reported that monensin alters the morphology of Golgi cisternae, intravesicular pH and transport of certain proteins [1–3]. These cellular changes have been considered as a plausible basis for monensin action. The exact mechanism of uptake of liposomes by non-phagocytic CHO cells is not fully understood but it has been reported that non-phagocytic cells take up liposomes either by fusion [24] or endocytosis [25].

Our results show that monensin in neutral vesicles is more effective in enhancing cytotoxicity of ricin as compared to charged vesicles. The mechanism of this effect of liposomal composition on enhancing potency of monensin is not clear. However, binding and uptake studies of ^{125}I-gelonin entrapped in various liposomal formulations revealed that the uptake is higher from neutral vesicles (approx. 2.0-fold) as compared to charged vesicles (Fig. 5). This may be the plausible basis for more effective enhancing effect of neutral vesicle. Though the mechanism of this higher uptake is also not clear but it is known that interaction of liposomes with mammalian cells in culture is dependent on both the cell type as well as the liposomal lipid composition [26].

Several investigators have reported discrepant results on the effect of charge on liposome–cell interaction. Some workers have reported positively charged liposomes to have a greater interaction with cells [27–29] while others have reported the same for negatively charged liposomes [30]. Under our experimental conditions, neutral liposomes are found to be taken up more efficiently by CHO cells compared to negatively charged liposomes. Although the binding of positive vesicle at 4°C is 4-fold higher than the neutral vesicle, the uptake at 37°C is negligible. On the contrary several groups have reported that stearylamine liposomes are taken up more efficiently at 37°C [27–29]. This discrepancy may be due to the fact that the other investigators have not taken into consideration binding of liposomes at 4°C. Our results show that there is no difference in the extent of binding of stearylamine liposomes at 4°C and uptake at 37°C indicating thereby that most of stearylamine liposomes remain adsorbed on the cell surface. A substantial proportion of positively charged vesicle remain adsorbed outside the cells without being internalized has also been reported by Dijkstra et al. [17]. This finding is tacitly supported by our observation that monensin in stearylamine liposomes is less potent in enhancing the cytotoxicity of ricin as compared to other liposomes.

We also observed that serum strongly modulates the binding of positive vesicles and enhancing potency of monensin in these vesicles. This may be due to the interaction of positive surface charge with negatively charged constituents in the serum leading to reduction in the net surface positive charge for interaction with the cells. This result support our earlier observation that incorporation of stearylamine in asialoganglioside liposome results in inhibition in galactose specific uptake of liposomes by the hepatocytes in vivo [19]. This result has been attributed to the masking of the galactose residue on the liposomal surface due to the binding of negatively charged plasma constituents.

A significant reduction in the lag period of ricin action by liposomal monensin suggests a more rapid and efficient release of ricin from intracellular compartment into the cytosol. This result is consistent with our earlier observation [7,21] and those reported by other investigators [14,31,32] that pretreatment of cells with carboxylic ionophore results in a reduction of lag period in the inhibition of protein synthesis by ricin and immunotoxins.

Attempts have been made to potentiate the cytotoxicity of immunotoxins both under in vivo and in vitro conditions by delivering monensin through DMSO, HSA or as water/oil emulsion [8,33,34]. This delivery vehicle, i.e., liposome has a number of advantages over the above carriers. For example, it is constituted from natural lipids and its structure could be altered with appropriate ligand for malignant cell specific delivery.

This present study has added to our contention that by suitable alteration of liposomal lipid composition and tailoring of liposomal surface with appropriate ligand it would be possible to direct liposomal monensin to specific tissues or cells for their selective elimination in combination with hybrid toxins.

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