Volume 234, number 2, 321–325 FEB 06072 July 1988

Fc receptor-mediated transcytosis of IgG-coated liposomes across epithelial barriers

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Received 20 April 1988; revised version received 17 May 1988

Drug carriers such as liposomes are not readily transported across cellular barriers that constitute epithelia. However, certain epithelia (rabbit yolk sac endoderm and enterocytes of suckling rat gut proximal small intestine) are well known to transcytose maternal IgG by Fc receptor-mediated endocytic events. We have shown that coating liposomes with appropriate IgG enhances their transport across these epithelia, as measured both by radioactivity indicative of liposomal membrane or entrapped ^{125}I-PVP and [^{3}H]inulin, and by the hypoglycemic effect of entrapped insulin. It is suggested that these transported liposomes follow a pathway of transcytosis in clathrin-coated vesicles, thus escaping lysosomal degradation.

Liposome; Epithelium; Transcytosis; Immunoglobulin G

1. INTRODUCTION

In recent years a number of drug carriers (liposomes, nanoparticles and microspheres) have been developed in order to improve target specificity and reduce toxicity of drugs used in chemotherapy. One of the major problems with these carriers is that they are not transported readily across cellular barriers, in particular epithelial cells lining the gut. Various investigators have shown that biodegradable substances like insulin or factor VIII entrapped in liposomes are transported across the mammalian gut [1–4] but the liposomes themselves are not transcytosed [4,5]. Although intact liposomes are taken up by the epithelial cells they are degraded intracellularly, thus releasing the entrapped drug of which only a small amount escapes intracellular digestion and is eventually transported across the cell into the blood circulation [5].

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Certain epithelia, as constituted by human placental syncytiotrophoblast, rabbit yolk sac endoderm and enterocytes of suckling rodent proximal small intestine, are well known to transcytose IgG derived from maternal blood, uterine fluid, and colostrum and milk, respectively [6]. Transport of IgG is highly selective and depends on the species of origin of the IgG. The transport mechanism involves binding of IgG to Fc receptors present on the epithelial cells followed by receptor-mediated endocytic events and eventually transcytosis in clathrin-coated vesicles [7,8].

Various studies [9,10] have indicated that liposomes coated with antibodies are taken up more readily by cells expressing Fc receptors (e.g. macrophages and lymphocytes) but there have been no investigations to show the possibility of specific receptor-mediated transcytosis of intact carriers across epithelial cells. We have, therefore, examined the possibility that IgG itself can act as a carrier of liposomes by causing their transcytosis across the yolk sac into the blood of the foetal rabbit and across the gut into the blood of the suckling rat. Our initial experiments provide encouraging

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

data in this respect and suggest that these epithelial barriers could be used as model systems for the analysis of similar transport processes in other types of cells.

2. MATERIALS AND METHODS

Negatively charged small unilamellar liposomes containing ^{125}I-polyvinylpyrrolidone (PVP) of molecular mass 40–60 kDa, [^{3}H]inulin or ox pancreas insulin (from Wellcome) were prepared as described earlier [5]. For use in the suckling rat experiments (see below) IgG was covalently coupled to liposomes by the method described by Leserman et al. [11] and for the pregnant rabbit experiments, IgG was nonspecifically attached onto liposomes by the sonication method [12]. Liposomal membranes were labelled with cholesteryl-[^{14}C]oleate as described by Kao and Juliano [13].

New Zealand white rabbits at 24–26 days of pregnancy and Wistar suckling rats 12–22 days old were used. Rabbits were operated on using nembutal/ether anaesthesia and after laparotomy, the exposed uterine horns were ligated so as to isolate each individual conceptus; 1 ml of liposomes (= 20 mg lipid) or other test material was injected into the uterine lumen between each ligature. The uterine horns were replaced into the abdominal cavity, the incisions closed, and the rabbits allowed to recover for 4 h before being given a lethal injection of nembutal into the marginal ear vein. Each conceptus was then removed from the uterine lumen by detachment of the placenta basalis. The yolk sac was dissected free, washed in saline to remove adherent debris and the paraplacental chorion trimmed away; amniotic fluid and foetal heart blood were also collected. Radioactivity was counted in appropriate aliquots of tissue and fluids and expressed as a concentration quotient (CQ) as shown in the results.

Suckling rats were isolated from the mother 1 h prior to feeding 0.5 ml (= 30 mg lipid) of liposomes or other test material by stomach tube. After 3 h, blood, liver and kidneys were removed and the radioactivity of the injected substances was measured. The glucose concentration in the blood was measured by the method described previously [1].

3. RESULTS

The results in table 1 show that when ^{125}I-PVP is injected into the uterine lumen of the pregnant rabbit in liposomal form it is subsequently transported into fetal blood and amniotic fluid more readily than when given in the free form. Transport was considerably enhanced, particularly to the blood, when liposomes were coated with human IgG. However, when liposomes were coated with bovine IgG, this enhancing effect of IgG on the transport of ^{125}I-PVP entrapped in liposomes was reduced to only 10% in the blood and 33% in the amniotic fluid when compared with results obtained for human IgG-coated liposomes. The amount of radioactivity still retained in the yolk sac was greatest for ^{125}I-PVP entrapped in human IgG-coated liposomes but coating with bovine IgG, as opposed to no coating, or administration in free form, also caused much more uptake of PVP. Comparison of the CQs for the radioactivity in the blood and the yolk sac reveals that in the case of human IgG-coated liposomes nearly twice as much radioactivity reaches the blood as is retained in the yolk sac. In the case of bovine IgG-coated liposomes however, the situation is reversed, with almost four times the amount of radioactivity being retained in the yolk sac as is transported to the blood.

Table 1

Transport of ^{125}I-PVP across rabbit yolk sac endoderm expressed as concentration quotients (CQs)

^{125}I-PVP administered	^{125}I-PVP radioactivity $\left(CQ^{a} = \frac{\text{cpm/ml or g tissue}}{\text{total } ^{125}\text{I-PVP cpm injected}} \times 10^{-3}\right)$		
	Yolk sac	Amniotic fluid	Fetal blood
Free	4.40 ± 0.7	0.29 ± 0.08	0.07 ± 0.02
Liposomes	6.00 ± 0.6	2.70 ± 0.8	3.40 ± 1.5
Human IgG-liposomes	32.00 ± 5.0	18.50 ± 1.2	61.00 ± 1.4
Bovine IgG-liposomes	25.60 ± 3.0	6.50 ± 0.9	6.00 ± 0.7

[a] Mean ± SE of 4 to 5 conceptuses

That the enhanced transport of PVP entrapped in liposomes coated with human IgG is a consequence of liposome uptake and transport as such, and not some other process, is indicated by the results shown in table 2. Here the liposomal membranes were labelled by incorporation of cholesteryl-[^{14}C]oleate, and it is clear that much more liposomal material has been taken up into the yolk sac and transported to the fetal blood when coated with human IgG than when liposomes were uncoated. Indeed, similar values for the CQs are apparent when comparison is made between results in tables 1 and 2, even though different compartments of the liposomes have been labelled.

The results in table 3 show that the transport of liposomally entrapped [^{3}H]inulin is greater than for the free substance when administered intragastrically to suckling rats. Covalent attach-

Table 2

Transport of liposomes labelled with lipid marker, cholesteryl-[^{14}C]oleate, across rabbit yolk sac endoderm expressed as concentration quotient (CQ)

Preparation	Cholesteryl-[^{14}C]oleate radioactivity $\left(CQ^a = \frac{\text{cpm/ml or g tissue}}{\text{total cpm injected}} \times 10^{-3}\right)$		
	Yolk sac	Amniotic fluid	Fetal blood
Liposomes	5.0 ± 1.2	2.9 ± 0.8	6.8 ± 2.2
Human IgG-liposomes	38.0 ± 4.0	22.0 ± 1.0	64.0 ± 3.0

[a] Mean ± SE of 5 conceptuses

ment of rat IgG onto liposomes containing [^{3}H]inulin almost doubled the transport of [^{3}H]inulin into the blood and when free rat IgG was administered together with IgG-coated liposomes, inhibition of transport of radioactivity was observed. Some of the absorbed radioactivity became localised in liver and kidney and more so when administered in liposomes than in free form. Results similar to these were obtained when transport of ^{125}I-PVP, either free, entrapped in liposomes, or entrapped in liposomes coated with IgG, was administered to suckling rats (not shown).

The results in table 4 show that when 2.5 mU of insulin entrapped in rat IgG-coated liposomes was administered intragastrically to suckling rats of different age groups, a significant reduction in blood glucose level occurred in those at 12 and 17 days but no change occurred in those at 22 days. On the other hand, 5.5 mU of insulin in non-IgG-coated liposomes produced no significant change in blood glucose levels in any of the rats except for a small reduction at 12 days. Insulin in free form given in a dose as large as 5 U did not reduce the blood glucose level in any animals at any of the three ages.

Table 3

Absorption of intragastrically administered [^{3}H]inulin in 12-day-old suckling rats

Preparation	[^{3}H]Inulin radioactivity (% of the dose[a])		
	Blood[b]	Liver	Kidney
Empty liposomes + [^{3}H]inulin	1.37 ± 0.2	1.57 ± 0.1	0.83 ± 0.1
Liposomal [^{3}H]inulin	2.77 ± 0.7	2.64 ± 0.4	1.65 ± 0.1
Rat IgG-coated liposomal [^{3}H]inulin	5.39 ± 0.5	2.79 ± 0.2	1.46 ± 0.2
Free rat IgG + IgG-coated liposomal [^{3}H]inulin	3.40 ± 0.4	2.32 ± 0.3	1.51 ± 0.3

[a] Mean ± SE of 5 rats
[b] % per ml of blood, or % per whole organ

Table 4

Effect of intragastrically administered insulin on blood glucose level in 12-, 17- and 22-day-old suckling rats

Preparation	[a]Blood glucose concentration (% of the initial value)		
Age:	12 days	17 days	22 days
Free insulin + lipids (5 U)	105 ± 7	101 ± 8	103 ± 7
Insulin in liposomes (5.5 mU)	87 ± 3	94 ± 6	93 ± 5
Insulin in rat IgG-coated liposome (2.5 mU)	67 ± 4	79 ± 4	95 ± 7

[a] Mean ± SE of 7 animals

4. DISCUSSION

Our results show that transport of the non-degradable molecules ^{125}I-PVP and [^{3}H]inulin to the blood of the fetal rabbit and suckling rat across epithelial barriers, constituted by yolk sac endoderm and gut enterocytes, is enhanced when these substances are incorporated into liposomes compared to presenting them to cells in their free form. Such transport is further increased (18-fold for ^{125}I-PVP across the yolk sac and almost doubled for [^{3}H]inulin across the gut) when appropriate IgG is either non-specifically attached or covalently linked to the liposome surface. That the transcytosis of IgG-coated liposomes is probably Fc-receptor mediated, is supported by several observations emerging from our results. Firstly, coating liposomes with bovine IgG, whilst enhancing the uptake of liposomes as indicated by the entrapped ^{125}I-PVP, led to much less transport across the yolk sac endoderm to the fetal blood compared to coating with human IgG. It is known that bovine IgG is readily endocytosed by yolk sac endoderm, but unlike human IgG is poorly

transported to the blood [14] and binds less readily to Fc receptors detected on isolated endodermal cells in rosette assays [15,16]. Secondly, it was found that co-administration of free rat IgG with IgG-coated liposomes produced competitive inhibition in the transport of entrapped [^{3}H]inulin across the gut wall of the suckling rat and which can be interpreted as competition for binding to receptors. Thirdly, we found that insulin entrapped in IgG-coated liposomes produced hypoglycemic effects in 12- and 17-day-old suckling rats, but not in those at 22 days. After 21 days of age the suckling rat is known not to transport IgG across the gut wall to the blood circulation [17] and beyond this age Fc receptors can no longer be detected on gut enterocytes as shown by a variety of assays [18–20].

An important question to be answered is whether liposomes were endocytosed and transported intact across the epithelial cells. Evidence that this is the case is suggested by the similar CQs obtained when cholesteryl-[^{14}C]oleate was used as a non-exchangeable liposomal membrane marker [13] and ^{125}I-PVP was entrapped in liposomes. Where entry of liposomes into yolk sac endodermal cells has taken place by other means, for example membrane fusion, such similar exiting of the different markers into the fetal blood and accumulation within the cells, would not have been expected. Furthermore, free [^{3}H]inulin and ^{125}I-PVP have very short half-lives in the blood [21] and the relatively high radioactivities found in this compartment at the end of our experiments suggest that intact liposomes are indeed present. The hypoglycemic effect produced in suckling rats by a much smaller dose of insulin entrapped in IgG-coated liposomes, provides further evidence that IgG-coated liposomes escape intracellular digestion and are transported intact into the circulation. Liposomes containing entrapped insulin have previously been shown to be more effective than free insulin in that they produce more prolonged and greater hypoglycemic effect when injected subcutaneously into rats and dogs [22].

We can only conjecture as to how IgG-coated liposomes escape digestion during their transcytosis. Transport of IgG across rabbit yolk sac endoderm involves selective events thought to take place at the cell surface [23] and in which IgG destined for transport is transcytosed in clathrin-coated vesicles bound to Fc receptors, whilst IgG destined for proteolysis is carried in endosomes which fuse with lysosomes. Transport of IgG across suckling rat gut takes place in the duodenum and jejunum by a similar mechanism except that selection has also been shown to occur in apical vesicles [8]. Very little or no free ^{125}I-PVP is transcytosed across yolk sac endoderm and gut enterocytes as shown in previous studies [24,25] and the present investigations; it merely enters the cells through fluid phase endocytosis and accumulates undegraded in phagolysosomes. A similar route probably is followed by [^{3}H]inulin but the enhanced transport of these molecules following entrapment in liposomes might reflect some degree of non-specific transcytosis in the coated vesicle pathway. By coating liposomes with human and rat IgG, a greater proportion of them might be expected to enter the coated vesicle pathway through specific binding to Fc receptors, which would explain why so much more ^{125}I-PVP, [^{3}H]inulin, or insulin (as deduced from its hypoglycemic effect) traverses the epithelial barriers. Coating liposomes with IgG might also be expected to increase 'non-selective' uptake into the endosome system through interaction with less specific binding sites on the apical plasma membrane and therefore greater accumulation in phagolysosomes. This would explain the increased radioactivity found in the yolk sac when liposomes were also coated with bovine IgG.

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