Selective uptake of liposomes by different cell types of liver through the involvement of liposomal surface glycosides

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In recent years liposomes have gained wide acceptance as carriers of biologically important molecules (Papahadjopoulos, 1978; Ghosh & Bachhawat, 1980a; Gregoriadis, 1980). Earlier work from this laboratory has opened up a potential application of glycolipids in their use as ligands for specific targeting of liposomes and also the phase transition temperature of the phospholipid used for making liposomes (Surolia et al., 1975; Surolia & Bachhawat, 1978). These facts and the information available concerning the presence of hepatic lectin on the surface of parenchymal cells (Ashwell & Morell, 1974) led us to design the experiment performed in vivo with the help of C₃₄ ganglioside where we had incorporated the ganglioside in such a way that the oligosaccharide portion of the glycolipid was at high density on the surface of liposomes (Surolia et al., 1977). A rapid uptake of these liposomes by the liver was observed after intravenous administration. This uptake was found to be primarily through the endocytotic process mediated by galactose receptor. However, a number of investigators were unable to confirm this observation and had suggested that the terminal glycoside residue on the liposome may not have any particular specificity (Gregoriadis & Neerunjun, 1974; Jonah et al., 1978). This led us to re-investigate further the role of different glycoside residues on the surface of liposomes on their uptake by various organs, and it was observed that enhanced liposome uptake by liver could be achieved by grafting galactoside and mannoside on the liposomal surface (Ghosh & Bachhawat, 1980b). Anomeric form as well as the density of the glycoside residues on the surface of liposomes were found to be
the determining factors for the uptake of glycosylated liposomes by the liver (Ghosh & Bachhawat, 1980b; Ghosh et al., 1981). Moreover, as observed in studies performed in vitro (Surolia & Bachhawat, 1978) and in vivo (Bachhawat, 1980a). The chain length of the oligosaccharides of glycolipids has an important role in the uptake of liposomes (Ghosh & Bachhawat, 1980b). These observations led us to conclude that other investigators had not taken into consideration the critical receptor density on the surface of liposomes in designing their experiments, and as such primarily the low density of the surface sugar on liposomes may be the reason for their inability to confirm our previous results.

Further studies (P. Ghosh, P. K. Das & B. K. Bachhawat, unpublished work) on the uptake of liposomes by isolated cell types of rat liver after administration in vivo revealed that hepatocytes were much more efficient than non-parenchymal cells in taking up liposomes having β-galactoside on their surface, whereas α-mannoside-containing liposomes were taken up preferentially by non-parenchymal cells.

Considering the efficacy of glycosylated liposomes in the targeting towards specific liver cell types, it was thought to be of great interest to see whether therapeutic substances enclosed in these types of liposomes can be effective in reversing the diseases conditions of liver. For this purpose galactosamine-induced hepatitis in rats was chosen as the model disease, since it is a type of liver injury that closely resembles human viral hepatitis. The severity and duration of this disease can be experimentally controlled by the amount of D-galactosamine, and it can also be effectively reversed by the administration of uridine (Decker, 1975; Keppler, 1975). The course of D-galactosamine-induced hepatitis was monitored by the estimation of liver glycogen, which has been shown to be decreased to less than 5% of the controls a single injection of D-galactosamine (Decker, 1975; Keppler, 1975). Earlier work from our laboratory has shown that the effective dose of phosphatidic acid-liposome-entrapped uridine required for the reversal of this experimental hepatitis was much less than that of free uridine (Mathias et al., 1977). Since there is a selective uptake of glycosylated liposomes by different liver cell types, a comparative study has been undertaken on the protective effect of uridine entrapped in liposomes having different glycosides on their surface and that entrapped in phosphatidic acid liposomes.

The rats were first injected intraperitoneally with D-galactosamine (150 mg/kg body wt.), and then after 30 min free uridine and uridine entrapped in different liposomes were given intravenously. Rats were killed and glycogen content of the liver was estimated after 3 h of D-galactosamine administration. Uridine was entrapped in different glycosylated liposomes and phosphatidic acid liposomes that were prepared by the methods described previously (Ghosh & Bachhawat, 1980b). In the competition studies, 10 mg of asialofetuin was administered intravenously before injection of liposomes. In this study, the dose of liposome-entrapped uridine was fixed at 80 mg/kg body wt., since the maximum possible amount of liposome that can be administered per rat cannot entrap more than the specified dose of uridine. Free uridine given to D-galactosamine-treated rats in high dosage (1.28 g/kg body wt.) completely reverses the D-galactosamine toxicity. Table 1 shows the effect of various types of liposome-entrapped uridine (80 mg/kg body wt.) on the liver glycogen content of D-galactosamine-treated rats. The liposome-entrapped uridine was found to be more effective in reversing the D-galactosamine toxicity compared with free uridine at the same dosage. It is evident from Table 1 that asialoganglioside-liposome- and phosphatidic acid-liposome-entrapped uridine produces more regenerating effect compared with other liposomes studied. At this dose, asialoganglioside-liposome- and phosphatidic acid-liposome-entrapped uridine produces 50% and 30% depletion respectively of the liver glycogen content. The regenerating effect of α-mannoside-liposome-entrapped uridine is slightly higher than free uridine at the same dosage. The more protective effect of asialo-galactosame-entrapped uridine on the D-galactosamine-treated rats compared with α-mannoside liposome indicates that the asialo-galactoside-liposomes are taken up preferentially by the parenchymal cells of liver as a direct consequence of the specific interaction of the exposed galactosyl residues with the galactose-binding protein present on the parenchymal cells as described by Ashwell & Morell (1974). The inhibition of the protective action of asialoganglioside-liposome-entrapped uridine by asialofetuin further confirms this observation. The difference in the regenerating effect of phosphatidic acid liposome- and asialoganglioside-liposome-entrapped uridine on the D-galactosamine toxicity may be due to the fact that the negatively charged liposomes are taken up by both parenchymal and non-parenchymal cells of liver (Gregoriadis & Ryman, 1972), thus diminishing the effectiveness of uridine, whereas asialoganglioside liposomes are predominantly taken up by the liver parenchymal cells, which are mainly affected by D-galactosamine (Hofman et al., 1976). Therefore it may be suggested that liposomes having β-galactoside on their surface may provide an effective way of delivering therapeutic substances of biological interest to diseased liver, as in the cases of viral-induced hepatitis, hepatitis A, etc.


Table 1. Effect of various types of liposome-entrapped uridine on the liver glycogen content of D-galactosamine-treated rats

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Liver glycogen content (mg/g of liver)</th>
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<tbody>
<tr>
<td>Normal value</td>
<td>45.0 ± 5</td>
</tr>
<tr>
<td>D-Galactosamine alone</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>Free uridine</td>
<td>5.8 ± 2.1</td>
</tr>
<tr>
<td>Dicetyl phosphate-liposome</td>
<td>4.3 ± 2.1</td>
</tr>
<tr>
<td>Phosphatidic acid-liposome</td>
<td>12.5 ± 2.5</td>
</tr>
<tr>
<td>Asialoganglioside-liposome</td>
<td>19.1 ± 3.1</td>
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<tr>
<td>α-Mannoside-liposome</td>
<td>7.5 ± 2.2</td>
</tr>
<tr>
<td>Asialoganglioside-liposome + asialofetuin</td>
<td>12.2 ± 2.3</td>
</tr>
<tr>
<td>Phosphatic acid-liposome + asialofetuin</td>
<td>11.5 ± 1.2</td>
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