Polyprenol Content in Primary Human Liver Carcinoma *

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The high dolichol content of the human liver is well established. Most of this polyprene is in membrane bound form and probably contributes to the structural make-up of the membrane.[1] In model membranes dolichol destabilizes the bilayer structure and induces hexagonal H_{II} phase, and also increases fluidity of the phospholipid fatty acids. Therefore, the amount of dolichol in a certain tissue is of considerable interest and pathological changes in the distribution pattern or quantity of polyprenes may influence the tissue function to a large extent. Primary hepatocellular carcinoma in human liver, like those in experimental systems, develops gradually and the malignant cells resemble in many aspects the hepatocytes.[2] In this study we have collected a number of primary liver carcinomas and analyzed their polyprene content and distribution pattern.

Biopsy and autopsy samples were subjected to surgical pathological investigation and only those samples with well established diagnoses were used. The samples were homogenized and the dolichol content of the lipid extract was determined as described previously.[3] The complex and tedious procedure employed previously for dolichyl-P determination was modified. The number of chromatographic steps employed earlier led to a poor recovery which made quantitation uncertain. The modified procedure which is outlined in Fig. 1 gives reproducible, reliable and relatively rapid estimation of dolichyl-P. The homogenate was extracted with chloroform—methanol—water (1:1:0.3) followed by filtration with Whatman 1 filter paper.

The extract was acidified and after incubation and washing KOH was added for alkaline hydrolysis. The acid hydrolysis liberated the sugar residues from dolichyl-pyrophosphate and the alkaline hydrolysis saponified phospholipids. The samples was placed on a Sep-PAK containing silica gel and washed first with chloroform saturated with ammonia followed by chloroform–methanol–ammonia (90:10:5). Dolichyl phosphate was eluated with chloroform–methanol–water (1:1:0.3) and the fraction after washing contained beside dolichyl-P only limited amounts of cholesterol. This isolated fraction was injected to hypersil column with a particle size of 3 μm (Hewlett Packard). The phosphorylated lipid was separated and identified by high performance liquid chromatography (HPLC). Appropiate synthetic standards proved that the appearing dolichyl phosphate peaks represented only these lipids without disturbing contaminations.

Human liver contains 0.5 mg dolichol per gram wet weight which is 40–50 times more than the amount of the total dolichyl-P which is about 10 μg per gram liver (Table 1). In human liver cirrhosis the amount of dolichol on gram basis decreases drastically while dolichyl-P content decreases in a more moderate extent. In developed human hepatocellular carcinoma the decrease of polyprenols is striking. The content of the free alcohol is decreased eight times and the phosphorylated lipid intermediate is only 30 % of that of the control.

The major dolichol in human liver, similarly to other organs, is that of 19 isoprenes closely followed by dolichol with 20 residues (Table 2). D17, D18, D21 and D22 are also present in smaller amounts. No major deviations occur in cirrhosis in contrary to the primary hepatocellular carcinoma where the polyprenol pattern moves to shorter regions. The amount of the dolichols above D19 is decreasing while the amount of the dolichols with shorter chain length is increasing. This change in distribution pattern is very characteristic and we have found it in all samples analyzed. The polyprenol pattern is very stabile and there are very few examples of deviation from the normal pattern. The distribution of the

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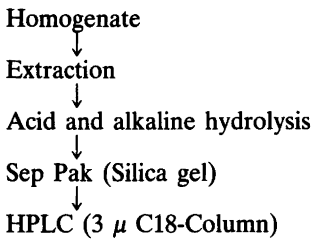

Fig. 1. Schematic representation of dolichyl-P determination.

individual dolichyl-P in the various samples was also determined (Table 3). In the control samples the pattern was similar to that of the free alcohol with some modification. The two main polyprene-P are dolichols with 19 and 20 isoprene residues making up 70 % of the total. Consequently, the remaining individual dolichyl-P has a low concentration. The distribution is not changed in any larger extent in cirrhosis. Similarly to the free alcohol the dolichyl-P isoprenoid pattern in primary liver cancer is also shifted to an increase of shorter

Table 1. Dolichol and dolichyl-P content in human liver. Samples of human liver, cirrhosis (Laennec type) and primary carcinoma (hepatocellular type) were diagnosed histologically parallel to determination of dolichol and dolichyl-P on HPLC. The values are the mean of 5–9 experiments, μg/g wet weight.

	Dolichol	Dolichyl-P
Control	457	10.2
Cirrhosis	56	8.0
Hepatocellular carcinoma	60	3.9

Table 2. Distribution of individual dolichols in human liver. The samples were used for histological diagnosis before measurement of HPLC.

	Composition, % of total					
	D17	D18	D19	D20	D21	D22
Control	9	12	39	30	11	4
Cirrhosis	5	14	41	26	11	3
Hepatocellular carcinoma	6	33	48	11	2	1

Table 3. Distribution of individual dolichyl-P in human liver.

	Composition, % of total					
	D17	D18	D19	D20	D21	D22
Control	1	10	34	38	8	9
Cirrhosis	2	8	30	41	9	10
Hepatocellular carcinoma	3	14	42	30	7	4

chain length polyprenes and a decrease of the longer ones, but with comparison with the free alcohol the changes are more moderate.

It is generally believed that dolichyl-P is a precursor of the free alcohol and that phosphorylation of the free alcohol may occur with the involvement of a CTP-specific kinase.[4] Consequently, close interrelationship is existing between the two forms of this lipid. Recent biosynthetic studies arrived to the conclusion that there are probably two different biosynthetic pathways for the phosphorylated and the non-phosphorylated forms, and the extent of transformation from one form to another occurs only to a limited extent.[5] This means that the amount and distribution pattern of one form is not dependent on or regulated by the amount of the other lipid. The independent regulation is also obvious from the studies in this work. Changes in dolichol and dolichyl-P content do not strictly follow each other but follow an individual pattern.

A decrease of the phosphorylated lipid intermediate may have substantial consequences. Since dolichyl-P appears to be the rate limiting factor in some glycosylation reactions, the decreasing amount may cause a reduction of the glycosylation. The low amount and the different type of phosphorylated intermediate may be responsible for the biosynthesis of the shorter and more branching oligosaccharide chains known to occur in cell membrane glycoproteins of tumor cells. These proteins are suggested to produce specific properties present only in tumor cells, such as the abscence of contact inhibition or high net negative charge. Dolichol is proposed to influence membrane fluidity and stability. The decreased dolichol content, may therefore hinder movement of metabolites through both plasma and intracellular membranes and thereby induce those metabolic changes which are characteristic of the malignant cell. It is also claimed but never proved that individual dolichyl-P may exhibit some specificity concerning different sugars. A change in the pattern may restrict the type of the monosaccharide transfered to the growing oligosaccharide chain, and the change in the dolichyl-P distribution pattern may contribute to the synthesis of the *N*-glycosidically linked oligosaccharides in tumor cells which are characterized by deviation in sugar sequences and also by deviating functions.

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