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0014-4754/87/040435-03$1.50 + 0.20/0

Prevention of experimental liver metastases by D-galactose

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22 July 1986

Summary. The metastasis of malignant tumors from a primary site to near and distant secondary sites is probably the most important event in the pathogenesis of cancer and it accounts for most cancer deaths[1]. Whereas advances in the treatment of primary cancer have led to increased patient survival, metastatic cancers are still the most difficult group of diseases to treat successfully[2]. As organ-characteristic lectins play an important role in the organ manifestation of metastatic islets[3,4], it might be possible (e.g. during surgical operations on malignant tumors) to block those organ-characteristic lectins with the appropriate receptor-bearing glycoconjugates in order to inhibit the metastatic spread. Recent experiments have demonstrated that neuraminidase treatment of tumor cells (mouse sarcoma-1) alters in vivo (Balb/c-mice) the organotropic distribution of metastases; instead of being found exclusively in the lung, they are found both in lung and liver. However, pre-injection and regular application of D-galactose – the same holds for arabinogalactan[5,6,13] – prevents the settling of metastases in the liver but does not influence the metastatic process to the lung, whereas mannan – as a galactose-free control substance – does not alter the initial pattern of metastasis to lung and liver.
Key words. Liver lectins; metastases; D-galactase.

In the course of our studies on the role of lectins as tools for tumor marking, we postulated in 1979 that the process of metastasis is analogous to that of bacterial infection and that the organotropy of metastasis, like that of infection, is mediated either by lectins in the invaded organs or by lectins on the invading tumor cells or bacteria[7,13,15]. After the discovery of vertebrate lectins by Ashwell and his group[10] we advanced the hypothesis that especially those organ-characteristic lectins may act as acceptors of malignant tumor cells in the metastatic process by interacting with cryptic precursor carbohydrate structures on the surfaces of metastatic tumor cells[6,7]. Because of the galactose specificity of those vertebrate lectins (e.g. of the Hepatic-Binding-Protein, HBP) we suggested that blockage with competitive receptor-bearing glycoconjugates may inhibit metastatic spread into the liver [4–6]. In the meantime we have obtained experimental evidence that certain metastasizing animal tumors treated in this way show no spreading of metastases into the liver. Recently, a similar inhibition model for infection proved that the lectin mediated organotropy of bacterial infections may also be inhibited by blocking the bacterial lectins with appropriate receptor-specific carbohydrates[14]. Our results concerning the inhibition of liver metastasis by the blocking of hepatocyte lectins with D-galactose can be summarized as follows.

In vitro, rosette formation between freshly isolated human organ cells (hepatocytes) and metastasizing tumor cells can be completely inhibited by low concentrations (5 mg/ml) of single sugars (D-galactose) or glycoconjugates (arabinogalactan) in the growth medium, demonstrating the participation of mitogenic lectins (e.g. the galactose specific HBP) in the adhesion phenomenon. Addition of glucose, mannose or galactose-free glycoconjugates (pullulan) does not inhibit this rosette formation. Neuraminidase treatment of the tumor cells, which removes the terminal sialic acid and exposes penultimate galactose residues, leads to a highly increased adhesion[8].

In vivo, the capacity of D-galactose to block the liver lectin (HBP) was tested by intravenous administration of tritiated α_1-acid-(asialo)glycoprotein to Balb/c-mice (100 µg solubilized in 0.1 ml PBS). In accordance with the results of Ashwell and Morell[10] this glycoprotein was rapidly cleared (within 15 min) from the circulation and taken up by the liver. Pre-injection of D-galactose (15 min before glycoprotein injection) caused a markedly delayed elimination of asialoglycoprotein from the serum. After 30 min an increase of radioactivity of more than 90% was present in the serum (105 dpm/µl serum after α_1-acid-(asialo)glycoprotein injection without receptor blocking compared to 200 dpm/µl after receptor blocking by pre-injection of

Experientia 43 (1987), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Metastatic distribution (number of tumor nodules) of sarcoma L-1 tumor in Balb/c-mice after i.v. inoculation and treatment of the animals with different substances

Balb/ C-mice	Neuraminidase treated L-1 sarcoma + PBS		+ D-Gal I		+ D-Gal II		+ Mannan		L-1 sarcoma + PBS	
N = 15	Lung	Liver	Lung	Liver	Lung	Liver	Lung	Liver	Lung	Liver
1	×	12	×	–	×	–	×	4	56	–
2	×	33	×	–	×	–	×	3	85	–
3	×	4	×	–	×	–	×	4	72	–
4	×	12	×	–	×	–	×	3	86	–
5	×	7	×	1	×	–	×	4	74	–
6	×	8	×	–	×	–	×	8	85	–
7	×	18	×	–	×	–	×	15	92	–
8	103	14	×	–	×	–	×	6	×	–
9	98	8	×	–	×	–	×	24	44	–
10	×	12	×	–	×	1	×	13	98	–
11	73	13	×	2	×	–	×	8	×	–
12	68	13	×	–	×	–	×	14	×	–
13	×	14	×	–	×	2	×	9	×	–
14	×	5	×	–	×	1	×	21	×	–
15	×	7	×	–	×	–	×	19	×	–

D-Gal I (1 mg/g body weight), D-Gal II (2 mg/g body weight). × = multiple confluent and uncountable tumor nodules.

D-galactose). Blockage was decreased by approximately 60% after 60 min. Thus we succeeded in blocking the liver lectin (HBP) by injection of the monosaccharide D-galactose. The same holds for arabinogalactan, which even leads to a longer lasting receptor blocking because of its greater molecular weight. This leads to a slower metabolism and elimination[5] in vivo.

In order to prove our hypothesis that the settling of a malignant tumor in the liver (e.g. binding of the tumor cells by hepatocytes) is associated with a recognition process, involving a D-galactose-specific lectin carbohydrate interaction[6,7,16] which can be inhibited by competitive glycoconjugates, we investigated the metastatic process of mouse sarcoma L-1 tumor cells in Balb/c-mice[9]. Neuraminidase-treatment of tumor cells alters the organotropic distribution of metastases; instead of occurring exclusively in the lung, they are found in both lung and liver. However, pre-injection (1 mg/g b. wt, i.p., 1 h before tumor cell inoculation) and regular application of D-galactose (for 3 days after tumor cell inoculation, at 8-h intervals) nearly completely prevents the settling of metastases in the liver. However, the rapid metabolism and elimination of D-galactose prevents complete receptor-blocking over a period of 8 h. Although the i.p. injection of D-galactose results in a prolonged presence in the circulation, this non-continuous application may be responsible for the negligible number of metastases which were found in the D-galactose treated animals (table). However, this treatment did not influence the homing to the lung. The number and average diameter of the tumor nodules in the lung did not differ from the control group. There might be a greater variety of lectins on the lung parenchyma cells which could not be blocked with single sugars such as D-galactose or D-mannose. On the other hand the lung capillary system could be an effective filter for trapping circulating tumor cells (or cell clumps) mechanically, and metastatic lesions might thus be induced without a specific recognition process.

D-Mannan, a galactose-free control polysaccharide, does not alter the initial pattern of metastasis to lung and liver (table). The most favorable period for D-galactose application, and the optimal D-galactose concentration (as descriobed above), were obtained from various in vivo experiments which we performed in the course of our studies.

Preliminary results of experiments with another, more clinically relevant, tumor model (mammary carcinoma HB in C3H mice) are in accordance with the results of the sarcoma L-1/Balb/c-mice model. Intravenous injection of the mammary carcinoma cells (without neuraminidase treatment) leads to multiple metastases in lungs and livers of the C3H mice which obviously can be completely inhibited by a D-galactose (or arabinogalactan) regimen as described above.

In summary, adhesion of bacteria and adhesion of tumor cells have much in common, especially the participation of lectins in the process[4,13,14]. In the future it might be possible to inhibit the metastatic process into the liver (e.g. during surgical operations of malignant tumors) by blocking organ lectins with D-galactose, galactans or appropriate neo-glycoconjugates of various polysaccharides as suggested for hepatotropic non-immunogenic drug carriers[11]. Also certain bacteria or similar bacterial polysaccharides, for instance asialopolysaccharides of B-streptococci, may compete as blocking agents for the receptor lectin involved in the metastatic adhesion of tumor cells. In this connection it is noteworthy to mention that viruses may also attach to the liver lectin (HBP) as an alternative route of infection[12] and may interfere with metastases.

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