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# ALTERATION OF GANGLIOSIDES IN PLASMA AND RED CELLS OF HUMANS BEARING MELANOMA TUMORS

Jacques Portoukalian[1], Georges Zwingelstein[1], Nabil Abdul-Malak[1] and Jean-François Doré[2]

[1] Laboratoire de Physiologie Générale et Comparée Université Lyon-I, 69621 Villeurbanne, France

[2] Laboratoire d'Immunologie et de Cancérologie Expérimentale, Centre Léon Bérard, 69373 Lyon Cedex, France

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## SUMMARY

In melanoma tumor-bearing humans, the levels of lipid-bound sialic acid were significantly elevated in both plasma and erythrocytes. Disialosyllactosylceramide in the plasma and sialosyllactosylceramide in red cells were the gangliosides mainly concerned by the increase, as compared to their concentration in the blood of healthy humans. In surgically treated patients, the plasma gangliosides remained higher than the controls, whereas a downward trend was noticeable in red cells. It is suggested that the occurrence of increased amounts of disialosyllactosylceramide in patients' plasma reflects the previously shown presence of this ganglioside as a major component in the sialic acid-containing glycolipid fraction of malignant melanocytes.

## INTRODUCTION

In a previous study, we have demonstrated the presence of large amounts of $GM_3$*, $GM_2$ and $GD_3$ gangliosides in human melanoma tumors, as well as in three malignant melanocyte cell lines freshly established from this type of tumor ( 2 ). Preliminary data indicated some immunoreactivity between several patients' sera and the high molecular weight ganglioside fraction purified from their melanoma tumors ( 3 ). These gangliosides were found to be mostly associated with the membrane-enriched fractions prepared from the tumors ( 4 ). Recently, a significant elevation in the sialic acid level in sera of melanoma patients has been reported ( 5 ), and in humans bearing various types of tumor, the lipid-bound sialic acid content of the serum has been shown to increase ( 6 ). An earlier study on hepatoma-bearing rats evidenced an alteration in the serum gangliosides

*Abbreviations: the nomenclature of Svennerholm ( 1 ) was used throughout. $GM_3$, sialosyllactosylceramide, NeuNac-Gal-Glc-Cer; $GM_2$, GalNac-(NeuNac)-Gal-Glc-Cer; $GD_3$, disialosyllactosylceramide, NeuNac-NeuNac-Gal-Glc-Cer; $GD_{1a}$, NeuNac-Gal-GalNac-(NeuNac)-Gal-Glc-Cer; $GD_{1b}$, Gal-GalNac-(NeuNac-NeuNac)-Gal-Glc-Cer; SPG, sialosylparagloboside, NeuNac-Gal-GlcNac-Gal-Glc-Cer.

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( 7 ). These observations led us to investigate a possible modification in the ganglioside pattern of the plasma and red cells from melanoma patients.

## MATERIALS AND METHODS

Blood samples were obtained from humans bearing melanoma tumors at various clinical stages of the disease. The blood taken after tumor excision was from patients who underwent surgery within three months before each experiment without any indication of further tumor recurrence. None of the patients received any therapy known to alter the lipid metabolism. Samples of blood were also obtained from healthy humans. The blood was treated with heparin in order to prevent clotting and the samples were immediately separated into plasma and red cells by centrifugation, then frozen at -70°C for no more than two days until utilization. Each plasma was extracted with 20 volumes of chloroform-methanol ( 1:1, v/v ) at 4°C for 4 hr . The red cell pellet was taken up with 10 volumes of ice-cold methanol for 30 mn, then 10 volumes of chloroform were added and the mixture was stirred vigorously. The extraction was allowed to proceed 4 hr at 4°C . After filtration, the solid residues from plasma and red cells were reextracted with 10 volumes of the same solvent mixture for 1 hr at room temperature, following homogenization with a Polytron homogenizer. Both extracts were pooled and evaporated under reduced pressure in a rotary evaporator at 45°C . The lipid residues were dissolved in a small amount of chloroform-methanol ( 2:1, v/v ), filtered, evaporated and weighed.

The extracts were freed from non-lipid contaminants on Sephadex G-25 ( Pharmacia, Uppsala, Sweden ) ( 8 ). The lipids were then applied, in diethyl ether, to a silicic acid column ( 100 mesh AR, Mallinckrodt, St Louis, Mo, USA ) previously washed with acetone and diethylether, kept at 15°C by a Haake NK 22 cryostat ( Haake, Berlin, Germany ). Neutral lipids were eluted by diethyl ether, then a mixture of diethyl ether-methanol ( 1:1, v/v ) eluted glycosphingolipids and phospholipids, except choline-containing phospholipids which were eluted by pure methanol. All fractions were evaporated and weighed. The fraction containing the gangliosides was applied to a DEAE Sephadex A-25 column and the gangliosides were purified according to Yu and Ledeen ( 9 ), then desalted on a Sephadex G-25 column. The sialic acid assay was performed on the purified gangliosides with the periodate-resorcinol method of Jourdian et al. ( 10 ). By this procedure, the recovery of $^{14}$C-labeled $GM_3$ added to plasma samples averaged 90%.

The purified gangliosides were taken up with a small amount of chloroform methanol ( 2:1, v/v ) and applied under a stream of nitrogen to silica gel 60 precoated chromatoplates ( Merck, Darmstadt, Germany ) along with known gangliosides extracted from beef brain. Following development in chloroform-methanol 0.2% aqueous potassium chloride ( 60:35:8, by vol.) ( 11 ), and staining with resorcinol-hydrochloric acid ( 12 ), quantification was carried out by scanning with a Vernon integrator ( Vernon, Vitry, France ). Alternatively, the gangliosides were visualized with primuline under ultra-violet light ( 13 ), the spots were scraped off, eluted and rechromatographed in chloroform-methanol-water-28% ammonia ( 60:35:6:2, by vol.) to ascertain their purity. Each purified component was subjected to neuraminidase hydrolysis ( EC 3.2.1.18., Type V from Clostridium perfringens, Sigma, St Louis, Mo, USA ) with and without taurocholate. Products of hydrolysis were chromatographed in chloroform-methanol-0.2% aqueous potassium chloride, along with known neutral glycolipids extracted from human erythrocytes ( 14 ). The major ganglioside of red cells was termed sialosylparagloboside, since its asialo moiety was the "paragloboside", as analysed by Siddiqui and Hakomori ( 15 ). $GM_3$, $GD_{1a}$ and $GD_{1b}$ were tentatively identified in red cells as other major components, whereas $GM_3$, SPG and $GD_3$ were found in plasma. Several minor gangliosi

TABLE I

Content of gangliosides in plasma of melanoma patients.

Results are expressed in nanomoles sialic acid per ml plasma ± SD

| | gangliosides | | | total lipid-bound sialic acid |
|---|---|---|---|---|
| | $GM_3$ | SPG | $GD_3$ | |
| normal (5*) | 10.8 ±2.1 | 0.7 ±0.1 | 0.7 ± 0.1 | 12.3 ±0.2 |
| melanoma (7) | 13.3 ±1.7 | 1.8 ±0.5 | 2.8 ± 0.6 | 18.0 ±2.2 |
| after surgery (3) | 14.7 ±1.7 | 2.2 ±0.2 | 1.4 ± 0.1 | 19.2 ±2.5 |

* Number of individuals. Each sample was analyzed twice. The results are the average of all determinations ± SD.

TABLE II

Content of gangliosides in red cells of melanoma patients.

Results are expressed in nanomoles sialic acid per g of packed red cells ± SD.

| | gangliosides | | | | total lipid-bound sialic acid |
|---|---|---|---|---|---|
| | $GM_3$ | SPG | $GD_{1a}$ | $GD_{1b}$ | |
| normal (5*) | 5.5 ± 0.6 | 17.0 ± 2.8 | 1.8 ± 0.2 | 0.5 ± 0.1 | 24.8 ± 3.3 |
| melanoma (7) | 16.2 ± 2.2 | 18.1 ± 2.0 | 3.2 ± 0.8 | 0.5 ± 0.1 | 37.9 ± 3.7 |
| after surgery (3) | 12.3 ± 0.3 | 17.6 ± 0.7 | 2.3 ± 0.6 | 0.6 ± 0.1 | 32.6 ± 1.1 |

* as in Table I

des were detected in both plasma and red cells, without further attempts to identify them.

## RESULTS AND DISCUSSION

Tables I and II show the amounts of lipid-bound sialic acid assayed in the plasmas and red cells obtained from melanoma tumor-bearing patients, surgically

treated patients and healthy humans. The values found for normal plasmas were somewhat lower than those reported by Kloppel et al. ( 6 ), although they were consistent with the data given by Yu and Ledeen ( 8 ), whereas the levels determined in red cells were high as compared to earlier studies ( 16-18 ). The gangliosides in both plama and red cells increased significantly ( as controlled by Student's t-test ) in melanoma tumor-bearing patients. After surgery, a slight downward trend was noticeable in the erythrocytes, whereas the plasma gangliosides remained at a high concentration. Since Kloppel et al. found the glycolipid-bound sialic acid content to decrease after surgery in the sera of carcinoma-bearing humans ( 6 ), it seems that the effect of malignancy upon the serum gangliosides depends on the type of tumor. Such an observation was already drawn by the comparison of the data concerning the levels of total sialic acid in the sera of humans bearing two different types of tumor: no alteration was found in breast cancer patients ( 19 ), whereas a significant elevation was observed in melanoma patients ( 5 ). It is noteworthy that the extent of variation shown in the latter on total sialic acid was similar to that determined on lipid-bound sialic acid in the present study.

Our findings indicate that the increase in the ganglioside content concerns mainly $GD_3$ in the plasma and $GM_3$. As pointed out by Skipski et al. ( 7 ), this alteration could be related to the type of tumor, since we have previously shown the ganglioside composition of three melanocyte cell lines, freshly established from human melanoma tumors, to comprise $GM_3$, $GM_2$ and $GD_3$ as major components ( respectively 30%, 15% and 50% of the total gangliosides ) ( 2 ). One possible mechanism by which the blood gangliosides are altered in tumor-bearing humans has been suggested by the report of cell surface shedding from tumor cells ( 20 ). In the course of our study, we had an additional indication about this phenomenon by the analyses of the ganglioside pattern of two ascitic fluids removed from melanoma patients and centrifugated to discard the ascitic cells. The lipid-bound sialic acid levels were respectively of 31.2 and 28.2 nmoles/ml in the fluids, with a very high concentration of $GD_3$, up to 50% of the gangliosides in both cases, although the fluids lacked any detectable amount of $GM_2$ which was a major component of the ascitic cells. Hence, it might be suggested that the elevation of $GD_3$ in the plasma of melanoma patients reflects some release of this compound by the malignant melanocytes. To what degree such a mechanism can account for the changes in blood gangliosides remained unclear, since the elevation in the red cells lipid bound sialic acid concerned only $GM_3$, without traces of $GD_3$. The hypothesis of a selectivity in the accumulation of $GM_3$ by the red cells is supported by the study of Keenan et al. ( 21 ) who showed some specificity in the binding of exogenous gangliosides to erythrocytes. An investigation is now in progress in our laboratory

in order to understand the nature of the ganglioside accumulation presently found in the red cells of melanoma patients. Moreover, a long-term study on blood gangliosides will determine whether the observed changes could be valuable in monitoring the variations of the tumor burden.

## REFERENCES


1. Svennerholm, L. (1963) J. Neurochem., 10, 613-623.
2. Portoukalian, J., Zwingelstein, G., and Doré, J.F. (1977) 4th International Symposium on Malignant Melanoma, Lyon, France.
3. Portoukalian, J., Zwingelstein, G., Doré, J.F., and Bourgoin, J.J. (1976) Biochimie, 10, 1285-1287.
4. Portoukalian, J., Zwingelstein, G., and Doré, J.F. (1976) Symposium on Recent Advances in Biology of Melanoma and Leukemia, Brussels, Belgium.
5. Silver, H.K.B., Rangel, D.M., and Morton, D.L. (1978) Cancer, 41, 1497-1499.
6. Kloppel, T.M., Keenan, T.W., Freeman, M.J., and Morré, D.J. (1977) Proc. Natl. Acad. Sci. USA, 74, 3011-3013.
7. Skipski, V.P., Katopodis, N., Prendergast, J.J., and Stock, C.C. (1975) Biochem. Biophys. Res. Commun., 67, 1122-1127.
8. Wells, M.A., and Dittmer, J.C. (1963) Biochemistry, 2, 1259-1263.
9. Yu, R.K., and Ledeen, R.W. (1972) J. Lipid Res., 13, 680-686.
10. Jourdian, G.W., Dean, L., and Roseman, S. (1971) J. Biol. Chem., 246, 430-435.
11. Van den Eijnden, D.H. (1971) Hoppe-Seyler's Z. Physiol. Chem., 352, 1601-1602.
12. Svennerholm, L. (1957) Biochim. Biophys. Acta, 24, 604-611.
13. Skipski, V.P. (1975) Methods in Enzymology, 35, 396-445 ( Lowenstein, J.M., ed.) Academic Press, New York.
14. Hakomori, S., Siddiqui, B., Li, Y.-T., Li, S.-C., and Hellerqvist, C.G. (1971) J. Biol. Chem., 246, 2271-2277.
15. Siddiqui, B., and Hakomori, S. (1973) Biochim. Biophys. Acta, 330, 147-155.
16. Ando, S., and Yamakawa, T. (1973) J. Biochem., 73, 387-396.
17. Wherrett, J.R. (1973) Biochim. Biophys. Acta, 326, 63-73.
18. Li, Y.-T., Månsson, J.-E., Vanier, M.-T., and Svennerholm, L. (1973) J. Biol. Chem., 248, 2634-2636.
19. Ip, C., and Dao, T. (1978) Cancer Res., 38, 723-728.
20. Kim, U., Baumler, A., Carruthers, C., and Bielat, K. (1975) Proc. Natl. Acad. Sci. USA, 72, 1012-1016.
21. Keenan, T.W., Franke, W.W., and Wiegandt, H. (1974) Hoppe-Seyler's Z. Physiol. Chem., 355, 1543-1558.