QUANTITATIVE DETERMINATION OF N-GLYCOLYLNEURAMINIC ACID IN CHICKEN MAREK'S DISEASE LYMPHOMA-DERIVED CELL LINE AND HUMAN VARIOUS CANCER TISSUES BY GAS CHROMATOGRAPHY-MASS FRAGMENTGRAPHY (GC-MF)

Tōru KAWAI

Department of Biochemistry
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

N-glycolylneuraminic acid (NeuGc) is distributed in many animals except in human and chicken. However, human and chicken produce a heterophile antibody [Hanganutziu-Deicher (H-D) antibody] which recognizes this type of sialic acid either in cancer status or when immunized with NeuGc-containing gangliosides. This occurrence of NeuGc was indicated as a cancerous marker (NAIKI et al., 1982, Adv. Exp. Med. Biol., 152 : 445–456 ; IKUTA et al., 1982. Biken J., 25 ; 47–50). In this thesis, a quantitative determination method of this sialic acid as well as normally found N-acetylneuraminic acid (NeuAc) using GC-MF was first established to prove physicochemically, the occurrence of NeuGc in the cancerous tissues.

Prior to determination, each d_3-methylester d_3-methylglycoside of each sialic acid as internal standards was made by methanolysis of each sialic acid-containing ganglioside with 0.05N HCl/CD_3OD. NeuAc was determined by peak area ratio of ions, m/e 420 to 426 while NeuGc was by peak area ratios of ions of both of m/e 386 to 389, and m/e 508 to 514. The detection limit for NeuGc was 20 pg using a mass-marker pair of 386 and 389 and 200 pg using that of 508 and 514 respectively.

In the Marek's disease lymphoma-derived cell line (MSBl), a H-D antigenic glycosphingolipid (GSL) was detected to be contained 3% of the total GSL fraction in sialic acid content by ELISA inhibition test, and the value fairly agreed with NeuGc content (1%) by GC-MF.

In total of 19 cases of human cancerous tissues, the contents of the GSL fractions were analyzed for both sialic acids. In comparison with those from normal tissues obtained from several different organs of a healthy donor (lung, gastric, liver, spleen, colon and rectum), total sialic acid contents of cancerous tissues were approximately 3 times higher. In respect of NeuGc detection, 15 cases (79%) were positive by using a mass-marker pair of 386 and 389, and 7 cases (37%) by a pair of 508 and 514. However, NeuGc was also detected in normal colon and rectum. These cancerous tissues and their GSL fractions were also examined immunologically by membrane immunofluorescence, ELISA and thin-layer chromatography enzyme immunostaining. Detection percentage of NeuGc in cancerous tissues by the GC-MF method was higher than that of H-D antigen(s) by any of those immunological methods.