PRELIMINARY STUDIES ON EXPRESSION OF HANGANUTZIU AND DEICHER (HD) ANTIGENS ON HUMAN CANCER CELLS

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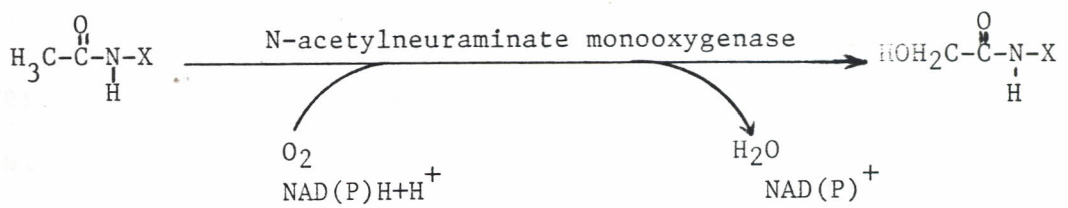
A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF SCIENCE IN THE UNIVERSITY OF NAIROBI.

1991

SUMMARY

N-glycolylneuraminic acid (NGNA), a sialic acid common in animals, is foreign to humans who possess only N-acetylneuraminic acid (NANA) on their cell membranes. Cancerous tissues occasionally possess NGNA-containing glycosphingolipids and glycoproteins referred to as Hanganutziu and Deicher (HD) antigens. Investigation with surviving slices and crude extracts of porcine submandibular gland, a tissue rich in NGNA, suggest that it is synthesized by an NAD(P)H-dependent hydroxylation of free or bound NANA catalysed by the enzyme N-acetylneuraminate monooxygenase (EC.1.14.99.18). Antibodies against NGNA have also been demonstrated in serum of cancer patients and not in normal humans. The fact that NGNA has been detected in cancerous tissues and fluids means that the enzyme involved is either newly generated by the cancerous cells or its activity is increased as a result of malignancy. This has not been determined in humans.

I therefore propose the following as what happens on the cell membrane of the cancerous tissue;



Where X is a glycosphingolipid or glycoprotein moiety.

In the course of malignancy, there is an increase in synthesis of sialic acids namely, NGNA, NANA or both. Following synthesis of NGNA, the immune system responds by producing high titer antibodies. These antibodies, called Hanganutziu and Deicher (HD) antibodies are of heterophile type because they occur in humans who have never been injected with NGNA-containing glycoconjugates.

In order to demonstrate the presence of the enzyme in human cancerous tissues, a biochemical enzyme assay procedure has to be developed. This study describes a spectrophotometric assay method that is simple to perform in an ordinary laboratory. For this purpose, porcine submandibular gland, a tissue known to have a high activity of the enzyme N-acetylneuraminate monooxygenase, was used as a model to establish the technique. The previously used radiolabeling technique is expensive, dangerous and tedious to perform.

A homogenate of the porcine submandibular gland was first spun at 15,000 x g to remove the mitochondrial and other heavy fractions. The resultant supernatant was again spun at 106,000 x g to obtain a cytosolic and microsomal fractions.

At first, the cofactor specificity was investigated using endogenous substrate and exogenous substrates in case of the cytosolic fraction. Several

divalent cations were singly incorporated in the assay system. Only Fe^{2+} ions were found to enhance the enzyme activity. The enzyme's dependence for the divalent cation was confirmed by addition of a chelating agent whereby the enzyme activity was completely abolished. Secondly, the coenzyme specificity was studied. NADH and NADPH were used on both fractions. The cytosolic enzyme showed a high K_m for NADH than the microsomal enzyme while the reverse was true when NADPH was used suggesting that the cytosolic enzyme prefers NADPH while the microsomal enzyme NADH. In order to maximise the detection of the enzyme activity, a reduced environment was created by incorporating 2-mercaptoethanol or reduced glutathione. An enhanced activity was observed in both fractions although the microsomal fraction had a relatively higher specific activity. The effect of different concentrations of exogenous NANA and cytidine 5'-monophosphate-N-acetylneuraminic acid (CMP-NANA) as substrates was investigated. CMP-NANA was recently labeled as the enzyme's authentic substrate. Only the cytosolic enzyme was investigated because the endogenous substrate contained in the fraction was exhausted after 20 minutes. The endogenous substrate in the microsomal fraction was not exhausted even after 60 minutes of incubation making it difficult for the exogenous substrate to be used. Reciprocal plots of

activity versus substrate concentrations of NANA and CMP-NANA gave K_m values of 0.28 mM and 0.34 mM respectively using NADPH.

The above results indicate that there exists a cytosolic and microsomal form of N-acetylneuraminate monooxygenase which has a bi-specificity for both coenzymes. Though the two forms of the enzyme showed affinities for the above mentioned two coenzymes, the cytosolic enzyme's K_m for NADPH was 2 times lower than that for NADH while the microsomal fraction had a 2.4 times lower K_m for NADH than NADPH. Similar affinities for the exogenous substrates were shown by the cytosolic enzyme though a preference for NANA was evident. Fe^{2+} ions were shown to be an important inorganic cofactor for the enzyme and a reduced environment was observed to be conducive for the activity of the enzyme.

Elevated levels of total sialic acid in serum of cancer patients as compared to normal controls have been reported. As mentioned earlier, heterophile HD antibodies have also been demonstrated in patient's serum. This shows that a certain portion of the sialic acid in the patient's serum is of the N-glycolylneuraminic acid type which elicits immunogenicity. To demonstrate any relationship between elevated total sialic acid levels and presence of HD antibodies, serum from 7 hepatoma patients was

assayed for the above on weekly basis for a period of 2 months. 33 normal serum samples were used for comparison. A spectrophotometric method of assay was used to determine total sialic acid and an Enzyme-linked Immunosorbent assay (ELISA) procedure was established for HD antibodies, using a pure N-glycolyl-containing ganglioside. All sera showed elevated total sialic acid content while 6 out of the 7 patients had a raised ELISA antibody titers for at least 4 weeks of the 2 months follow up study. Haemagglutination (HA) titers of the same serum samples were determined using horse erythrocytes to confirm that the antibodies determined by ELISA were specifically oriented to the purified N-glycolyl-containing antigen. A positive correlation was observed between ELISA and HA titers in 2 out of the 7 patients indicating that antibodies were mainly N-glycolylneuraminic acid specific. However, comparison between sialic acid levels and HD antibody titers showed that most of the sialic acid produced by the cancerous tissue is mainly of the N-acetylneuraminic acid type and which has no relationship with the antibodies in the serum.

The above described work was necessary for the future follow up of cancer patients. It is important that a follow up on sialic acid levels and HD antibody titers is carried out on cancer patients to diagnose a possible recurrence of the tumour or metastasis after

surgery and during chemotherapy, radiotherapy or immunotherapy. These studies would also be of significance in evaluating effects of a new drug or form of treatment on a tumour. Enzyme studies are also important in that during follow up studies, the removed cancerous tissues will be screened for N-acetylneuraminate monooxygenase activity. The demonstration of any activity indicates possible conversion of non-immunogenic NANA to immunogenic NGNA. The presence of NGNA can be demonstrated by detection of HD antibodies in serum.