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ELEVATED ACTIVITY OF CYTIDINE 5'-MONOPHOSPHO-N-ACETYLNEURAMINIC ACID HYDROLASE IN SERUM OF OVARIAN CANCER PATIENTS AS A POSSIBLE INDICATOR OF MALIGNANCY

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SUMMARY: Cytidine 5'-monophospho-N-acetylneuraminic acid hydrolase activity has been demonstrated in homogenates of normal ovary and ovarian epithelial adenocarcinomas, as well as in the sera of normal individuals and ovarian cancer patients. The specific activity of the enzyme in ovarian tumors is significantly reduced compared to normal ovaries. In pre-operative sera of these patients, the activity is elevated about 2 fold in comparison with age and blood group matched controls. The patients with lower tissue enzyme activity have higher serum values. After tumor reductive surgery, serum levels of this enzyme are diminished, but are still higher than controls. Clinical use of this assay for detection and management of ovarian neoplasia is promising.

INTRODUCTION:

Cytidine 5'-monophospho-N-acetylneuraminic acid (CMP-NANA) is the common substrate for the enzyme N-acetylneuraminic acid (NANA) transferase and CMP-NANA hydrolase. The later enzyme is a phosphodiesterase specific for CMP-NANA which is completely resistant to hydrolysis by alkaline phosphatase of Escherichia coli and intestine, venom phosphodiesterase, 5'-nucleotidase, phosphodiesterase and the phosphatases together, as well as neuraminidase (1). The level of CMP-NANA hydrolase in a cell may control in part the formation of NANA containing heteropolymers by NANA transferases.

CMP-NANA hydrolase has been detected in a number of animal organs (1-3), but its existence in human tissue has not as yet been established. In this communication, we demonstrate that this enzyme is present in human ovary, as well as serum, and propose that a part of this enzyme is shed from the malignant cells of the ovary into the systemic circulation, resulting in the elevation of CMP-NANA hydrolase in patients' sera. Possible use of this assay in the early diagnosis and management of malignancy is discussed.

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MATERIALS AND METHODS: CMP- [4-^{14}C]- NANA (1 Ci/mole) was obtained from New England Nuclear, Boston, MA and was purified by paper chromatography before use. All other chemicals of analytical grade were obtained from various commercial sources. Methods for the preparation of ovarian homogenate and collection of serum have been described earlier (4). The enzyme was assayed essentially by the procedure of Kean and Bighouse (2). The incubation mixture contained 100 nmoles of CMP- [4-^{14}C] - NANA (200,000 d.p.m.), 7.5 μmoles of tris-HCl, pH 9.0, 0.25μmoles of $CaCl_2$, 0.2% triton X-100, and 25 μl of serum or tumor homogenate in a total volume of 50μl. The incubation was performed at 37°C for 1 hour and terminated by 50μl of 0.4M EDTA, pH 9.0. The reaction mixture was immediately frozen in dry ice and kept frozen until separation of the products (5). Three control tests were run in parallel which contained 1) 25μl of 0.85% NaCl or 2) boiled enzyme instead of the enzyme and 3) zero time incubation. This assay was also confirmed by the method of Van Dijk et al (3).

RESULTS:

CMP-NANA hydrolase in homogenates from normal and malignant ovaries. Linearity of the reaction rate with respect to incubation time and amount of enzyme protein was established using normal and malignant ovarian tissue homogenate as well as sera. In 7 specimens of normal ovarian homogenate the specific activity (nmoles/hour/mg protein) of the enzyme varied in the range 56-206, with a mean $\pm$ 1 SD of 113 $\pm$ 58. In parallel experiments, the CMP-NANA hydrolase was determined in homogenates from 13 ovarian cancer specimens and the data summarized in Table 1. The specific activity of the enzyme was lower in all 13 samples than the normal mean. In 2 of the patients (BV and WM) the specific activity was within normal range. BV had a recurrent borderline serous cystadenocarcinoma of the ovary, while WM had a benign Brenner's tumor of the ovary. Excluding these 2 samples, the mean $\pm$ 1 SD of the 11 established malignant specimens was 27 $\pm$ 11, and all the normal values are higher than mean $\pm$ 2 SD. There seemed to be no correlation between the age and blood group of the donors and their tissue levels of CMP-NANA hydrolase.

CMP-NANA hydrolase activity in pre- and post-operative sera of cancer patients. To compare the enzyme levels in the malignant tissue and serum, CMP-NANA hydrolase was assayed in the pre- and post-operative sera and tissue of the same patient. The normal serum level of this enzyme was established in a group of 10 healthy women matched with the patients with respect to age

Table 1. CMP-N-ACETYLNEURAMINIC ACID HYDROLASE ACTIVITY IN HOMOGENATES OF NORMAL AND MALIGNANT OVARY

Donor	Diagnosed Ovarian Carcinoma	Stage	Age	Blood Group	Enzyme Activity (nmoles/hr/mg Protein)
-	Normal[+]		-	-	113[*]
SR	Papillary Serous Adenocarcinoma	III	61	A Rh^+	8
CR	Recurrent adenocarcinoma	II	56	B Rh^-	15
CP	Serous cystadenocarcinoma	III/IV	48	O Rh^+	17
LB	Mucinous cystadenocarcinoma	III	55	A Rh^+	23
HS	Serous cystadenocarcinoma	III	63	A Rh^+	23
CJ	Adenocarcinoma	III	47	A Rh^-	32
IG	Mixed serous and clear cell adenocarcinoma	III	67	O Rh^+	34
TL	Adenocarcinoma	IV	72	A Rh^+	34
BG	Serous cystadenocarcinoma	III	81	O Rh^+	37
LE	Mesonephroid adenocarcinoma	IIC	64	A Rh^+	37
YM	Serous cystadenocarcinoma	III	57	A Rh^+	41
WM	Benign Brenner's tumor	-	70	A Rh^+	65
BV	Recurrent borderline serous cystadenocarcinoma	-	57	A Rh^+	96

[+]Homogenates from 7 ovaries removed during radical hysterectomies and oophorectomies were used, which were confirmed normal by histopathological examination.

[*]Mean of 7 values ranging from 56 to 206 with 1 SD of 58. All values are the average of duplicate determinations.

and blood group. The enzyme activity in nmoles/hour/ml of serum ranged from 28-67 in the control group with mean $\pm$ 1 SD of 52 $\pm$ 12. In all patients, the pre-operative sera showed higher activity than the normal mean $\pm$ 2 SD (Table 2). When serum was drawn 4-7 months after surgery, the level of CMP-NANA

Table 2. CMP-N-ACETYLNEURAMINIC ACID HYDROLASE ACTIVITY IN PRE- AND POST-OPERATIVE SERUM OF CANCER PATIENTS

Donor**	Interval+ (months)	Enzyme Activity* (nmoles/hour/ml serum) Pre-operative	Post-operative	% Change
SR	7	122	114	-6
CR	6	139	119	-14
CP***	4	110	114	+4
HS	6	104	92	-11
CJ	6	87	74	-15
TL	6	82	68	-17
BG	5	93	75	-19
LE	5	92	82	-11
BV	-	142	-	-

*Values are average of duplicate determination.

+Interval between drawing pre- and post-operative serum.

**Pre-operative sera of LB, IG, YM, WB and post-operative serum of BV were not available for assay.

***Expired.

hydrolase was lower than pre-operative values in all cases except patient CP. Her disease progressed despite treatment and she expired 4 months after surgery. Although the differences in the CMP-NANA hydrolase level between pre- and post-operative sera was small, this reduction in the activity was reproducible. These values are still higher than the normal mean, possibly due to the presence of some residual disease.

Results of mixing experiments with tissue and serum ruled out the possibility of any inhibitors or stimulators.

CMP-NANA hydrolase in sera of ovarian cancer patients with no clinical

Table 3. CMP-N-ACETYLNEURAMINIC ACID HYDROLASE ACTIVITY IN SERUM OF PATIENTS WITH NO CLINICAL EVIDENCE OF DISEASE

Donor	Diagnosed Ovarian Carcinoma	Stage	Enzyme Activity* (nmoles/hour/ml serum)
HE	Dermoid cyst with epidermoid carcinoma	-	50
PJ	Recurrent endometroid adenocarcinoma	III	54
BB	Adenocarcinoma	III	59
RE	Serous adenocarcinoma	III	62
HE	Adenocarcinoma	III	66
AS	Dysgerminoma	IV	67
DE	Adenocarcinoma	III	68
LS	Adenocarcinoma	II	75
MJ	Papillary adenocarcinoma	III	79
KG	Adenocarcinoma	III	80

*Values are average of duplicate determinations.

evidence of the disease. The enzyme was assayed in a group of ovarian cancer patients who had no clinical evidence of the disease for at least a year. The enzyme levels in this group were still somewhat higher than normal with a mean $\pm$ 1 SD of 66 $\pm$ 10. In 3 patients, KG, LS, and MJ, the enzyme levels are higher than even this mean value (Table 3). It is interesting that one of them (MJ) had a recent recurrence of the disease, although KG and LS are still free of disease. This raises the possibility of using this enzyme assay to determine recurrence of the disease.

DISCUSSION

Serum enzymes have been explored for many years as possible early biochemical indicators of neoplasia and as aids in following the progression and regression of the disease (6,7). There are a number of possible mechanisms for the appearance of abnormal serum enzyme activities (7). Among

these possibilities, change in the permeability of the cell allowing leakage of soluble enzymes or shedding of cell surface bound enzymes into the circulation (8) are likely to result in the elevation of serum level with concomitant reduction of the enzyme activity in the tumor cells.

Comparison of the data in Tables 1 and 2 indicate that the patients with lower specific activity in the tissues have higher serum levels, with 1 exception (BV). This patient had a recurrent borderline serous cystadenocarcinoma of the ovary. Reduction of the serum level following removal of tumor further suggests that the tumor cells might be the source of part of the serum enzyme.

Cell surface constituents are shed *in vitro* and *in vivo* into the surrounding medium after they terminate their sojourn as part of a functional cell structure. The shedding of these molecules keeps the cell surface free to accept new stimuli or messages (8). In neoplasia this is an important means of tumor-cell escape from immune destruction. The shed components make up a family of so-called "blocking factors" that interfere with host immunity against neoplasia (9-14).

CMP-NANA hydrolase in rat liver is an enzyme of the plasma membrane (2) and this enzyme is located at the surface of the cell membrane, with its functional group directed to the outside (15). Higher activity of CMP-NANA hydrolase in the serum of ovarian cancer patients may be a result of cell surface shedding by the tumor cells, although other mechanisms cannot be ruled out. Galactosyl transferase, which has also been reported to be an ectoenzyme in other systems (16,17) has a higher level in the serum of ovarian cancer patients, although the specific activity of the enzyme in tumor tissues were also elevated (4).

We have not yet investigated whether elevation of this enzyme takes place in the serum of patients with other malignant and non-malignant diseases. It is also not clear whether this increased activity in serum is due to the synthesis of a new species of the enzyme by the proliferating tumor

cells. Purification of the enzyme from tumor tissue and development of immunological procedures will make the assay more sensitive. Possible use of elevated CMP-NANA hydrolase as a diagnostic screening tool or as a monitor of therapeutic response is presently under investigation.

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REFERENCES

1. Shoyab, M., and Bachhawat, B.K. (1967) Biochem J. 102, 13-14C.
2. Kean, E.L., and Bighouse, K.J. (1974) J. Biol. Chem. 249, 7813-7823.
3. Van Dijk, W., Maier, H., and Van den Eijnden, D.H. (1976) Biochem. Biophys. Acta 444, 816-834.
4. Bhattacharya, M., Chatterjee, S.K., and Barlow, J.J. (1976) Cancer Res. 36, 2096-2101.
5. Kean, E.L. (1972) Methods Enzymol. 28, 983-990.
6. Bodansky, O. (1961) Advan. Cancer Res. 6, 1-80.
7. Schwartz, M.K. (1973) Clin. Chem. 19, 10-22.
8. Doljanski, F., and Kapeller, M. (1976) J. Theor. Biol. 62, 253-270.
9. Hellström, K.E., and Hellström, I. (1974) Adv. Immunol. 18, 209-277.
10. Proctor, J.W., Rudenstam, C.M., and Alexander, P. (1974) J. Natl. Cancer Inst. 53, 1671-1676.
11. Coggin, J.H. Jr., Ambrose, K.R., Dierlam, P.J. and Anderson, N.G. (1974) Cancer Res. 34, 2092-2101.
12. Kim, U., Baumler, A., Carruthers, C., and Bielat, K. (1975) Proc. Natl. Acad. Sci. U.S. 72, 1012-1016.
13. Chatterjee, S.K., Kim, U., and Bielat, K. (1976) Br. J. Cancer 33, 15-26.
14. Chatterjee, S.K., Kim, U. (1977) J. Natl. Cancer Inst. 58, 273-280.
15. Van Dijk, W., Maier, H., and Van den Eijnden, D.H. (1977) Biochem. Biophys. Acta 466, 187-197.
16. Roth, S., and White, D. (1972) Proc. Natl. Acad. Sci. U.S. 69, 485-489.
17. Porter, C.W. and Bernacki, R.J. (1975) Nature (London) 256, 648-650.