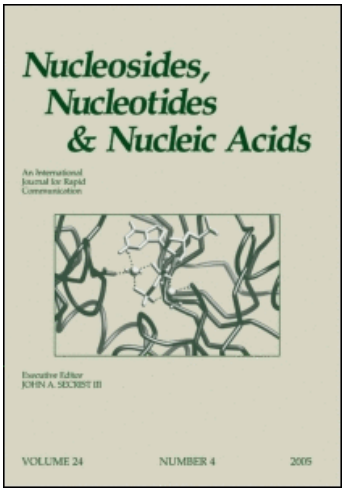

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Dual Column HPLC Analysis of Modified Ribonucleosides as Urinary Pathobiochemical Markers in Clinical Research

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DUAL COLUMN HPLC ANALYSIS OF MODIFIED RIBONUCLEOSIDES AS URINARY PATHOBIOCHEMICAL MARKERS IN CLINICAL RESEARCH

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ABSTRACT. - The quantitative determination of the urinary ribonucleosides m1Ado, m22Guo and t6Ado of breast cancer patients was performed by use of an automated high performance liquid chromatography analyzer with on-line sample processing and analysis by a dual column switching technique. M22Guo and t6Ado showed elevated excretion values (normalized to creatinine) in all of our 24 perioperatively examined breast cancer patients. Studies of these two urinary ribonucleosides 3 years after primary surgery showed markedly reduced excretion values in case that no relapse had occurred.

Modified ribonucleosides are mainly found in transfer RNA. They orginate from cellular RNA breakdown and are excreted in the urine. Since these metabolic products are not reutilized but renally eliminated, the relative proportion of modified ribonucleosides is higher in urine than in serum. Evidence has been provided that the profile of modified ribonucleosides in human urine is altered in individuals suffering from neoplastic diseases [1-8]. Several of the following compounds pseudouridine, m1Ado, m1Ino, m2Guo, m22Guo, PCNR and t6Ado represent in each case pathobiochemical marker molecules for different types of cancer diseases. These findings imply the analysis of modified ribonucleosides in urines to be useful as a non-invasive screening test.

Over the last five years, we have developed a dual column HPLC method with systems integrated sample processing [7,8]. The apparatus consists of a modular automated high performance liquid chromatography (SEC-HPAC/RPLC) system combining chemoselective affinity and size exclusion properties in the pre-column (SEC-HPAC) with a reversed phase analytical column (RPLC) for quantifying the ribonucleosides in urine samples. The two columns are connected via an automated six-port switching valve. The pre-column is filled with a modified phenylboronic acid substituted vinyl polymer and has, under HPLC conditions, 2 functions: (1) Since they contain cis-diol groups, the ribonucleosides are bound chemoselectively and reversibly by forming under slightly alkaline conditions a cyclic diester with the phenyl boronate functional groups. The ribonucleosides are thus separated from the rest of the urine matrix. (2) The gel permeation properties of the pre-column material allow complete separation of the biological residual matrix, even if it contains proteins.

We have analyzed the excretion pattern of N1-methyladenosine (m1Ado), N2-dimethylguanosine (m22Guo) and N6-carbamoyl-threonyladenosine (t6Ado) in the urine of patients with breast cancer (Fig.1). These 3 nucleosides have been chromatographically and spectrometrically characterized

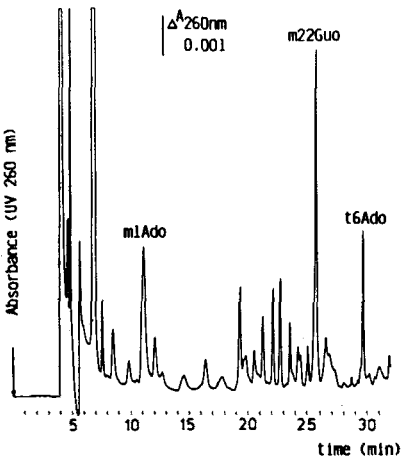

Fig.1 shows as an example a typical HPLC run of 20 µl of a urine from a patient with breast cancer.

including chemical peak-shifting experiments. The urinary levels of m22Guo and t6Ado reflect the tRNA-turnover and allow conclusions to be drawn about whole-body metabolic activities [9].

In all of the 24 samples collected perioperatively m22Guo (control collective 2.26, SD = 0.29 μmol per mmol creatinine) was increased [10]. 21 of the 24 values, i.e. about 90 %, exceeded the means of our healthy control group [11] by more than 2 standard deviations (SD). A cut-off value for pathological findings is normally estabilished in this manner, when metabolites - in this case biochemical tumor markers - are measured which are also present in urines of healthy people. Three years after surgery 7 patients, i. e. one third, still had a urinary m22Guo-index above the mean + 2 standard deviations of a group of normal subjects. One of the patients was currently suffering a local relapse at the time of investigation, another had suffered a relapse the year before. 21 of the 24 patients had perioperatively increased urinary t6Ado-values (mean in our control group of healthy normal subjects 0.95; SD = 0.25 μmol t6Ado per mmol creatinine) [10], 13 of the values exceeding the 2-fold SD-range. Three years later only 1 patient - who is relapse-free - still had such a high urinary t6Ado index. Levels above the mean but below the cut-off value were estabilished for 16 patients. Of the 4 patients with local relapse 3 had increased t6Ado-indices. The urinary nucleoside indices of the other modified ribonucleoside analyzed , m1Ado, of breast cancer patients and of healthy subjects did not differ significantly. These findings support the idea of using specific modified ribonucleosides as non-invasive tumor markers in clinical diagnosis. Especially m22Guo may prove successful in identifying patients having a high relapse risk.

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