The Use of ^{1}H-NMR Spectroscopy for Predicting the Efficiency of Neoadjuvant Chemotherapy of Breast Cancer

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 7, pp. 102-105, July, 2000
Original article submitted February 21, 2000

The pool of low-molecular-weight metabolites was studied in patients with breast cancer by high-resolution ^{1}H-NMR spectroscopy. In order to predict the efficiency of treatment, mathematical regression analysis was carried out with consideration for some clinical morphological characteristics of patients, chemotherapy protocols, and the degree of therapeutic pathomorphosis. The efficiency of drug therapy was largely determined by metabolic status of tumors in untreated patients with breast cancer.

Key Words: *^{1}H-NMR; low-molecular-weight metabolites; breast cancer; neoadjuvant chemotherapy; degree of therapeutic pathomorphosis*

Current concepts on the mechanisms underlying the effects of antitumor drugs widely used in clinical practice are contradictory and do not fully explain the efficiency of drug therapy of malignant tumors. The purpose of this study was to develop new approaches to individual treatment of cancer patients and prediction of its efficiency. A pool of low-molecular-weight (below 400 D) metabolites can be investigated by ^{1}H-NMR spectroscopy *in vitro* and *in vivo*. This pool includes essential amino acids (choline, myoinositol), creatine, and lactate [1,4,5]. A pool of low-molecular-weight metabolites was investigated by ^{1}H-NMR spectroscopy in biopsy specimens from patients with breast cancer, who were then treated by neoadjuvant chemotherapy (NCT). The degree of therapeutic pathomorphosis (TP) was chosen as the criterion of breast cancer sensitivity to the treatment.

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MATERIALS AND METHODS

Twenty-seven patients with breast cancer aged 33-76 years were examined, most of them had locally disseminated tumors (Table 1); all patients were treated by NCT according to protocols presented in Table 2.

The degree of TP was evaluated by pathomorphological analysis of biopsy specimens. Four degrees of TP are distinguished. They reflect changes in tumor tissue under the effect of drug therapy: I) minimal changes in tumor cells (ugly polynuclear cells, solitary foci of sclerotic cells); II) replacement of 50% tumor tissue by connective tissue; III) virtually complete replacement with connective tissue with solitary foci of tumor cells; and IV) complete absence of tumor cells (Table 2).

Low-molecular-weight metabolites in biopsy specimens of breast cancer were analyzed by ^{1}H-NMR spectroscopy. Tumor samples collected from patients during trephine biopsy were immediately frozen in liquid nitrogen, after which the samples for ^{1}H-NMR spectroscopy were treated according to the international protocol [3].

0007-4888/00/0007-701$25.00

Tumors were weighed frozen and thoroughly crushed in a ceramic mortar in liquid nitrogen. The powder (1 g) was homogenized in 4 ml cold 6% $HClO_4$ in a glass homogenizer. The mixture was centrifuged at 3000*g* and 4°C for 10 min. Supernatants were collected and neutralized by 20% KOH. For complete precipitation the samples were left in an ice bath for at least 1 h and then recentrifuged at 3000*g* and 4°C for 10 min. Supernatants were frozen and lyophilized (Freeze-dry System, Labconco). Directly before recording of NMR spectra lyophilized samples were dissolved in D_2O, centrifuged for 3 min to remove unsolved material, and pD of supernatants was adjusted to 6.80±0.05 by adding NaOD or DCl.

^{1}H-NMR spectra were recorded on an AM-400 WB NMR spectrometer (Bruker). The signal of residual water protons was suppressed by presaturation at the corresponding frequency (exposure 1 sec). The use of a 90° probing impulse and 30-sec relaxation delay helped to rule out the effects of saturation of studied signal and to measure the metabolite concentrations. Depending on the sample weight, the spectra were obtained by summing 64-3000 impulse presentations. The free induction fading signal was treated using exponential multiplication with a constant 2 in order to increase the signal/noise ratio. Chemical shifts were estimated using an internal standard (4,4-dimethyl-4-sodium silanenthane sulfate, δ=0 m.d.) added directly before recording. The signal intensity is proportionate to the concentration of the respective compound. For quantitative evaluations the intensity of each signal was standardized to the intensity of the internal standard and weight of wet tissue.

The results were processed by regression analysis using TP criterion. The specific contribution of the factor was evaluated in units equivalent to pathomorphosis units (EPU).

RESULTS

Spectra of biopsy specimens differed in all patients and reflected individual metabolic characteristics of each patient (Fig. 1). We plotted a regression-factor weight function and determined the significance of the effect on the TP criterion of the tumor for 13 factors characterizing the quantitative and qualitative composition of low-molecular-weight metabolites in tumor cells and the relationship between the chosen criterion and some clinical characteristics of the patients and NCT protocols (Table 3).

Analysis of regression function showed that quantitative composition of metabolite pool in tumor biopsy specimens determined the efficiency of chemotherapy by the TP criterion. If the maximum specific significance of relative intensity of the peak at δ=1.75 m.d. (14.09 EPU) is taken for 100%, the specific significance of the rest variables will be 11.21-100%, while specific contribution of chemotherapy protocols is only 3.19-55.22%.

The following metabolites are the most significant: lactate (1.3 m.d.), choline-containing compounds (3.2 m.d.), creatine+creatine phosphate complex (3.0 m.d.), and amino acids characterized by signals δ=2.0-2.2 and δ=2.4-2.5 m.d.

Accumulation of lactate reflecting the degree of tumor tissue hypoxia negatively affects the therapeutic effect; the specific significance of this factor is -9.5 EPU.

TABLE 1. Distribution of Patients by TNM Stages (According to Classification of International Anticancer Union)

TNM stage	Number of patients	
	abs.	%
T2N0M0	3	11.11
T2N1M0	3	11.11
T3N0M0	3	11.11
T3N1M0	2	7.41
T3N2M0	1	3.70
T4N0M0	4	14.82
T4N1M0	7	25.93
T4N2M0	3	11.11
T4N1M1	1	3.70

TABLE 2. Distribution of Patients by Treatment Protocols and TP Degree

NCT protocols	Number of patients		TP degree	Number of patients	
	abs.	%		abs.	%
Cyclophosphamide+doxorubicin+5-fluorouracyl (No. 1)	5	18.52	I	4	41
5-Fluorouracyl+epirubicin+cyclophosphamide (No. 2)	2	8.33	II	7	26
Cyclophosphamide+methotrexate+5-fluorouracyl (No. 3)	2	8.33	III	4	15
Taxoter+cisplatin (No. 4)	8	33.33	IV	5	18
Cyclophosphamide+doxorubicin+cisplatin (No. 5)	7	25.93	Total	27	100

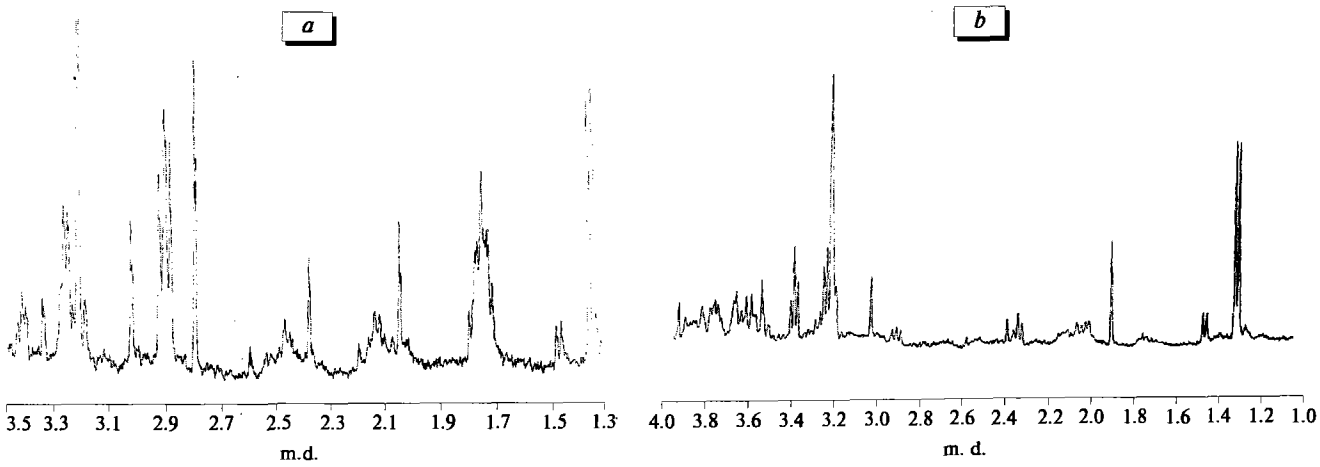

Fig. 1. ^{1}H-NMR spectra of metabolites in breast cancer biopsy specimens from patients P. (*a*) and K. (*b*).

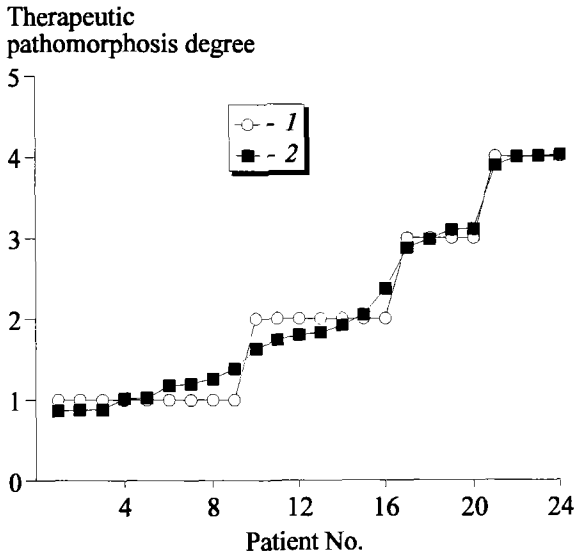

Fig. 2. Prognostic accuracy of specific function of ^{1}H-NMR metabolites, characteristics of patients, and polychemotherapy protocols by therapeutic pathomorphosis (TP) criterion. *1*) TP evaluated histologically; *2*) estimated TP.

We obtained a persuasive evidence that the increased level of choline-containing compounds in the tumor playing an important role in phospholipid metabolism has a favorable impact on NCT effect irrespective of the dose; specific significance of choline-containing metabolites is 3.39 EPU.

Proton spectroscopy allowed us to evaluate the total concentration of creatine and creatine phosphate. The significance of this signal for TP criterion is high (40.03%, Table 3). High content of creatine+creatine phosphate complex in breast cancer cells has a negative impact on the therapeutic effect. The content of this complex in tumor cells differs in different patients; cells with low content of this complex are more susceptible to drug therapy, which indicates that the creatine kinase system is involved in the realization of the therapeutic effect.

In vitro experiments demonstrated that the content of amino acids in tumor cells is an essential factor de-

TABLE 3. Distribution of Patients by TP Degree

Factor, characteristic	Prognostic value EPU
Metabolites	
1.3 m. d. (lactate)	-9.51
1.75 m. d.	-14.09
1.9 m. d. (acetate)	5.58
2.0 m. d.	5.01
2.0-2.2 m.d.	12.4
2.4-2.5 m. d.	2.78
2.7 m. d.	-2.3
2.8 m. d.	-1.58
2.9 m. d. (triplet)	5.08
2.9 m. d. (triplet)	4.44
3.0 m. d. (creatine/creatine phosphate)	-5.64
3.2 m. d. (choline-containing compounds)	3.39
3.4 m. d.	-2.14
Age	-4.07
Number of estrogen receptors	-1.37
Tumor size	-2.12
Number of metastasis sites	1.12
NCT protocol:	
No. 1	0.45
No. 2	3.2
No. 3	7.78
No. 4	0.94

Note. *See table 2. Adequacy of F/F_{CR}=0.161/2.8.

termining their sensitivity to adriamycin [2]. Our results indicate that the higher amino acid concentration in breast cancer biopsy specimens, the more effective is drug therapy, the significance of amino acids being 12.40 and 2.78 EPU.

Hence, the metabolic status of tumors in untreated patients with breast cancer largely determines the efficiency of drug therapy. Differences in the treatment protocols, patient's age, tumor size, number of estrogen receptors, and metastases in the lymph nodes are less significant for the treatment efficiency.

The curve of estimated values of TP degree virtually completely coincided with the curve plotted on the basis of experimental data (Fig. 2), *i. e.* the effect of NCT can be predicted with high probability on the basis of certain data.

The study was supported by Noncommercial Partnership ASGL — New Antitumor Drugs.

REFERENCES

1. P. F. Daly and J. S. Cohen, *Cancer Res.*, **49**, 770-779 (1989).
2. E. Hanushovska, I. Dovinova, and I. Tkach, *Neoplasma,* **45,** 187-197 (1998).
3. S. L. Howeless, R. J. Maxwell, A. C. Peet, and J. R. Grifiths, *Magnetic Resonance Med.*, **28,** 214-236 (1992).
4. D. Leibfritz, *Anticancer Res.*, **16,** 1317-1324 (1996).
5. F. Podo and J. D. Certaines, *Ibid.*, 1305-1316.