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THE POSSIBILITY OF THE ENZYMATIC TEST APPLICATION FOR EFFICACY ESTIMATION OF 5-FLUOROURACIL IN PATIENTS WITH GASTROINTESTINAL CANCER

O.M.Bakurova, K.O.Mironova, B.G.Borzenko

Donetsk National Medical University named after M.Gorky

Key words: gastrointestinal cancer; thymidine phosphorylase; 5-fluorouracil

A comparative study of peculiarities of the thymidine phosphorylase activity in the blood plasma and tissues of patients of different age with gastrointestinal cancer ($T_{3-4}N_{0-x}M_{0-y}$) has been carried out. The aim was to evaluate the diagnostic relevance of different types of the thymidine phosphorylase (TP) activity and their association with tumor growth and the treatment efficacy of 5-fluorouracil. In this study 96 patients aged 35-70 years old with gastrointestinal cancer of $T_{3-4}N_{0-x}M_{0-y}$ stages were included. The control group consisted of 60 non-cancerous patients of the same age without the gastroduodenal zone pathology. The research protocol for studies with human materials was approved by the Ethical Committee of the Donetsk National Medical University named after M.Gorky. The peculiarities of enzyme activities were studied in the blood plasma and tissues. Activities of tissue enzyme were studied in the surgically removed material in 58 tumors and in non-cancerous stomach mucosa (the mucosa margin of resection distant from the carcinoma was as a control). The types of the TP activity were determined spectrophotometrically [2] according to the amount of thymine (thymidine) formed. Statistical analysis of results has been performed using of parametric and nonparametric methods Medstat software package. It has been demonstrated that the TP catabolic activity in tumors is 2.6 times lower compared to the non-neoplastic mucosa of the resection margin and is accompanied with decrease of the activity in the blood plasma. The increase of the transferase activity has been found both in tumor tissues (more than 1.7 times) and in the blood plasma. Changes in the tissues activity in the postoperative material depended on the type of tumor and efficacy of 5-fluorouracil. The value of different types of TP activities can be used as a test for proliferative activity changes (the criteria of Willcoxon, $W = 28.0$, $p<0.001$). Control of individual dynamics of the enzymes activity in the blood may be used as an informative test for monitoring of patients and drug treatment optimization. A practical application of the present study is that targeting TP for therapeutic purposes would be more desirable in individualization of anticancer therapy depending on tumor spreading.

Gastric cancer and colorectal cancer are the more common cancer localizations. Unfortunately, more than 50% of gastrointestinal cancer cases are diagnosed at advanced stages. It is now clear that cancer may be more or less aggressive and that several prognostic and predictive factors may play a major role in the choice of the most appropriate therapy and for final results. Since last century 5-fluorouracil (5-FU) with other anticancer agents and prodrugs from the group of fluoropyrimidines are widely used in the drug treatment of gastric cancer and colorectal cancer [3]. The importance of tumor proliferation in response determining chemotherapy has been clearly proven. The intensity of cellular DNA synthesis and, thus, cell division, depends on the level of deoxythymidine triphosphate (dTTP, the key precursor for DNA synthesis). It can be synthesized following one of the two possible pathways. There are both *de novo* synthesis (which can be inhibited by 5-FU) and *"salvage pathway"*, for example, by recycling thymine, which can be reincorporated into DNA. The intensity of "salvage pathway" is regulated by the activity of two enzymes; one of them is thymidine phosphorylase (TP; EC 2.4.2.4.). A high expression is related to malignant angiogenesis and invasion, and therefore, it is associated with a poor prognosis [2]. It has been postulated that the angiogenic effect of PD-ECGF/TP is related to the enzymatic activity of TP, which catalyses the reversible phosphorolytic cleavage of thymidine to thymine and 2′-deoxyribose-1-phosphate [4]. TP has a moderate or even negligible role in the activation of antimetabolite 5-fluorouracil (5-FU) to fluoro-deoxyuridine-5′-monophosphate by the transferase activity (TPan), but its phosphorolytic activity (catabolic, TPc) is essential for the activation of a prodrug 5′-deoxy-5-fluorouridine (5′-DFUR, furtulon) to 5-fluorouracil. The various complex interactions of TP/PD-ECGP give it an essential role in cellular functioning, and hence, it is an ideal target in cancer chemotherapy. The aim of our study was to investigate the diagnostic relevance of different types of the thymidine phosphorylase activity and their association with tumor growth and the treatment efficacy of 5-fluorouracil.

Materials and Methods

In this study 96 patients aged 35-70 years old with gastrointestinal cancer of $T_{3-4}N_{0-x}M_{0-y}$ stages were included. The control group consisted of 60 non-cancerous patients of the same age without the gastroduodenal zone pathology.

O.M.Bakurova – Candidate of Medicine, associate professor of the Chemistry Department of the Donetsk National Medical University named after M.Gorky

Table

The activity of thymidine phosphorylase in the blood plasma of healthy people and patients with $T_{3-4}N_xM_0$ stages of gastrointestinal cancer at the age of 35-70 years old (nmol/mg·min) (n = 156)

Age	TPan		TPc		TPan/TPc	
	Control	GIC	Control	GIC	Control	GIC
35-45	15.35±2.45	28.52±4.56**	41.61±3.12	38.85±2.85	0.37±0.09	0.73±0.25*
46-60	14.33±3.01	25.03±5.15*	47.88±2.62	15.26±2.55**	0.30±0.14	1.64±0.52**
61-70	14.58±3.45	26.58±2.98**	52.01±2.89	18.31±2.08**	0.28±0.12	1.45±0.71*

Note: Authentic differences versus the control: * – $p<0.05$, ** – $p<0.001$.

The research protocol for studies with human materials was approved by the Ethical Committee of the Donetsk National Medical University named after M.Gorky. The peculiarities of enzyme activities were studied in the blood plasma and tissues. Activities of tissue enzyme were studied in the surgically removed material in 58 tumors and in non-cancerous stomach mucosa (the mucosa margin of resection distant from the carcinoma was as a control). The types of the TP activity were determined spectrophotometrically [2] according to the amount of thymine (thymidine) formed.

Statistical analysis of results was performed using of parametric and nonparametric methods Medstat software package.

Results and Discussion

The peculiarities of enzyme activities have been studied both in the tissues and blood plasma of patients with gastrointestinal cancer (GIC), and in the blood plasma of the control group (Table). It has been demonstrated that the TP catabolic activity in tumors is 2.6 times lower compared to the non-neoplastic mucosa of the resection margin It has been determined that the TPc activity decrease in the gastric cancer tissues up to 21.92±8.60 nmol/mg·min in comparison with non-transformed control tissues (57.82±7.99 nmol/mg·min, $p<0.01$) is accompanied by its transferase activity (TPan) increasing in the tumors (from control 76.86± ±11.65 nmol/mg·min, to 128.01± ±9.82 nmol/mg·min, $p<0.05$). The similar tendency has been found in tumors of colorectal cancer – the TPc activity decrease up to 17.96± ±3.48 in comparison with the control (45.22±6.76 nmol/mg·min, $p< 0.01$); the TPan activity increase up to 187.48±22.03 nmol/mg·min in comparison with the control (106.71±15.21 nmol/mg·min, $p< 0.05$). The statistical differences between the mean values of the TP activity in tumors and non-neoplastic mucosa have been shown by Willcoxon W-criteria calculation – for TPan $W = 66.0$, $p<0.001$; for TPc $W = 190.0$, $p<0.001$. This disorder may be one of the causes of dTTP synthesis increasing and a higher rate of proliferation.

In the present study the age-dependent enzymes activity values in the blood plasma of patients with GIC, and in the blood plasma of the control group have been determined (Table). In the healthy organism the age-dependent activity increase is characteristic for TPc (the index of Spearman's rank correlation, i.e. $\rho = 0.874$, a positive correlation between the TPc activity and age), but not for TPan. In case of cancer development a tendency is lost – for TPc $\rho = -0.189$, but for TPan $\rho = 0.405$. Therefore, the possibility of dTMP synthesis increasing by the "salvage pathway" in patients with GIC have been determined. The TP activity changes in the blood plasma of patients demonstrate their activity peculiarities in tissues.

For the better consideration of the individual role of the TP activity peculiarities for proliferation we propose to use the index of TPan/TPc. It also decreases in the age-dependent manner in the plasma of GIC patients ($\rho = 0.349$).

To reveal prognostic possibilities of the enzyme activities studied for the drug treatment efficacy we created 2 groups of two different pathomorphologycal types of gastric cancer tumors samples from patients with (and without, comparison group) 5-FU treatment before surgery. The values of TPan/TPc in the samples of the intestinal type and the diffuse type of gastric cancer in the comparison group were similar – 3.40±0.51 and 2.20±0.82, $p>0.05$, respectively. In cases of 5-FU treatment in the intestinal type of cancer it was minimal 0.58±0.15, $p<0.01$. Decrease in the index was associated with tumor necrosis development and thus with 5-FU efficacy. In the diffuse type of the GC samples the authentic differences versus the comparison group index were absent (1.82±0.23, $p>0.05$) and the area of necrosis was not determined.

CONCLUSIONS

A practical application of the present study is that targeting TP for therapeutic purposes would be more desirable in individualization of anticancer therapy depending on tumor spreading.

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Address for correspondence:
16, Il'icha av., Donetsk, 83003, Ukraine.
Tel. (67) 621-25-75. E-mail: borzenko.bg@rambler.ru.
Donetsk National Medical University named after M.Gorky

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МОЖЛИВІСТЬ ЗАСТОСУВАННЯ ФЕРМЕНТАТИВНОГО ТЕСТУ ДЛЯ ПРОГНОЗУ ЕФЕКТИВНОСТІ 5-ФТОРУРАЦИЛУ У ХВОРИХ НА ГАСТРОІНТЕСТИНАЛЬНИЙ РАК

О.М.Бакурова, К.О.Миронова, Б.Г.Борзенко

Донецький національний медичний університет ім. М.Горького

Ключові слова: гастроінтестинальний рак; тимідинфосфорилаза; 5-фторурацил

Тимідинфосфорилаза метаболізує 5-фторурацил та його похідні. З прогностичною метою ми вивчали особливості видів її ферментативної активності в тканинах та плазмі крові хворих на рак шлунка і кишечника різного віку. Матеріалом служили тканини раку шлунка, кишечника та нетрансформованої слизової країв їх резекції (контроль) 58 хворих з верифікованим діагнозом $T_{3-4}N_xM_0$ стадій, плазма крові 96 хворих на гастроінтестинальний рак (ГІР) у віці від 35 до 70 років. Як контроль використовували плазму крові 60 умовно здорових осіб того ж віку, які не мали патології гастродуоденальної зони. Спектрофотометрично визначали активність ТФк та ТФан [2] на СФ-46. Інкубаційні суміші містили фосфатні буфери оптимальних pH, субстрати та косубстрат для ТФан. Реєстрували зміни оптичної щільності тиміну (тимідину) в 0,01 н NaOH. Статистичну обробку результатів проводили з використанням параметричних та непараметричних методів програм пакету «MedStat». Встановлено зростання трансферазної активності в пухлинах шлунка та кишечника в 1,7 рази порівняно з нетрансформованою слизовою країв резекції та зниження катаболічної (фосфорилазної) її активності в середньому в 2,6 рази (W-критерій Вілкоксона – для ТФ ан W = 66,0, p<0,001; для ТФк W = 190,0, p<0,001). Для моніторингу змін проліферативної активності запропоновано показник співвідношення трансферазного та фосфорилазного видів активності (ТФан/ТФк), доведено його зв'язок з патологією (W = 28,0, p<0,001). Показано можливість застосування для прогнозу ефективності 5-фторурацилу. Активність ферменту в плазмі крові відбиває зміни активності в тканинах, може допомогти у вирішенні питань контролю індивідуальної ефективності препаратів групи 5-фторурацилу. Існує пряма кореляція між змінами видів активності ТФ у плазмі і тканинах. Визначення індивідуальних особливостей видів активності ТФ у плазмі крові у хворих на рак шлунка, кишечника в динаміці може допомогти у вирішенні питань прогнозу індивідуальної ефективності препаратів групи 5-фторурацилу.

ВОЗМОЖНОСТЬ ПРИМЕНЕНИЯ ФЕРМЕНТАТИВНОГО ТЕСТА ДЛЯ ПРОГНОЗА ЭФФЕКТИВНОСТИ 5-ФТОРУРАЦИЛА У БОЛЬНЫХ ГАСТРОИНТЕСТИНАЛЬНЫМ РАКОМ

Е.М.Бакурова, К.А.Миронова, Б.Г.Борзенко

Донецкий национальный медицинский университет им. М.Горького

Ключевые слова: гастроинтестинальный рак; тимидинфосфорилаза; 5-фторурацил

Тимидинфосфорилаза участвует в метаболизме 5-фторурацила. Изучены особенности ее видов активности как в тканях опухолей, так и в плазме крови больных различного возраста гастроинтестинальным раком. Материалом служили ткани рака желудка, кишечника и нетрансформированной слизистой краев резекции (контроль) 58 больных с верифицированным диагнозом $T_{3-4}N_xM_0$ стадий, плазма крови 96 больных гастроинтестинальным раком (ГИР) в возрасте 35-70 лет. В качестве контроля использовали плазму крови 60 условно здоровых лиц того же возраста, не имевших патологии гастродуоденальной зоны. Спектрофотометрически определяли активность ТФк и ТФан [2] на СФ-46. Инкубационные смеси содержали фосфатные буферы оптимальных pH, субстраты и косубстрат для ТФан. Регистрировали изменения оптической плотности тимина (тимидина) в 0,01 н NaOH. Статистическую обработку результатов проводили с использованием параметрических и непараметрических методов программ пакета «MedStat». В опухолях установлено повышение трансферазной активности в 1,7 раза и снижение ее катаболической (фосфорилазной) активности в среднем в 2,6 раза по сравнению с активностью в нетрансформированных краях резекции (W-критерий Вилкоксона – для ТФ ан W = 66,0, p<0,001; для ТФк W = 190,0, p<0,001). Для мониторинга пролиферативной активности предложен показатель соотношения ТФан/ТФк, установлена его связь с патологией (W = 28,0, p<0,001). Изменения активности в плазме крови отражают активность опухолевого процесса. Установлено снижение показателя соотношения активности тимидинфосфорилазы в тканях опухолей при развитии лечебного патоморфоза опухолей на фоне неоадъювантной терапии 5-фторурацилом. Предложенный биохимический тест может использоваться для индивидуального контроля эффективности препаратов его группы. Существует прямая корреляция между изменениями видов активности ТФ в плазме и тканях. Определение индивидуальных особенностей видов активности ТФ в плазме крови больных раком желудка и кишечника в динамике может помочь в решении вопросов прогноза индивидуальной эффективности препаратов группы 5-фторурацила.