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THE INFLUENCE OF 5-FLUOROURACIL ON ACTIVITY OF THYMIDINE PHOSPHORYLASE IN GASTRIC ADENOCARCINOMA AND NORMAL ADJACENT TISSUE

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The *aim* of the present study was to evaluate the change of thymidine phosphorylase (TP) activity in gastric adenocarcinoma and adjacent tissue upon the intraarterial administration of 5-fluorouracil (5-FU). *Matherials and methods*: The specimens of tumor and adjacent tissue were obtained by surgical operation on 36 patients (20 men and 16 women aged from 38 to 82 years) with gastric adenocarcinoma (stages II–IV). 5-FU was administered in the bolus dose to the gastroepiploic artery 2–60 min before the tumor resection. The concentration of 5-FU and activity of TP in both tissues were determined by high performance liquid chromatography method. *Results*: The concentration of 5-FU was decreased in a time-dependent manner in both tissues, though the interpatient variability of this value was much less in tumor tissue. The activity of TP was decreased with the course of the time after 5-FU administration in tumor and normal adjacent tissue. There were statistically significant differences in the TP activity in both tissues between 2–20 min and 40–60 min after 5-FU injection ($p < 0.05$). *Conclusion*: 5-FU administration results in the decrease of TP activity in tumor and normal adjacent tissues that might have importance for chemotherapy with fluoropyrimidines.
***Key Words*: 5-fluorouracil, thymidine phosphorylase, gastric adenocarcinoma, normal mucosa.**

Chemotherapy with fluoropyrimidines — 5-fluorouracil (5-FU) and its derivatives (capecitabine, tegafur, etc.) is nowadays the standard for the treatment of gastric adenocarcinoma (GAC) [1]. Thymidine phosphorylase (TP) is a key enzyme of these drugs activation which catalyzes transformation of capecitabine to 5-FU and further — 5-FU — to 2′-deoxy-5-fluorouridine. This enzyme plays an important role in the pathogenesis of tumor growth and in many respects determines the efficacy of the chemotherapy of oncological diseases, meanwhile the role of TP is dual.

On the one hand, TP is considered as a negative prognostic factor for GAC and other cancer types because of its participation in the processes of angiogenesis and apoptosis. Various studies testify it's identity with the platelet-derived endothelial growth factor [2, 3]. It was shown that TP induces endothelial cells migration that intensifies vascular invasion in tumor [4, 5]. Inhibitory effect of TP on cancer cells apoptosis was demonstrated on the different cell lines [6]. In the solid tumors the hyperproduction of TP comparing to normal tissues is observed [3, 7].

On the other hand, at the chemotherapy with fluoropyrimidines the hyperexpression of TP in tumors of different localizations guaranties the increase of the production of active metabolites of these drugs in tumor as compared to normal tissue. Thus, due to the catalytic activity of TP capecitabine promotes the maximal cytostatic effect on the tumor under the minimal influence on normal tissues [8]. Therefore the determinant factor of fluoropyrimidines pharmacodynamics is the activity of TP both in tumor and normal tissues.

The aim of the present study was to investigate TP activity in GAC and adjacent tissue upon the intraarterial administration of 5-FU.

MATERIALS AND METHODS

Patients. The specimens of tumor and normal adjacent tissue (gastric mucosa without signs of malignant transformation or inflammation) obtained in surgical operation from 36 patients (20 men and 16 women aged from 38 to 82) with GAC on different stages (II–IV) were used. Study was approved by the Ethic Committee of M. Gorky Donetsk National Medical University.

Patients were treated with the intraarterial chemotherapy in the neoadjuvant regimen. 5-FU was administered in the bolus dose to the gastroepiploic artery 2–60 min before the tumor resection. Tissue specimens were frozen and stored under −70 °C till use. The mucosa samples were taken at a distance of 5–7 cm from the tumor node edge.

Tissues were homogenized on ice with 35 mM KH_2PO_4/Na_2HPO_4 buffer, pH = 7.4 (ratio of specimen weigh to buffer was 1:20). All reagents used in the present study were intended for high performance liquid chromatography (HPLC).

Determination of 5-FU concentration. The concentration of 5-FU in tissues was determined by the method of N. Christophidis [9] which involved the double extraction of 5-FU from the homogenate and further measurement of concentration by means of HPLC. Ethylacetate was added to the tissue homogenate, vortex mixed over 90 s and centrifuged during 3 min at 855 g. The upper organic layer was collected to the tube with alkaline buffer (Na_2HPO_4 mixed with NaOH till pH = 11.0). The tube content was vortex mixed again and centrifuged as described above. The supernatant was collected and used for 5-FU concentration measurement.

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Abbreviations used: 5-FU – 5-fluorouracil; GAC – gastric adenocarcinoma; HPLC – high performance liquid chromatography; TP – thymidine phosphorylase.

5-FU concentration was determined with HPLC-system (Konikrom Plus, Konik Group, Spain) with PDA-detector and chromatographic column (250x4.6 mm, YMC Europe GmbH, Germany). For elution 20 mM ammonium chloride solution with 2% of acetonitrile was used. For the calculation of 5-FU concentration the calibration curve (correlation coefficient R = 0.99) after the above described preparation was used.

Determination of TP activity. TP activity in the tissues homogenates was determined by the accumulation of thymine — the product of thymidine phosphorolysis, by the method of van Kuilenburg [10].

The reaction mixture consisted of 35 mM KH_2PO_4/Na_2HPO_4 (pH = 7.4), 1 mM of mercaptoethanol and 2 mM of thymidine. Reaction was started by the addition of homogenate to the reaction mixture. At once after (1 probe) and in 45 min of incubation at 37 °C (2 tube) reaction was stopped by addition of acetonitrile. Proteins were removed after centrifugation at 12 000 g during 15 min, after that acetonitrile was removed by chloroform. The concentration of thymine was determined by means of HPLC as described above for 5-FU. The total protein concentration in homogenate was measured by Lowry method [11]. TP activity was calculated as the difference between the level of thymine on 45 min (2 probe) and 0 min (1 probe) with account of protein concentration in probe and presented in μmol/mg per min.

The statistical data processing was made with the help of Statistica 6.0 software (Statsoft Inc.).

RESULTS

5-FU concentration in tissues. In tumor tissue there was the negative correlation between the concentration of 5-FU and the time after its administration ($r = -0.83$, $p < 0.05$) (Fig. 1).

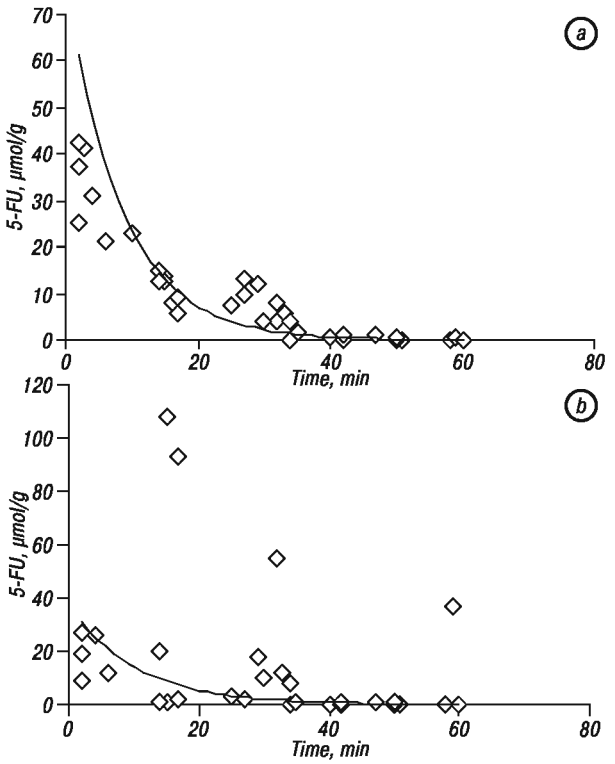

Fig. 1. Concentration of 5-FU in gastric tumor (*a*) and adjacent mucosa (*b*) in different periods after 5-FU administration

The maximal value of 5-FU concentration was 41.35 μmol/g on the 2 min, minimal — 0.24 μmol/g in 1 h after injection. The two fold decrease of 5-FU in tumor was detected already in 10 min. In the first 30 min after injection 5-FU level was decreased by 80%, the rest 20% was degraded during next 30 min.

In adjacent mucosa the correlation between drug concentration and time was lower than in tumor ($r = -0.39$, $p < 0.05$). The peak 5-FU concentration was 108.3 μmol/g (in the tissue resected in 15 min after 5-FU injection), the lowest value of 5-FU concentration was 0.01 μmol/g (in 60 min after 5-FU injection).

TP activity in tissues. In the initial period TP activity in tumor was much higher than in adjacent mucosa. With the course of time after 5-FU injection in both tissues the decrease of TP activity was observed (Fig. 2).

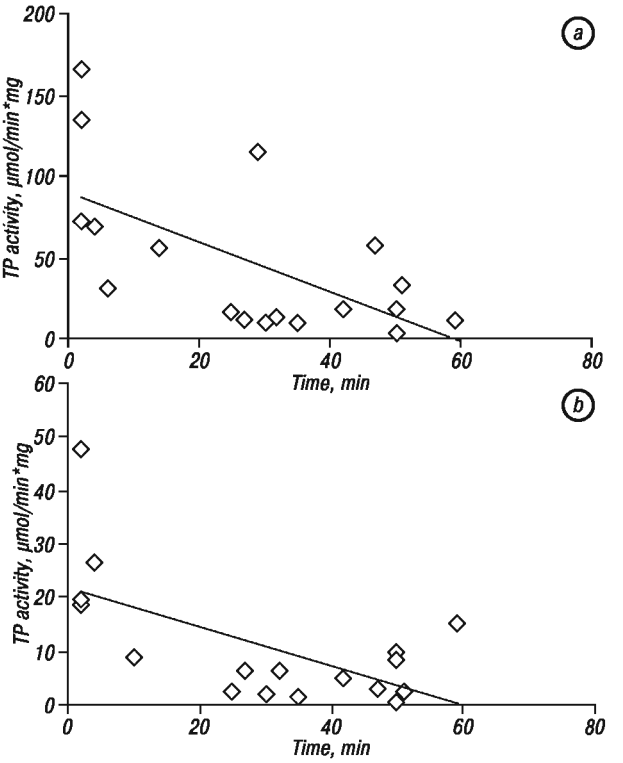

Fig. 2. TP activity in tumor (*a*) and adjacent mucosa (*b*) in different periods after 5-FU administration

There was a moderate correlation between TP activity and time after injection both in tumor ($R = -0.63$; $p < 0.05$) and adjacent mucosa ($R = -0.64$; $p < 0.05$). The mean value of TP activity (26.8 and 6.4 μmol/mg per min in tumor and adjacent tissue respectively) was significantly lower in the samples on 40–60 min after 5-FU injection as compared to the samples resected on 2–20 min (86.9 and 25.3 μmol/mg per min in tumor and adjacent tissue, respectively). In both cases the difference according to Mann — Whitney test was significant ($p = 0.012$ in tumor and $p = 0.008$ in adjacent tissue).

DISCUSSION

The obtained data testify that the intraarterial injection of 5-FU to gastroepiploic artery leads to the gradual decrease of 5-FU concentration in the gastric tumor tissue. The type of this dependence is similar

to the pharmacokinetic curves for 5-FU in the blood plasma; however in contrast to standard pharmacokinetic studies in the present study the data from different patients was used. Besides that the pharmacokinetic parameters are also similar: in the present study 5-FU concentration in tumor was decreased 2-fold in the first 10 min that approximately corresponds to 5-FU half-life time in blood [12].

In the adjacent tissue the interindividual difference of 5-FU degradation rate was more expressed but within one hour drug concentration was also significantly decreased. The fundamental differences between the metabolic processes in tumor and normal tissues might be a reason for the observed disappearance of the interindividual variability in tumor comparing to adjacent tissue.

It is notably that together with decreasing of drug concentration with the time after its injection TP activity lowered in both investigated tissues. According to the obtained data during the study period enzyme activity in tumor lowers 1.5-fold, in the adjacent tissue — 2.8-fold. There may be the following reasons below for such influence of 5-FU load on TP activity.

5-FU is an effective cytostatic agent which action leads ultimately to the suppression of protein synthesis in the cell. So one possible way of its action on TP may be the inhibition of this enzyme gene expression either on the stage of mRNA synthesis or on the stage of translation. These effects are characteristic for other antimetabolites. It was shown that cancer chemotherapy alters gene expression in tumor and adjacent tissue of patients with rectal cancer [7]. Some chemotherapeutic drugs (cisplatin, camptothecin, paclitaxel) when incubated with human colon cancer cell lines decrease TP expression [13]. In this context the fact that 5-FU influence on TP activity develops already in the first hour after drug administration to the patients assumes that the rate of this enzyme synthesis is high as compared to the synthesis of other cell proteins, i.e. it is possible that the half-life time of TP is not longer than one hour. Currently there is no data concerning TP protein turnover rate. But according to the data of research [14] dedicated to the study of more than 5 000 cell proteins the half-life time of proteins varies a lot at that the minimal half-life is about 45 min. Thus, decrease of TP amount in the cell under the action of 5-FU is theoretically possible in the case this enzyme has the short half-life time, i.e. high turnover rate.

Another possible mechanism of drug influence is that by means of direct interaction with TP the metabolites of 5-FU may inhibit its activity. The option that 5-FU itself interacts with TP protein is not in question because 5-FU is the substrate of TP. In this case enzyme activity might be low at once after drug injection when the concentration of the drug was on the peak level. The gradual non-sharp decrease of TP activity after 5-FU load testifies the decrease of its amount but not the activity.

The fact that 5-FU administration results in the decrease of TP level may be of importance for the efficacy of the chemotherapy. In the meantime this fact has the double meaning because TP plays a dual role in the pathogenesis of oncological diseases.

As mentioned above, TP participates in the activation of 5-FU and other fluoropyrimidines. In this regard suppression of TP may result in the decrease of the concentration of active metabolites of 5-FU in tissues. This will weaken the required "useful" effect of cytostatic on tumor.

In the meantime there are several reasons for considering TP inhibition as the aim of various anticancer chemotherapies. TP expression positively correlates with microvessel density, tumor grade, disease stage and metastasis. High TP level in tumor is associated with poor prognosis and reduction of overall survival [3, 15]. For the cell lines expressing TP the increase of migration and invasive activity is characteristic [16]. Besides that TP suppresses the hypoxia-induced apoptosis of cancer cells [4, 6]. Inhibition of TP by various agents (e.g., 5-bromo-6-aminouracil) induces tumor cells apoptosis [2]; and the antiproliferative action of metformin is supposed to be associated with the decrease of TP expression under the action of this drug [17].

In the present study patients received one dose of 5-FU that was followed by lowering of TP activity for at least 1 h after injection. Therefore, it's arguable that long-term infusions of 5-FU used in clinical practice may result in consistent suppression of TP activity. In this case the process of 5-FU activation will definitely slow down. At the same time the long-term inhibition of TP may stipulate decrease of metastasis and promote apoptosis of cancer cells. So for the result of the therapy the important role plays the balance between two effects: negative — decrease of fluoropyrimidines transformation to the active metabolites, and positive — suppression of TP as the negative factor of disease development.

Thus, in the present study it was found that intraarterial injection of 5-FU provides decrease of TP activity for up to 1 h after drug administration in both gastric tumor and normal adjacent mucosa.

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