Stashkevych M. Modeling of 5-fluorouracil infulence on activity of its' metabolic enzymes. Journal of Education, Health and Sport. 2015;5(11):62-67. ISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.33358
http://ojs.ukw.edu.pl/index.php/johs/article/view/2015%3B5%2811%29%3A62-67
https://pbn.nauka.gov.pl/works/666610
Formerly Journal of Health Sciences. ISSN 1429-9623 / 2300-665X. Archives 2011–2014
http://journal.rsw.edu.pl/index.php/JHS/issue/archive

Deklaracja.
Specyfika i zawartość merytoryczna czasopisma nie ulega zmianie.
Zgodnie z informacją MNiSW z dnia 2 czerwca 2014 r., że w roku 2014 nie będzie przeprowadzana ocena czasopism naukowych; czasopismo o zmienionym tytule otrzymuje tyle samo punktów co na wykazie czasopism naukowych z dnia 31 grudnia 2014 r.
The journal has had 5 points in Ministry of Science and Higher Education of Poland parametric evaluation. Part B item 1089. (31.12.2014).

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 05.09.2015. Revised 05.10.2015. Accepted: 30.10.2015.

MODELING OF 5-FLUOROURACIL INFULENCE ON ACTIVITY OF ITS' METABOLIC ENZYMES

M. Stashkevych

O. O. Bogomolets National Medical University

Abstract

This article presents the results of model experiment which involved intraperitoneal administration of 5-fluorouracil (5-FU) to rats with further determination of the activity of its' key metabolic enzymes: dehydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP), in various time intervals after 5-FU injection. These results were compared to ones from the control group of animals which did not receive 5-FU. It was revealed that 5-FU has the suppressive influence on TP up to 12 hours after injection and does not affect activity of DPD. As these enzymes are responsible for 5-FU activation (TP) and degradation (DPD) the observed influence of the drug is desired effect associated with lesser toxic impact on normal cells.

Keywords: 5-fluorouracil, dehydropyrimidine dehydrogenase, thymidine phosphorylase, rat liver.

Introduction

Anticancer drug 5-fluorouracil (5-FU) was implemented into clinical practice in the middle of the previous century, and since that time fluoropyrimidines have found wide utility as part of various schemes of chemotherapy [1, 2]. However, the usage of 5-FU is limited by its' relatively high toxicity [3]. One of the most important factors influencing results of 5-FU-based chemotherapy is the velocity of biotransformation of this drug in tissues which in its' turn depends on the activity of key metabolic enzymes of 5-FU. There are two principal pathways of 5-FU metabolism: activation by means of addition of various pentose phosphates resulting in formation of nucleotides, and degradation through biotransformation to fluorobetaalanine [4]. One of the key enzymes of 5-FU activation pathway is thymidine phosphorylase (TP) which is known to be hyperexpressed in solid tumors [5]. The breakdown of 5-FU is catalyzed by dihydropyrimidine dehydrogenase (DPD). The insufficiency of this enzyme which is manifested in 3-5% of population is associated with severe toxic effects of fluoropyrimidines [6]. In our previous study [7] it was shown that high doses of 5-FU have suppressing impact on TP which is possibly associated with the inhibition of cellular proteins synthesis due to cytostatic action of the drug. It is known that fluoropyrimidines administration leads to decrease of the activity of some enzymes involved in nucleotides metabolism, e.g. purine nucleoside phosphorylases [8]. Taking in account the influence of 5-FU high doses on the activity of its' activating enzyme it is of concern to study 5-FU influence on the enzyme responsible for its' degradation. The **aim** of the present study was evaluation and comparison of 5-FU influence on the activity DPD and TP in the model animal experiment.

Materials and methods

Liver specimens were obtained from male 3-month old Wistar rats. Animals of test group (n=16) received 5-FU (15 mg/kg) intraperitoneally and were decapitated upon ether anesthesia in different time intervals after 5-FU injection. Therefore test group was divided into sub-groups (n=4) according to the time after 5-FU administration: "1 hour", "3 hours", "12 hours" and "24 hours". Control group (n=8) did not receive 5-FU. Liver was excised and homogenized in phosphate buffer (pH=7.4).

DPD activity in homogenates was determined by the method [9] based on the measurement of reaction mixture optic density change at 260 nm due to the accumulation of DPD product uracil. Reaction mixture consisted of phosphate buffer (pH=7.4), mercaptoethanol (1 mmol/l), 5,6-dihydrouracil (25 mcmol/l), $NADP^+$ (750 mcmol/l), $MgCl_2$

(2.5 mmol/l). Change of the optic density was measured using UV-spectrometer “Specord-200”.

TP activity in homogenates was determined according to the method of van Kuilenburg [10] by means of measurement of the product of thymidine phosphorolysis – thymine accumulation. Reaction mixture consisted of phosphate buffer (pH=7.4) and thymidine (2 mmol/l). Reaction was initiated by addition of homogenate. Mixture was divided into two parts. Immediately after the addition of homogenate acetonitrile was added to one part, second part was incubated at 37ºC and reaction was stopped in the same fashion. Denaturated protein was removed by means of centrifugation at 12000 g for 15 minutes and acetonitrile was extracted from solution with the help of chloroform. Thymine concentration was determined using HPLC-system (Konikrom Plus, Konik Group, Spain) with PDA-detector and column YMC TriartC18 (250x4,6 мм, YMC Europe GmbH, Germany) in the reversed-phase regimen. The solution of ammonia chloride with acetonitrile was used as mobile phase. Enzymes activity was expressed as U/mg of protein (U=nmol/min), protein concentration in homogenates was determined by Lowry. Data was analyzed using «Statistica 6.0» software. Study was approved by the Ethic Committee of M.Gorky Donetsk National Medical University.

Results

Obtained results regarding activity of liver DPD and TP in control group and in test groups of animals (in 1, 3, 6, 12 and 24 hours after 5-FU injection) are presented on fig.

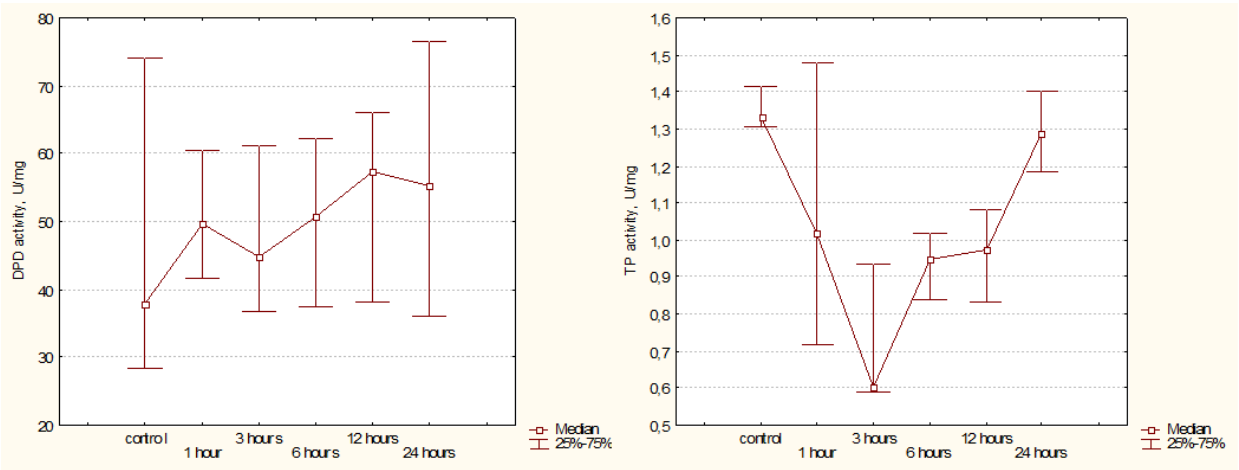

Fig. Activity of dihydropyrimidine dehydrogenase and thymidine phosphorylase in rat liver in control group and in groups after 5-fluorouracil administration

Despite that liver specimens were obtained from the animals of the same line, gender and age, high interindividual variability was peculiar for DPD activity in all groups of animals. The variation coefficient for DPD activity in different groups of animals ranged from 25 to 60%. There were no statistical differences between median DPD activity in the control group and in all test groups according to Mann-Whitney test. Spearman rank analysis confirmed the absence of correlation between time after 5-FU injection and DPD activity ($R=0.02$, $p<0.05$).

As distinct from DPD, TP activity gradually decreased with the time after the administration of 5-FU reaching its' minimum in the test group "3 hours". There was strong correlation in the sequence of groups "control" – "1 hour" – "3 hours" – "6 hours" with Spearman correlation coefficient 0.7 ($p <0.05$). In 6 and 12 hours after 5-FU injection TP was also lower than in control group ($p <0.05$) but 24 hours after 5-FU administration TP activity recovered to the initial level.

Discussion

Results of the present study testify that the load of tissues with 5-FU has different impact on the activity of key enzymes of its' metabolism. Previously obtained data regarding suppressive action of 5-FU on TP activity both in tumor and normal adjacent mucosa in patients with gastric adenocarcinoma [7] were proven in the present model experiment. On one hand such influence of 5-FU on its' activating enzyme may lead to decrease of production of 5-FU active metabolites. On the other hand TP is the negative factor of tumor progression, it stimulates angiogenesis and inhibits cancer cells apoptosis [5], so suppression of this enzyme may contribute to the total cytostatic action of 5-FU. It cannot be excluded that 5-FU administration had an influence on DPD activity which was not detected in the present study due to high variability of the data in all groups of animal. But even if this influence is present it is obviously far less than the impact of 5-FU on TP.

Our previous studies have shown that 5-FU load leads to decrease of TP activity after its' intraarterial administration which allows creating high local concentration of the drug in tumor of the affected organ as well as in normal tissue. To be noticed is that intraarterial chemotherapy is mostly used for treatment of liver cancer [11]. Design of present experiment also allowed for the saturation of rat liver with high dose of 5-FU as the drug was injected intraperitoneally.

It stands to reason that the accumulation of high doses of 5-FU in normal tissues may stipulate the increased toxicity of the chemotherapy. At the systemic administration more than 80% of 5-FU undergoes degradation catalyzed by DPD which is most active in blood

mononuclear cells and in liver. It was shown [6, 12] that decrease of DPD activity is directly associated with the enhancement of fluoropyrymidines effects. With that background the absence 5-FU high doses inhibiting influence on the enzyme of its' degradation and suppression of the enzyme of its' activation established in the present study is the desired effect associated with lesser toxic impact of this cytostatic on normal cells.

Conclusion

This study has shown that the intraperitoneal administration of single bolus dose (15 mg/kg) of 5-FU to rats does not alter activity of the enzyme of 5-FU degradation - DPD in liver whereas TP activity is suppressed that is manifested up to 12 hours after drug injection.

References

1. Orditura M., Galizia G., Sforza V. et al. Treatment of gastric cancer. World J Gastroenterol 2014; Vol. 20, № 7: 1635-1649.

2. Rustum Y.M. Fluoropyrimidines in Cancer Therapy. Humana Pres, Totowa 2003.

3. Matsusaka S., Lenz H.J. Pharmacogenomics of fluorouracil -based chemotherapy toxicity. Expert Opin Drug Metab Toxicol 2015; Vol.11, № 5: 811-821.

4. Thorn C.F., Marsh S., Carrillo M.W. et al. PharmGKB summary: fluoropyrimidine pathways. Pharmacogenet.Genomics 2011; Vol.21, № 4: 237-242.

5. Bronckaers A., Gago F., Balzarini J. et al. The dual role of thymidine phosphorylase in cancer development and chemotherapy. Med Res Rev 2009; Vol.29, № 6: 903-953.

6. Amstutz U., Froehlich T.K., Largiader C.R. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. Pharmacogenomics 2011; Vol.12, № 9: 1321-1336.

7. Stashkevych M., Khomutov E., Dumanskiy Y. et al. The influence of 5-fluorouracil on activity of thymidine phosphorylase in gastric adenocarcinoma and normal adjacent tissue. Exp Oncol 2014; Vol.36, № 4: 267-270.

8. Honda T., Inagawa H., Nishizawa T. et al. The relationship between clinicopathological factors and the reduction of pyrimidine nucleoside phosphorylase activity after preoperative administration of 5'-deoxy-5-fluorouridine. Anticancer Res 2010; Vol.30, № 8: 3207-3211.

9. Dolegowska B., Ostapowicz A., Stanczyk-Dunaj M. et al. Spectrophotometric methods as a novel screening approach for analysis of dihydropyrimidine dehydrogenase

activity before treatment with 5-fluorouracil chemotherapy. J Physiol Pharmacol 2012; Vol.63, № 4: 411-421.

10. Van Kuilenburg A.B., Zoetekouw L. Determination of thymidine phosphorylase activity by a non-radiochemical assay using reversed-phase high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2005; Vol.820, № 2: 271-275.

11. Lewandowski R.J., Geschwind J.F., Liapi E. et al. Transcatheter intraarterial therapies: rationale and overview. Radiology 2011; Vol.259, № 3: 641-657.

12. Del R.M., Di P.A., van Schaik R.H. et al. Dihydropyrimidine dehydrogenase polymorphisms and fluoropyrimidine toxicity: ready for routine clinical application within personalized medicine? EPMA J 2010; Vol.1, № 3: 495-502.