

ASSESSING BIOCHEMICAL AND OXIDATIVE STRESS PARAMETERS AFTER VAGINAL AND ORAL ADMINISTRATION OF 5-FLUOROURACIL IN LABORATORY ANIMALS

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Abstract

Intraepithelial vaginal neoplasias represent 0.4% of the intraepithelial diseases of the lower genital tract. In order to evaluate the advantages of vaginal administration for such disorders, we used an animal model and it was assessed: the hepatic function, by measuring the activity of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), the renal function, by assessing the plasma level of the urea, creatinine and uric acid and the modification of certain oxidative stress parameters, glutathione peroxidase (GPx), reduced glutathione (GSH) and the level of lipid peroxidation through the malondialdehyde concentration (MDA) for a new formulation of mucobioadhesive tablets with 5-fluorouracil (5-FU) *versus* the oral administration of equivalent dosages, 6 respectively 20 mg/kg body weight. The assessed parameters for the two doses compared to control were: ASAT [UI/L] 137.71 ± 12.85 and 172.57 ± 25.47 , control 103.43 ± 10.18 , uric acid [mg/dL] 0.92 ± 0.09 and 2.18 ± 0.12 , control 0.87 ± 0.47 , GPx [$\mu\text{mol GSSG/min/mL}$] 3.50 ± 0.63 and 4.03 ± 0.62 , control 3.01 ± 0.50 , GSH [$\mu\text{g/mL}$] 32 ± 2.51 and 21.5 ± 10.56 , control 40 ± 5.29 , MDA [$\mu\text{mol/mL}$] 0.81 ± 0.11 and 0.97 ± 0.24 , control 0.50 ± 0.09 . The intravaginal administration of 6 mg/kg body weight 5-FU generated small modifications at the renal and hepatic level compared to the oral administration of the equivalent dosages and induced a smaller level of oxidative stress.

Rezumat

Neoplaziile vaginale intraepiteliale reprezintă 0,4% din bolile intraepiteliale ale tractului genital inferior. Pentru a aprecia avantajele administrării vaginale de 5-fluorouracil în astfel de afecțiuni s-a folosit un model animal pentru evaluarea funcției hepatice prin măsurarea activității aspartat aminotransferazei (ASAT), alanin aminotransferazei (ALAT), funcției renale, prin determinarea nivelului plasmatic al ureei, creatininei și acidului uric și modificarea unor parametri de stres oxidativ, glutatation peroxidaza (GPx), glutatationul redus (GSH) și nivelul de peroxidare lipidică prin evaluarea concentrației malondialdehidei (MDA) pentru o nouă formulare de comprimate mucoadezive cu 5-fluorouracil (5-FU) *versus* administrarea orală a unor doze echivalente, 6, respectiv 20 mg/kg corp. Parametrii analizați pentru cele două doze față de lotul martor s-au modificat astfel: ASAT [UI/L] $137,71 \pm 12,85$ și $172,57 \pm 25,47$, martor $103,43 \pm 10,18$, acid uric [mg/dL] $0,92 \pm 0,09$ și $2,18 \pm 0,12$, martor $0,87 \pm 0,47$, GPx [$\mu\text{mol GSSG/min/mL}$] $3,50 \pm 0,63$ și $4,03 \pm 0,62$, martor $3,01 \pm 0,50$, GSH [$\mu\text{g/mL}$] $32 \pm 2,51$ și $21,5 \pm 10,56$, martor $40 \pm 5,29$, MDA [$\mu\text{mol/mL}$] $0,81 \pm 0,11$ și $0,97 \pm 0,24$, martor $0,50 \pm 0,09$. Administrarea intravaginală a 6 mg/kg corp 5-FU a produs modificări reduse la nivel hepatic și renal reduse față de administrarea pe cale orală a dozelor echivalente și a indus un nivel al stresului oxidativ mai redus.

Keywords: 5-fluorouracil (5-FU), mucoadhesive tablets, biochemical parameters, oxidative stress parameters

Introduction

Literature highlights that the 5-fluorouracil (5-FU) antitumor agent, administered systemically (orally or intravenously), displays cytotoxic effects as well as radio-sensitizing effects on the level of cancerous cells [1, 2]. Sometimes it is necessary to reduce the dosage or even to interrupt the therapy with 5-FU because of the occurrence of severe adverse effects [3-5]. All these aspects have led to several research works on animal models and

clinical investigations regarding the possibility of 5-FU topical administration [6]. On the vaginal mucosa 5-FU is actually administered as a cream-type ointment [7] or gel [8].

Materials and Methods

The objectives of the present study targeted the assessment of biochemical and oxidative stress parameters after the administration of a new formulation of vaginal mucoadhesive tablets with

5-FU [9, 10]. White Wistar female rats (weighing 250-300g) were used for the experiments. During the experiments we followed the international ethical regulations regarding working on laboratory animals and were approved by the local ethics committee [11]. The animals were divided in 5 groups of 7 and received the substances daily, in a single dose, at 9.00 a.m., for 5 days, as per the following schedule: Group 1 (L1, Control, coded NS): normal saline 0.5 mL/100g body weight rat, orally; Group 2 (L2, coded V5FUD1): 5-FU, 6 mg/kg body weight/day, vaginally; Group 3 (L3, coded V5FUD2): 5-FU, 20 mg/kg body weight/day, vaginally; Group 4 (L4, coded O5FUD1): 5-FU, 6 mg/kg body weight/day, orally; Group 5 (L5, coded O5FUD2): 5-FU, 20 mg/kg body weight/day, orally. The animals were kept in laboratory conditions with *ad libitum* access to food and water. After 5 days of treatment, the animals were anesthetized with ethylic ether and blood samples were drawn. The assessment of the hepatic function by measuring ASAT and ALAT activities, and of the renal function by measuring the plasmatic level of urea, creatinine and uric acid, were determined in the serum, the measurements being performed using an Imola RX automated analyser with *in vitro* diagnostic (IVD) commercial kits produced by RANDOX brand, Great Britain. The assessments of the oxidative stress parameters were performed on total blood for GPx and GSH and on serum for MDA, on a PICOSS 05 semi-automated analyser. The glutathione assessment was performed spectrophotometrically and was based on the ELLMAN method [12]. The assessment of glutathione peroxidase activity was performed using the Fukuzawa method [13]. The malonaldehyde assessment was performed using a

modified Ohkawa method, adapted for the serum determination of the parameter [14]. The data was statistically processed using the student t-test. The results were expressed as the mean values $\pm$ the standard deviation of the mean values determined for each parameter and for each of the studied substances. We also determined the p values between groups and if they were below 0.05 we considered the differences statistically significant.

Results and Discussion

For both used doses, 5-FU determined a statistically significant increase of ASAT, in the case of oral administration, as well as vaginal administration, compared to control ($p < 0.05$). The obtained results for this parameter in rats treated with 5-FU are presented in Table I. The increase of ASAT induced by 5-FU was statistically significant in animals which received the tested substance orally, compared to the values obtained for this parameter in animals that received 5-FU intravaginal. The ASAT increase was stronger for higher doses (20 mg/kg body weight/day), in both types of administration, the results not being statistically significant. Similar results were obtained for ALAT (Table I). For both used doses, 5-FU determined the increase of this parameter, in the case of oral administration as well as intravaginal administration, compared to control (L1), the results being statistically significant only for groups L2, L4 and L5. The increase of ALAT induced by 5-FU was statistically significant in the animals that received the tested substance orally, compared to the values obtained for this parameter in animals which received 5-FU intravaginal.

Table I

Values for ALAT (UI/L), ASAT (UI/L) in rats treated with 5FU

Biochemical parameter	L1 (NS)	L2 (V5FUD1)	L3 (V5FUD2)	L4 (O5FUD1)	L5 (O5FUD2)
ASAT (U/L)	103.43 (± 10.18)	137.71 (± 12.85)	146.57 (± 14.62)	172.57 (± 25.47)	192.29 (± 13.74)
ALAT (U/L)	39.57 (± 5.39)	49.86 (± 11.89)	43.00 (± 3.83)	62.71 (± 5.62)	77.57 (± 12.03)

Table II

Values for urea (mg/dL), creatinine (mg/dL), uric acid (mg/dL)

Biochemical parameter	L1 (NS)	L2 (V5FUD1)	L3 (V5FUD2)	L4 (O5FUD1)	L5 (O5FUD2)
Urea (mg/dL)	31.29 (± 1.80)	32.71 (± 4.35)	36.14 (± 2.41)	33.71 (± 3.35)	37.86 (± 5.05)
Creatinine (mg/dL)	0.57 (± 0.05)	0.58 (± 0.13)	0.62 (± 0.05)	0.63 (± 0.04)	0.65 (± 0.11)
Uric acid (mg/dL)	0.87 (± 0.47)	0.92 (± 0.09)	0.94 (± 0.14)	2.18 (± 0.12)	2.29 (± 0.38)

The results obtained following the determination of urea, creatinine and serum uric acid in rats treated with 5-FU are presented in Table II. For both used

doses, 5-FU determined the increase of these parameters, in the case of oral administration as well as intravaginal administration, compared to

control, the results being statistically significant ($p < 0.05$) only for the comparative analysis for L1/L3 and L1/L5 for the serum urea, respectively, L1/L4, L1/L5, L2/L4 and L3/L5 for the serum uric acid. The administration of 5-FU, in both and for both routes of administration, determined the modification of the oxidative stress parameters, highlighted by the increase in the values of GPx (Figure 1a) and MDA (Figure 1b) and the decrease in the values of GSH (Figure 1c). The results present statistical significance only after the comparative analysis. Short-term (5 days) use of 5-FU caused oxidative stress. Antioxidant systems showed a high activity expressed by the value of GPx, with the decrease of hepatic glutathione, a major antioxidant that acts both directly and as a substrate for peroxidase, as evidenced especially for the oral formulation, in a dose of 20 mg/kg body weight/day.

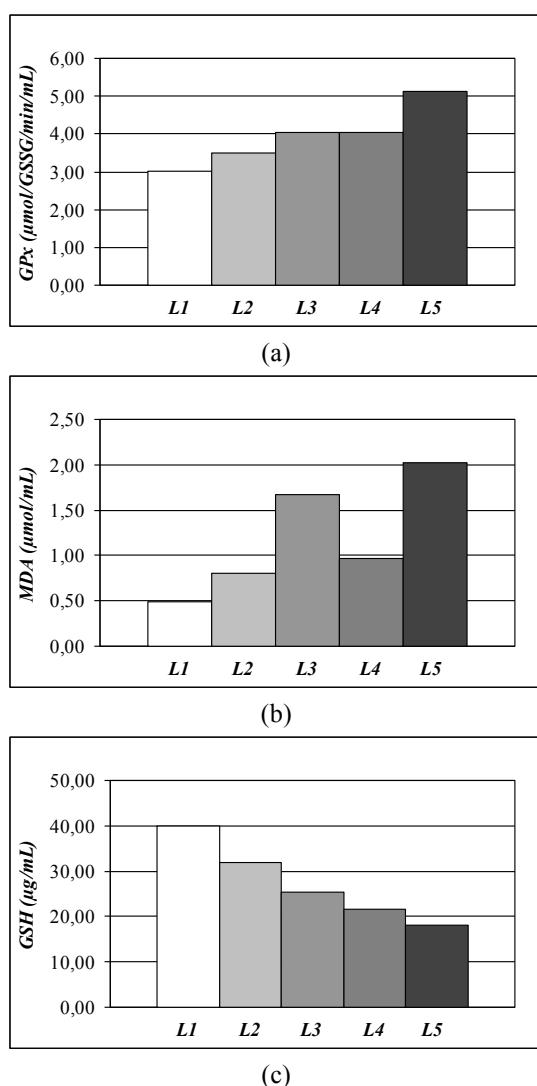


Figure 1.

Values for GPx (a), MDA (b) and GSH (c) in rats treated with 5-FU

It was noticed that 5-FU determines genotoxicity on the level of vaginal epithelial cells, as well as on the level of the cervix cells, contributing considerably to curing the disease, especially in the case of topical administration, with reduced progression rates to invasive cancer [15, 16]. Starting from the previously presented literature data, it was considered suitable to formulate vaginal mucoadhesive tablets containing 5-FU as the active ingredient, and to test *in vivo* several biochemical and oxidative stress parameters, after the administration of 5-FU various doses. The studies performed in laboratory animals have highlighted the fact that the intravaginal administration of 5-FU, in doses of 6 mg/kg body weight, determined a mild occurrence of systemic adverse effects demonstrated by the values of the hepatic enzymes activity, compared to the oral administration of some equivalent doses. For the administration schedule and doses, no modification of the values for urea and creatinine was recorded. The values of the uric acid increase in the case of oral administration for both doses, but this is due to the 5-FU mechanism, which is more likely responsible for the metabolism increase of the nitrogenous bases rather than renal damage. The activity of the GPx enzyme is significantly increased in the case of administration the formulation orally in the dose of 20 mg/kg body weight and is correlated with the decrease of the glutathione level, an aspect which is maintained in the case of the 6 mg/kg body weight dose. A decrease in the level of GPx with the maintaining of a GSH level close to the one of the control sample (32 μg/mL compared to 40 μg/mL), is highlighted in the case of intravaginal administration in a concentration of 6mg/kg body weight. The level of lipid peroxidation is obviously increased in the case of 20 mg/kg body weight doses administered orally as well as intravaginally, dose for which the peroxidation process is intensified on the level of cellular membranes, but significantly decreased in the case of vaginal administration of the 6 mg/kg body weight dose, respectively 0.81 μmol/mL compared to 0.50 μmol/mL the value of the control sample.

Conclusions

The obtained data show that the oral administration of 5-FU, in both doses, determined the increase of ASAT, ALAT, uric acid, urea and serum creatinine, the effects being improved in the case of intravaginal administration; the modifications on the level of oxidative stress parameters were less aggressive in the case of vaginal administration compared to the oral administration of 5-FU, especially for the concentration of 6 mg/kg body weight.

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