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Effect of Betulonic Acid and Its Derivatives on the Morphology of Kidneys in the Animals with Transplanted Lewis Lung Carcinoma at the Background of Polychemotherapy and without It

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Abstract

Effect of betulonic and [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acids and their methyl esters on the morphology of kidneys of the C57BL/6 mice with transplanted Lewis pulmonary adenocarcinoma at the background of cytostatic polychemotherapy (cyclophosphane, adriamycin, vincrystine, prednisolone) and without it was investigated. It was established by means of morphometry that the introduction of betulonic and [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acids and their methyl esters against the background of polychemotherapy and without it has a positive effect on the course of tubulointerstitial nephropathy as it reduces the degree of necrotic and dystrophic changes of epithelial cells of proximal tubules as well as reduces the edema of interstitial tissue.

Key words: polychemotherapy, Lewis lung carcinoma, nephroprotective effect, betulonic acid, [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acid and their methyl esters

INTRODUCTION

Among the toxic effects of anti-tumor therapy, one of important places is taken by nephrotoxicity, whose development is promoted by the following parameters: 1) intense blood supply and sensitivity of the organ with respect to hypoxia; 2) a high rate of metabolic and transport processes in the epithelial cells of proximal tubules, being accompanied in some cases by the formation of toxic metabolites; 3) the rate of excretion by means of glomerular filtration and tubular secretion [1]. It is known that the most nephrotoxic agent in the composition of CHOP scheme (cyclophosphane, adriamycin, vincrystine, prednisolone) is a cyclophosphane metabolite such as acrolein [2, 3]. Resulting from the action of urotoxic agents, as a rule, an acute tubular or interstitial damage can develop such as the necrosis of renal papillae, which could result in the development of renal insufficiency thus requiring for a dynamic observation and permanent correction within an intercourse period using preparations with protective properties [4]. In this aspect, individual substances synthesized on the base of betulin could be considered as promising, such as betulonic acid and its derivatives [5]. They exhibit low toxicity, hepato-, nephroprotective, immunomodulating, cytostatic action and pronounced antioxidative activity [6, 7]. Besides, it was demonstrated that betulonic acid and [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acid against the background of influencing polychemotherapy (PCT) in mice with lymphoma RLS cause decreasing the level of necrotic and dystrophic damages in epithelial cells, as well as exhibit antimetastatic properties [8, 9, 6].

The present work was aimed at studying the influence betulonic acid, [3-oxo-20(29)-lupen-28-oil]-3-aminopropionic acids and their methyl esters upon kidney morphology in mice with

transplanted Lewis lung carcinoma against the background of PCT and without its influence.

EXPERIMENTAL

The agents under testing were as it follows: betulonic acid **I**, its methyl ester **II**, [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acid **III** and its methyl ester **IV** synthesized at the Laboratory of Medical Chemistry, NIOCh of the SB RAS (Novosibirsk) [10]. The cytoprotective action of the compounds synthesized was studied in mice of C57BL/6 line with the initial mass of body amounting to 22–25 g, kept under the conditions of standard *vivarium*. All animals were transplanted with a culture of Lewis lung carcinoma into a hip muscle ($2 \cdot 10^6$ cells in 0.1 mL of physiological solution) taken from the Tumor bank of the Institute of Cytology and Genetics, SB RAS (Novosibirsk). In 10 days after transferring the tumor, the mice were divided into 10 groups (10 individuals being in each group). A half of the animal groups was intraperitoneally once introduced with a complex of cytostatic preparations simulating PCT: doxorubicin (Lens-Farm Ltd., Moscow) – 4 mg/kg, cyclophosphane (Biokhimik OJSC, Saransk) – 50 mg/kg, vincrystine (Gedeon Richter, Hungary) – 0.1 mg/kg and prednisolone (Gedeon Richter, Hungary) – 5 mg/kg of the body mass. Compounds **I–IV** were introduced through probe into stomach at a dose of 50 mg/kg every day for 8 days against the background of PCT and without it.

Reference group represented a mice group with transplanted tumor (V group), those were introduced with an equivalent amount of water-Tween mixture. The reference group (VI) consisted of animals after introducing the complex of cytostatic agents. In 8 days the mice were decapitated under ethereal anesthesia, kidneys were extracted, fixed in 10 % paraformaldehyde solution based on 0.1 M Sorensen's phosphate buffer (pH 7.4) during 4 days with the following standard treatment using a MICROM histological complex (Carl Zeiss, Germany). The sections 3–4 μm in thickness were dyed with hematoxylin and eosin, with performing PAS reaction and additional dying by hematoxylin and orange G. The preparations were studied using an Axioskop 40 microscope (magnification ×400).

The morphometric analysis of sections was performed with the use square-shape graticule with 289 points [11]. For kidneys, we calculated the volume density of epithelial cells with dystrophic and necrotic changes, as well as the volume of interstitial tissue and tubular lumen. Statistical data processing was performed using the methods of parametric statistics with the use of Microsoft Excel software package. The results considered reliable at the value Student criterion value $p < 0.05$. The variation of volume density in experimental groups was es-

TABLE 1

Effect of betulonic acid and its derivatives introduced against the background of polychemotherapy (PCT) on kidney morphometric parameters in mice with Lewis lung carcinoma

Groups	Volume density				
	Epithelial cells			Interstitial	Tubular
	Dystrophy (1)	Dystrophy (2)	Necrosis	tissue	lumen
I (PCT + **I**)	$0.50\pm0.007^{\#\#\#}$	$0.23\pm0.016^{\#\#\#}$	$0.012\pm0.005^{\#\#}$	0.12±0.008	0.13±0.009
II (PCT + **II**)	$0.46\pm0.016^{\#\#\#}$	$0.27\pm0.014^{\#\#\#}$	$0.009\pm0.004^{\#\#}$	0.13±0.007	0.14±0.013
III (PCT + **III**)	$0.46\pm0.009^{\#\#\#}$	$0.19\pm0.011^{\#\#\#}$	$0.0026\pm0.001^{\#\#}$	0.14±0.017	$0.21\pm0.012^{\#\#\#}$
IV (PCT + **IV**)	$0.42\pm0.004^{\#\#\#}$	$0.26\pm0.008^{\#\#\#}$	$0.017\pm0.003^{\#\#}$	0.13±0.004	$0.18\pm0.006^{\#\#}$
V (reference)	0.54±0.01	0.19±0.02	0.015±0.003	0.14±0.004	0.11±0.01
VI (PCT)	$0.18\pm0.015^{***}$	$0.49\pm0.023^{***}$	$0.07\pm0.011^{**}$	0.15±0.004	0.12±0.008

Note. Here and in Table 2: 1 – dystrophy of weak intensity; 2 – hydropic and ballooning degeneration.

$^{**}p < 0.01$, $^{***}p < 0.001$ with respect to VI group.

$^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ with respect to V group.

timated with respect to reference groups, whose parameters were taken to be 100 %.

RESULTS AND DISCUSSION

The main pathological changes in animal kidneys of the reference group were revealed mainly in proximal tubules in the form of moderately pronounced necrotic and dystrophic changes in epithelial cells. The fraction of cells with hydropic degeneration and necrosis amounts to 19 and 1.5 %, respectively (Table 1). One could observe a minimum level of pathological changes in glomeruli, interstitial tissue and the absence of metastasis. So, in separate glomeruli there were revealed only a small expansion of mesangial matrix and fibrin depositions in the form of PAS-positive substance between capillary loops (Fig. 1). Revealed kidney damages in animals with transplanted tumor (the absence of metastasis, the damage glomerular and interstitial tissue vessels, minimum pathological changes in tubules) allow one to conclude concerning paraneoplastic nature of nephropathy [12]. The development and pronouncing level of nephropathy depend on the type of the tumour and the conditions cellular and B-cell humoral immune response [13]. Earlier, for mice with transplanted lymphoma RLS there was noted development of severe necrotic nephrosis and tubulointerstitial nephritis [9]. In this experiment, mice with Lewis lung carcinoma were revealed to exhibit only a minimum damage of tubular cells in proximal tubules.

When undertaking PCT, the main pathogenetic mechanism of developing the drug kidney damage is presented by a direct dose-dependent nephrotoxic action of metabolite resulting from cytostatic agents. So, introducing the complex of cytostatic agents resulted in the development of tubulointerstitial nephritis in mice combined with membranous nephropathy. The main pathological changes were revealed in the epithelial cells of proximal tubules such as cytoplasm swelling, fragmentation of the brush limbus, focal hydropic dystrophy and ballooning degeneration (nuclear pycnosis, nuclear chromatin condensation at the periphery of a nucleus in the form of lumps with the following nuclei lysis and cells), necrotic changes

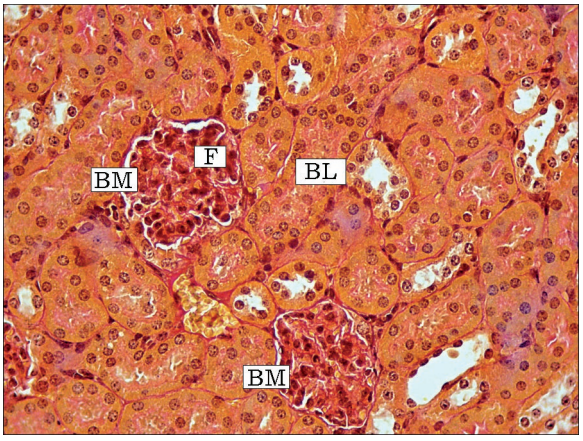

Fig. 1. Mouse kidney with transplanted Lewis lung carcinoma. There is dystrophy of epithelial cells in proximal tubules, mesangial matrix dilatation and fibrin deposits between capillary loops observed. Dying: PAS–hematoxylin–orange G. Here and in Figs. 2–6: BM – basal membranes, BL – brush lymbus, F – fibrin, NC – necrosis of cells, PE – perivascular edema. Magnification ×400.

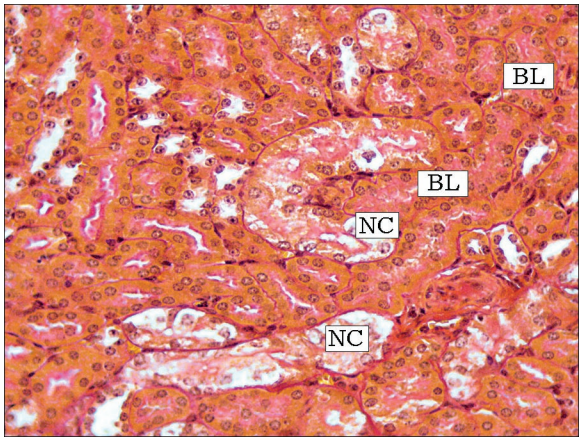

Fig. 2. Mouse kidney with transplanted Lewis lung carcinoma after introducing cytostatic agents. Hydropic dystrophy and ballooning degeneration of epithelial cells in proximal tubules, necrosis of cells, perivascular edema. Dying: PAS–hematoxylin–orange G. Magnification ×400.

of separate tubular formations (Fig. 2). According to the data of morphometric analysis, the volume of epithelial cells with hydropic dystrophy and ballooning degeneration exhibited a 157 % increase, whereas the fraction of epithelial cells with weakly pronounced dystrophic process demonstrated a 77 % decrease (see Table 1).

Against the background of cytostatic influence, one could note a significant increase (by 366 %) in volume density of necrotic changes

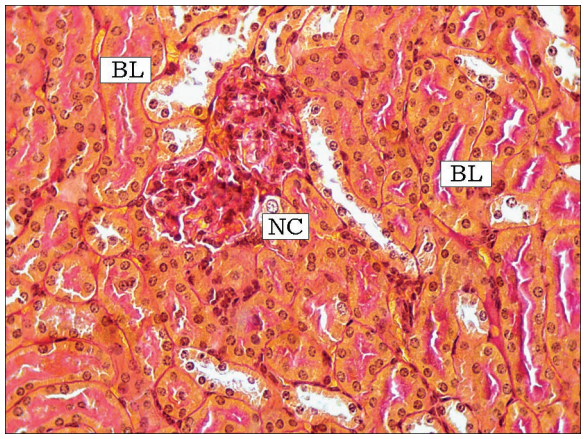

Fig. 3. Mouse kidney with transplanted pulmonary Lewis carcinoma after introducing cytostatic agents and [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acid. Moderate degeneration of epithelial cells in proximal tubules, reduction of brush lymbus edema. Dying: PAS–hematoxylin–orange G. Magnification ×400.

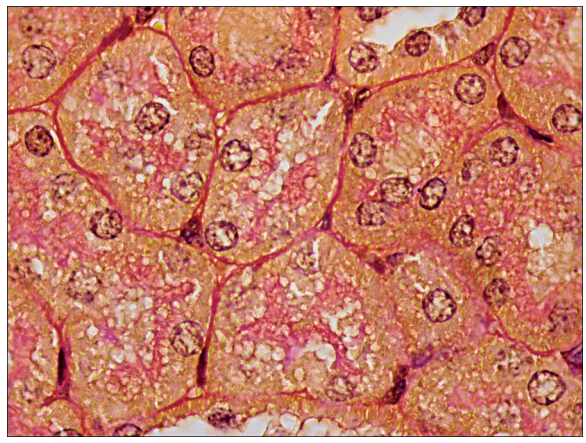

Fig. 4. Mouse kidney with transplanted Lewis lung carcinoma after introducing [3-oxo-20(29)-lupen-28-oyl]-3-aminpropionic acid. Vesicular lipid infiltration of epithelial cells in proximal tubules. Dying: PAS–hematoxylin–orange G. Magnification ×1000 with immersion.

in epithelial cells. In the lumen of receiving tubes we determined hyaline cylinders, whereas in the interstitial tissue a moderate hyperemia and perivascular edema was revealed. Resulting from the morphometric study it was revealed no considerable changing the parameters of interstitial tissue volume and tubular lumen in animals of reference group in contrast with reference. Thus, in this experiment, the introduction of cytostatic agents complicates the course of nephropathy being accompanied with an increase in dystrophic and necrotic damage of nephrocytes.

Introducing the compounds **I–IV** under testing against the background of PCT resulted in reducing the alteration level of epithelial cells in proximal tubules. Under the action of triterpenoids, we observed 80–98 % reducing the volume density of epithelial cells in the state of necrosis and 54–60 % decrease of that in the state of hydropic dystrophy and ballooning degeneration. Besides, we observed an increase in the volume epithelial cells with the symptoms of weakly pronounced degeneration (170–180 %) (see Table 1, Fig. 3). Reducing the edema of epithelial cells and brush limbus against the background of introducing the compounds **III** and **IV** is accompanied by a 50–75 % increase in volume density of the lumen of proximal tubules. The parameters of the volume density of the interstitial tissue in the group with introducing triterpenoids did not

TABLE 2

Effect of betulonic acid and its derivatives on kidney morphometric parameters in mice with Lewis lung carcinoma without polychemotherapy

Groups	Volume density				
	Epithelial cells			Interstitial tissue	Tubular lumen
	Dystrophy (1)	Dystrophy (2)	Necrosis		
I	0.46±0.01**	0.23±0.018	0.007±0.004	0.14±0.005	0.17±0.004**
II	0.48±0.007**	0.19±0.018	0±0**	0.14±0.009	0.17±0.004**
III	0.5±0.011**	0.22±0.017	0.0023±0.001*	0.11±0.011*	0.17±0.009*
IV	0.47±0.006**	0.21±0.011	0.0021±0.001*	0.15±0.011	0.18±0.005**
V (reference)	0.54±0.01	0.19±0.02	0.015±0.003	0.14±0.004	0.11±0.01

Note. For designation see Table 1.

*$p \le 0.05$, **$p \le 0.01$ for reference group.

differ reliably from the reference. The most pronounced positive dynamics of reparative processes was observed against the background introducing compound **III**.

Introducing the agents **I–IV** without PCT exerts a positive effect and on the course of paraneoplastic nephropathy: in proximal tubules one can observe the reduction of the volume density of epithelial cell necrosis, on the average 56 % (Table 2). The fraction of epithelial cells with edematic cytoplasm, vesicular lipid infiltration and fragmented brush lymbus decreases in these groups on the average by 8–13 % comparing with reference (Fig. 4). The morphometric study of mice kidney in the group with isolated introduction of triterpenoids did not result in revealing any significant difference from the reference in the fraction epithelial cells in the state of hydropic dystrophy and ballooning degeneration. All the group after introducing triterpen compounds demonstrate reducing the edema of epithelial cells in proximal tubules and brush lymbus resulting in a 54 % increase in the volume density of tubular lumen. Introducing compound **III** was accompanied by insignificant (22 %) reducing the edema of interstitial tissue.

It should be noted that the introduction of triterpene compounds, both against the background of PCT, and without its influence, does not result in any considerable positive changes in the condition of the glomerular apparatus.

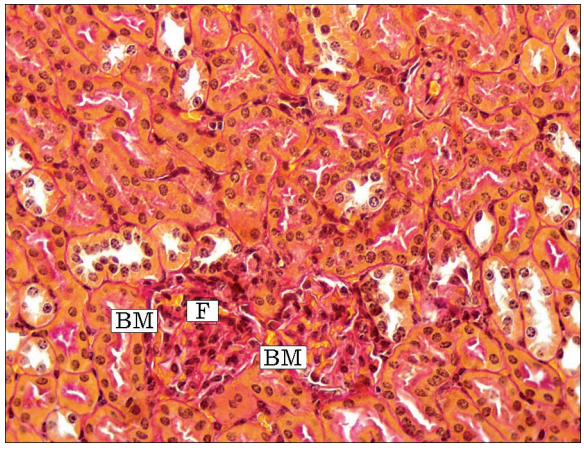

Fig. 5. Mouse kidney with transplanted pulmonary Lewis carcinoma after introducing cytostatic agents and methyl ester of betulonic acid. Thickening basal membrane of glomeruli, dilatation of mesangial matrix. Dying: PAS–hematoxylin–orange G. Magnification ×400.

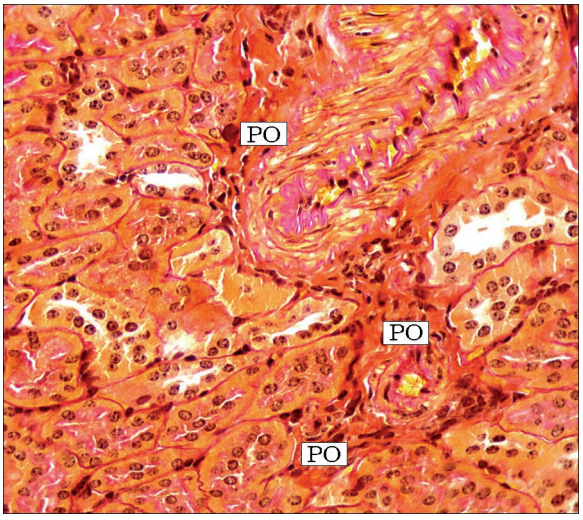

Fig. 6. Mouse kidney with transplanted Lewis lung carcinoma after introducing methyl ester of betulonic acid. Perivascular edema and plasmatic impregnation of arterial wall. Dying: PAS–hematoxylin–orange G. Magnification ×400.

For all the animals, as before, the symptoms of membranous nephropathy are pronounced: basal membranes are thickened in the glomeruli, the volume of mesangial matrix is increased with fibrin (PAS-positive substance) depositing in a capillary loop (Fig. 5) as well as with phenomena of system disorganizing the connective tissue in the form of plasmatic impregnation of arteriole walls (Fig. 6).

CONCLUSION

1. It is revealed that betulonic acid, [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acid and their methyl esters exert a protective effect on kidney morphology in animals, both against the background of polychemotherapy, and without it.

2. It is established that the base of pathological process in animals' kidneys with transplanted pulmonary Lewis carcinoma, to all appearance, consists in paraneoplastic nephropathy.

3. It is demonstrated that polychemotherapy in animals is accompanied by a toxic damage of epithelial cells with the development of tubulointerstitial nephritis.

4. All the triterpenoids under investigation exhibit the ability of reducing against the background of polychemotherapy the level of dystrophic and-necrotic processes in kidney tubules only.

5. Betulonic acid, [3-oxo-20(29)-lupen-28-oyl]-3-aminopropionic acid and their methyl esters in the mode of isolated introduction reduce the intensity of paraneoplastic nephropathy.

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