

THE HISTOPATHOLOGICAL EXAMINATION OF MICE TISSUES AFTER THE TREATMENT WITH NEW SYNTHESIZED PROSTAMIDES WITH ANTIGLAUCOMA ACTION

DENISA IOANA UDEANU¹, MANUELA ENACHE²,
DENISA MIHELE¹, FLOREA COCU³

¹University of Medicine and Pharmacy "Carol Davila", Bucharest,
Faculty of Pharmacy, Department of Clinical Laboratory and Food
Safety, Traian Vuia 6, Sect.1, Bucharest, 020956

²Sanitary Veterinary and for Food Safety Direction, Calea Feldioarei 20A, Brasov

³National Institute for Chemical and Pharmaceutical Research and
Development, Bucharest, Calea Vitan 112, Sect.3, Bucharest

*corresponding author: denisaudeanu@gmail.com

Abstract

Prostamides are nowadays considered as the most effective drugs used for the long term treatment of primary open angle glaucoma. New prostamides with potential antiglaucoma action were synthesized within the Department of Biosynthesis of Natural Products from the National Institute for Chemical and Pharmaceutical Research and Development, Bucharest. The current research is part of the preliminary toxicological studies and tries to assess the possible histopathological modifications of mice tissues after the treatment with new synthesized prostamides.

Rezumat

Prostamidele sau prostaglandin-etanolamidele sunt considerate la ora actuală cele mai eficiente medicamente antihipertensive oculare. În cadrul Departamentului de Sinteză Compuși Naturali de la Institutul Național de Cercetare și Dezvoltare Chimico-Farmaceutică, București, au fost sintetizați noi compuși analogi structurali ai prostamidei F2 α cu acțiune antihipertensivă oculară. Studiul face parte din testele preliminare toxicologice și își propune stabilirea eventualelor modificări histopatologice ce pot să apară la șoareci în urma tratamentului cu noile prostamide sintetizate.

Keywords: prostamides, glaucoma, histopathological examination

Introduction

Prostamides or prostaglandin-ethanolamides are the most effective currently available drugs for treating glaucoma. They are COX-2 derived oxidation products of the endocannabinoid/endovanilloid anandamide. In spite of their structure similarity with prostaglandins, the prostamides act through a different receptor not yet identified [4,6,7].

Generally, prostamides are local hormones and display slight systemic distribution. They are rapidly metabolized via oxidative and

conjugative pathways. The excretion of the systemically absorbed drugs and their metabolites predominantly occurs in urine. [1,2,3,6]

The study deals with the histopathological examination of some tissues after the treatment with two newly synthesized prostamides designed for topical ophthalmic administration in glaucoma therapy [5, 8].

Materials and methods

Experiments were performed on White Albino Swiss mice weighing 20 ± 2 g.

All animals used in the study were kept in standard laboratory conditions. They received water *ad libitum* and were not fed for 12h before the experiment. All experiments were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from February 2nd, 2002.

The animals were distributed in 4 groups of 10 individuals each and daily treated during 14 days as it follows:

- two control groups were intraperitoneally (i.p.) treated with 0.5mL/20g mouse physiological solution and 0.5mL/20g mouse of 0,01% Tween 80 solution, respectively
- one group was i.p. treated with 15mg/Kg bw D-cloprostenol-1-N-ethanolamide
- one group was i.p. treated with 15mg/Kg bw D-cloprostenol-1-N-ethylamide

Equipment:

- Tissue Processor TPC15 (Medité)
- TBS88 Paraffin Embedding System (Medité)
- Tissue Stainer TST 3 (Medité)
- Microtom LEICA RM 2155 (Leica)
- Microscope Nikon

Substances:

- D-cloprostenol-1-N-ethylamide
- D-cloprostenol-1-N-ethanolamide
- Formaldehyde
- Paraffin
- Ethylic alcohol

The new antiglaucoma prostamides (D-cloprostenol-1-N-ethanolamide and D-cloprostenol-1-N-ethylamide) were obtained by stereocontrolled synthesis within the Department of Biosynthesis of Natural Products from the National Institute for Chemical and Pharmaceutical Research and Development, Bucharest. These compounds are derivatives of

D-cloprostenol, a prostaglandine F_{2α} analogue already used in veterinary medicine [5].

The new synthesised prostamides are highly lipophylic compounds having very low solubility in water. In order to obtain a proper solution for intraperitoneal administration, both compounds were dissolved in water using a surfactant (Tween 80).

After 14 days, all animals were chloroform anesthetised and slaughtered.

Sampling and samples fixation

The livers, kidneys, brains, lungs and spleens were collected immediately in order to avoid diagnosis error and placed in formalin (10% formaldehyde in water) as fixative solution.

Tissue processing was performed according to the procedure normally applied by the Romanian Sanitary Veterinary and for Food Safety Direction, which involves the use of a Tissue Processor TPC15 and TBS88 Paraffin Embedding System. All samples were transferred into a cassette and immersed in multiple baths of progressively concentrated ethanol (to dehydrate the tissue), followed by toluene and finally hot paraffin. During the embedding process, additional paraffin was added to create a paraffin block which allowed the sectioning of the tissues into very thin slices (3-5µm) using a microtome (microtom LEICA RM 2155). The sections were deparaffinized and rehydrated.

Staining of processed histology samples

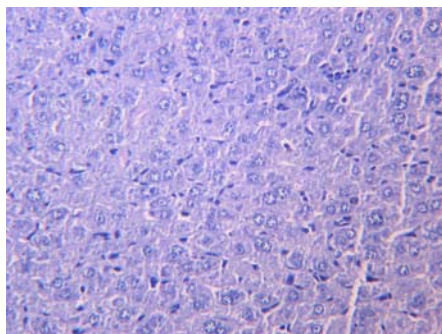
The microtome slices were hematoxylin – eosin - methylene blue stained using a TST3 Tissue Stainer. Hematoxylin and methylene blue were used to stain nuclei blue, while eosin was used to stain the cytoplasm and extracellular matrix in varying degrees of pink.

Results and discussion

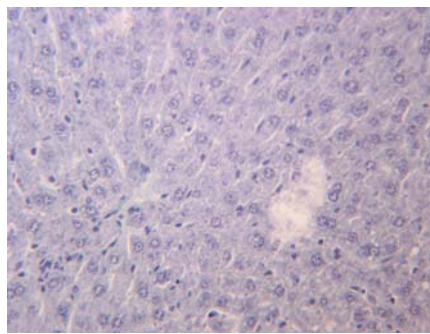
The 14 days treatment with i.p. 15mg/Kg bw dose of D-cloprostenol-1-N-ethylamide and D-cloprostenol-1-N-ethanolamide produced no significant histopathological modifications comparing to controls. No tumour cells were observed in any of the treated groups.

The two receptor-selective analogs of prostamide F_{2α} derivatives are specially designed for topic ophthalmic administration. Our unpublished data demonstrated that the 0.03% prostamides ophthalmic solutions have antihypertensive ocular action and the therapeutic daily dose is 1.5µg/drop/eye. The i.p. dose of 15mg/Kg bw used in this study is 10⁴ times higher than the therapeutic ophthalmic dose.

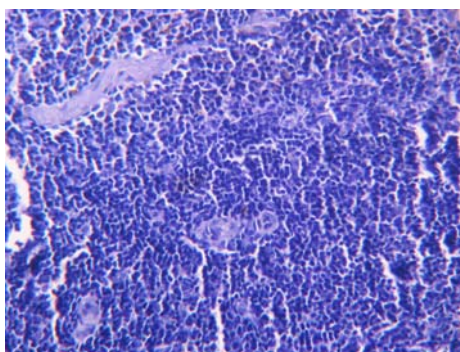
Some of the tissue sections were selected and presented in the following figures (Fig. 1- 10).

**Figure 1**

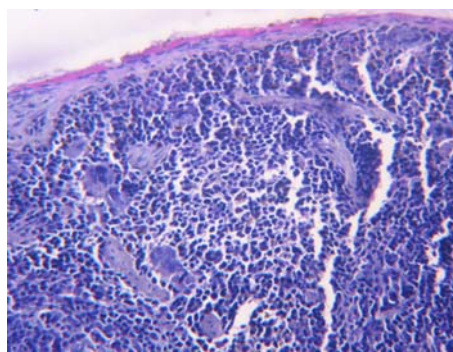
Liver – control group – liver cells in regeneration (binucleate liver cells); Kupffer cells; rare region of storage disease; normal bile ducts

**Figure 2**

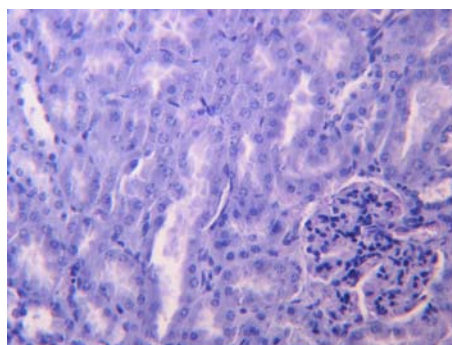
Liver- treated group - binucleate liver cells; rare limfocytes; Kupffer cells; no major histological destructions

**Figure 3**

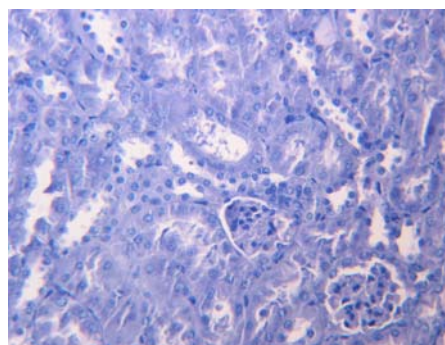
Spleen – control group - white pulp with trabecular architecture, normal sinusoides

**Figure 4**

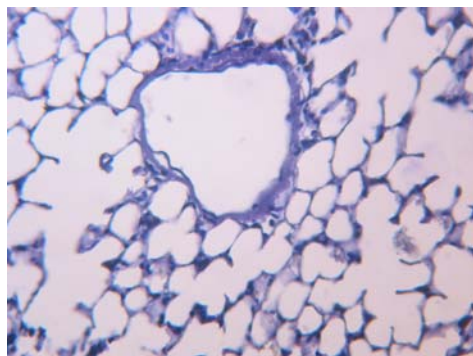
Spleen –treated group - spleen capsule with trabecular architecture, the sinus of the red pulp and the white pulp; megacariocyte specific for mice

**Figure 5**

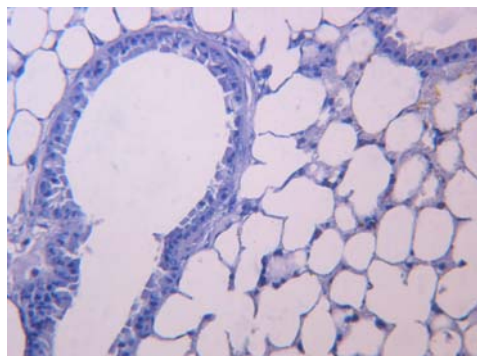
Kidney – control group: normal renal glomerula , proximal ducts with normal nucleus, rare limfocyte

**Figure 6**

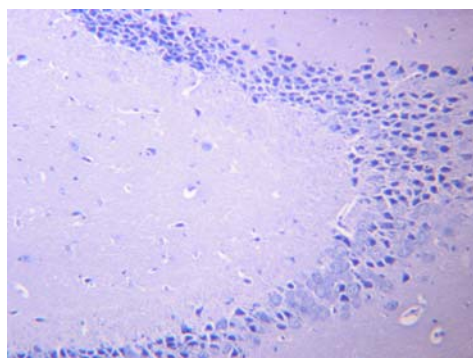
Kidney – treated group: normal renal glomerula without histological modification

**Figure 7**

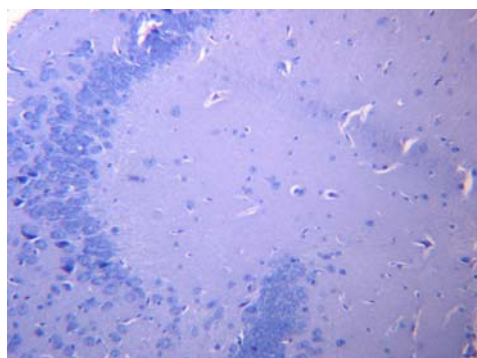
Lung – control group: central bronchioles, rare area of emphysema

**Figure 8**

Lung – treated group: bronchioles with normal epithelia, fibrine deposit

**Figure 9**

Brain - control group: hippocampus zone with molecular ledge, pericardial edema

**Figure 10**

Brain treated group - hippocampus zone with molecular ledge, pericardial edema; area without histological destructions

Conclusions

The newly synthesised prostamides produced no significant anatomic-pathological modifications of the liver, lung, kidney and spleen to mice after the daily administration for 14 days of an i.p. dose 10^4 higher than the ophthalmic therapeutic dose.

Acknowledgements

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