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GENERAL ARTICLE

FIGHTING MULTIPLE DRUG RESISTANCE: EFFECTS OF UV-ACTIVATED CHLORPROMAZINE ON RABBIT'S EYE PSEUDOTUMOURS

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Abstract
Introduction: Multiple drug resistance requires a flexible approach to find medicines able to overcome it. One method could be the exposure of existing medicines to UV laser beams to generate active photoproducts against bacteria and/ or malignant tumors.
Methods: The interaction of Chlorpromazine (CPZ) (irradiated with 266 nm pulsed laser beams) was studied at concentrations of 10 mg/ ml and 20 mg/ ml in ultrapure water, with pseudotumors of rabbits eyes.
Results: The use of CPZ water solution exposed to 266 nm in the treatment of pseudotumor tissues produced on rabbit eyes showed that treatment results depend on initial (before irradiation) CPZ concentration and exposure time. At this stage, one could not specify which out of the generated photoproducts, individual or as a group, was/ were efficient in pseudotumor cure but overall effects were observable. Application of CPZ irradiated solutions on rabbit eyes pseudotumors seemed to produce a faster recovery of tissues with respect to control, untreated eyes.
Conclusions: Histologic findings in the treated tissues showed a good anti-inflammatory response. The results obtained open perspectives to fight MDR and/ or development of pseudotumoral processes with substances that were not initially made for this purpose (non-antibiotics, for instance).
Keywords: MDR, CPZ, laser irradiation, photoproducts, pseudotumors

Introduction

Multiple drug resistance (MDR) became and remains a worldwide health issue, which requires a flexible approach to find medicines able to overcome it. One possible method could be the exposure of existing medicines to UV laser beams to generate photoproducts that seem to be efficient against bacteria and/ or malignant tumors. This is particularly applicable for substances that are not antibiotics (non-antibiotics) but may generate, by exposure to laser beams, isomers that might have antibiotic properties.

A particular class of medicines for these studies is represented by phenothiazines which are used as neuroleptics (Chlorpromazine – CPZ, Thioridazine – TZ) but which may work as

agents to treat malaria or tuberculosis (particularly TZ) as well [**1,2**]. Recent studies performed on larger volumes (bulk – 1.5 ml) of CPZ solutions in ultrapure water at several concentrations, showed that hundreds of photoreaction products are obtained by exposure to UV laser radiation, namely 266 nm, 337.1 nm or 355 nm and that the application of solutions containing mixtures of the obtained photoproducts on Gram-positive bacteria evidences a higher antibacterial effect of the mixture than that of the CPZ parent compound [**2-4**]. On the other hand, there is the MDR acquired by malignant tumors; in some cases, infections of MDR tumoral tissues with MDR bacteria are mentioned.

Materials and methods

The study was performed on rabbit eyes; rabbits were treated according to animal ethics regulations of "Carol Davila" University of Medicine and Pharmacy in Bucharest. The protocol was approved by the Ethics Committee of Scientific Research of "Carol Davila" University of Medicine and Pharmacy (Code PO-35-F-03, No. 34). All treatment injections were performed under anesthesia with Xylazine 4 mg/ kg im, Ketamine, 10-20 mg/ kg iv and Atropine 0.5 ml of solution 1‰ to minimize the suffering.

We started by producing pseudotumoral tissues on rabbit eyes by using the Schmidt-Erfurth method, which consists in the insertion of a propylene suture wire (0.5) at the scleroderma limbus (**Fig. 1**) [**5**].

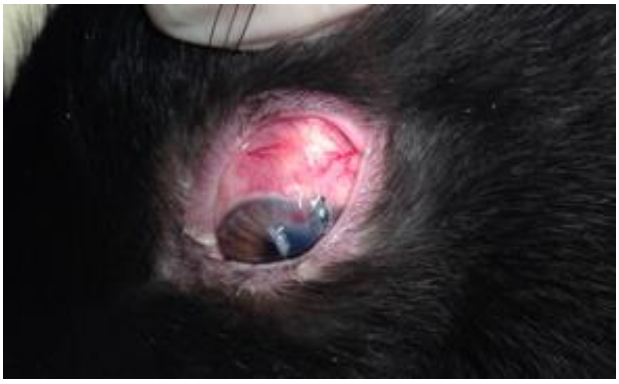

Fig. 1 Pseudotumor obtained on rabbit eye (Schmidt-Erfurth method)

The CPZ solutions in concentrations of 20 mg/ ml and 10 mg/ ml were exposed to 266 nm laser beam having 6.5 mJ average energy, for time intervals between 5 and 240 minutes, previous to treatment.

The treatment was applied on 5 rabbits; rabbits were injected with CPZ subconjunctivally, at the limbus, right near the pseudotumoral area; rabbit no. 1 was kept as control I and had pseudotumors on both eyes but not treated; rabbit no. 2 was control II and had pseudotumors treated with unexposed CPZ in two different concentrations (one eye 0.1 ml of solution at 20 mg/ ml in ultrapure water and the second 0.1 ml of solution at 10 mg/ ml). Rabbits no. 3, 4 and 5 were treated with CPZ solutions in two concentrations irradiated at 266 nm different time intervals as it follows: for rabbits no. 3 and 4, one eye was injected with 0.1 ml CPZ 20 mg/ ml solution and the second eye was injected with 0.1ml CPZ 10 mg/ ml solution, both irradiated for 20 minutes; these two rabbits were studied to observe the reproducibility of the results; as for rabbit no. 5, one eye was injected with 0.1 ml solution at 20 mg/ ml and the other with 0.1 ml solution at 10mg/ ml, the irradiation time being in both cases of 4 hours.

At the end of the treatment, the eyes were extracted and anatomopathological examination was performed. The results were organized on pairs of extracted eyes and were shown as images of parts of tissues selected in accordance with the dimensions and qualities of the effects produced on them. **Fig. 2** shows the histopathologic image of pseudotumoral tissue extracted from control rabbit I (rabbit no. 1). Areas A and B (**Fig. 2**) include cells of inflammatory type; rare, normal vascular elements may be seen in zone C (**Fig. 2**) at the end of the three days of observation.

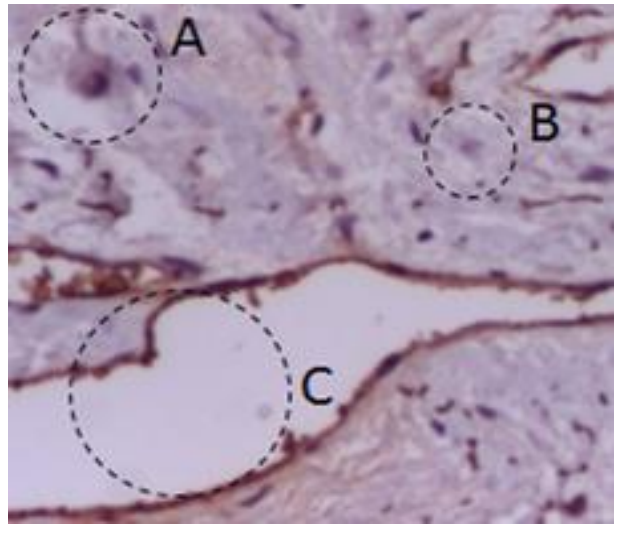

Fig. 2 Histological findings in pseudotumoral tissue of control rabbit I. Inflammatory cells (A, B), vascular elements (C)

Fig. 3 highlights the histological images from control rabbit II, in whom 0.1 ml of unirradiated CPZ solutions at 20 mg/ ml and 10 mg/ ml were injected; a granular inflammatory tissue (A) may be noticed as well as a moderate chronic infiltrate with frequent eozinophils (at treatment with 20 mg/ ml solution).

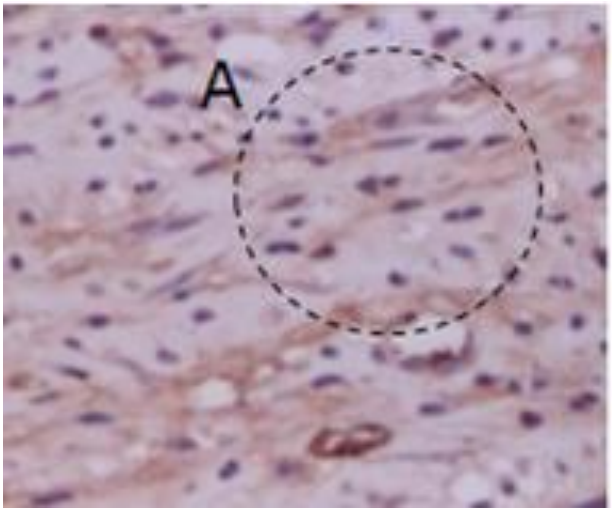

Fig. 3 Histological findings in control rabbit II; aria A shows granular inflammatory tissue (CPZ 20 mg/ ml)

Fig. 4 shows that the treatment was done with 10 mg/ ml solution, necrotic masses of tissues (area A) rich in eozinophils infiltrate (area B). There were no significant differences between control I and control II rabbit eyes, i.e. the unirradiated CPZ solutions did not contribute to a faster destruction of the pseudotumors.

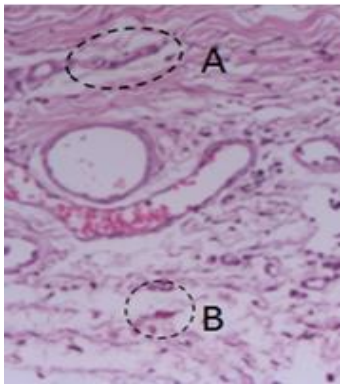

Fig. 4 Histological findings in control rabbit II (CPZ 10 mg/ ml) – necrotic tissue (A), eozinophils infiltrate (B)

For treated rabbits no. 3 and 4, CPZ solutions at the two concentrations were irradiated for 20 minutes. This time interval had a lag between 5 min and 37.5 min, for which the modifications of CPZ solutions were measured. The generated photoproducts at 20 min exposure to UV laser beam were the same as those reported in the above-mentioned measurements, the differences being their concentrations.

Fig. 5 shows the results after the treatment with CPZ solutions of rabbit no. 3. **Fig. 5a** presents the image of the pseudotumor tissue after treatment with CPZ 20 mg/ ml. One may observe rich chronic inflammations (A), frequent eozinofiles (B) and necrotic nodular tissue masses (C), as well as frequent blood vessels (D). In **Fig. 5b** the image of the eye treated with irradiated CPZ at 10 mg/ mL shows the same effects. However, the extension of the damages was lower in the treatment with 10 mg/ mL than with 20 mg/ ml, which recommended the use of CPZ solutions at 10 mg/ ml for treatment, after exposure to UV laser beam.

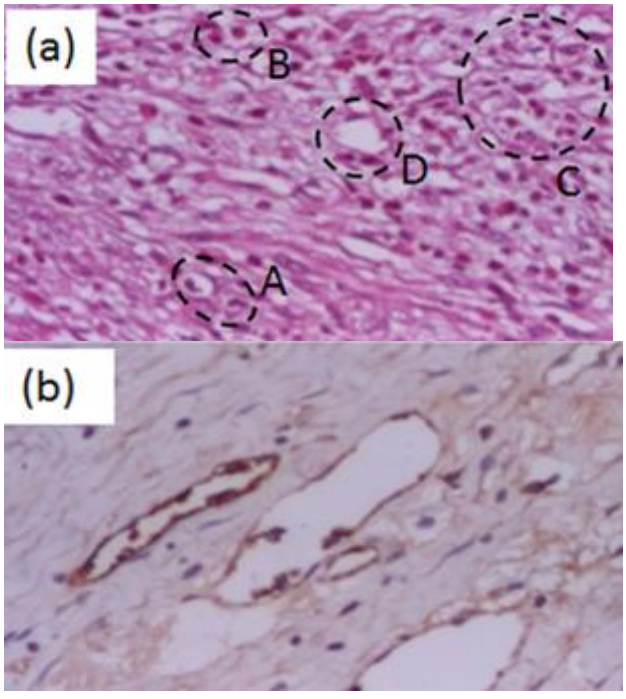

Fig. 5a chronic inflammations (A), frequent eozinofiles (B) and necrotic nodular tissue masses (C), and frequent blood vessels (D). **Fig. 5b** the same aspect at a different magnification

Fig. 6 shows the histopathologic pseudotumoral tissue for an eye extracted from

rabbit no. 4. The eye was treated with 10 mg/ ml CPZ solution exposed for 20 min to 266 nm laser beam.

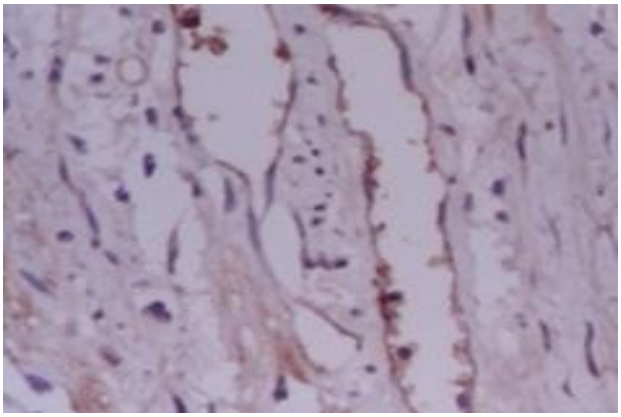

Fig. 6 Histological aspect from pseudotumoral tissue from rabbit no. 4. A similar aspect in rabbit no. 3; in addition, necrotic tissue was observed

The results in this case are comparable with those obtained in rabbit no. 3; one may also observe a nodular necrotic mass produced on the pseudotumor tissue.

Fig. 7 (**a and b**) shows the histopathologic images of samples of eye pseudotumor tissues obtained from rabbit no. 5.

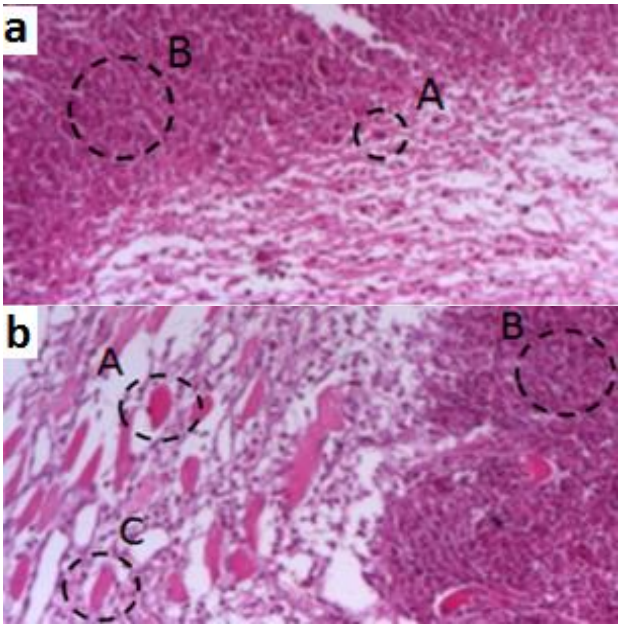

Fig. 7 a CPZ 20 mg/ ml exposed for 4h at 266 nm; eozinofiles (A) and nodular necrotic tissue (B) could be noticed**; Fig. 7b** shows frequent trombosis on vessels (C)

In **Fig. 7a**, the eye was injected with 0.1 ml CPZ solution at 20 mg/ ml in water, exposed for 4h at 266 nm and in **7b** the tissue was injected with 0.1 ml CPZ solution at 10 mg/ ml irradiated in the same conditions. In both images one may observe rich, chronically inflammated infiltrates which contain frequent eozinofiles (areas A) as well as nodular necrotic tissular masses (areas B) inserted in the striate muscle tissue. Relatively frequent blood vessels exhibiting trombosis (**Fig. 7b**, area C) were noticed.

The main conclusion that results out of **Figs. 5-7** is that the treatment with irradiated CPZ solution is most efficient in recuperating the qualities of the corneal tissue when the concentration 10 mg/ ml is used. Although the exposure time for the solution used to treat rabbit no. 5 is of 4 hours (i.e. 18 times longer than in the case of solutions used to treat rabbit no. 3), the effects are not spectacularly better than in the case of solutions exposed for 20 minutes.

Conclusions

The use of CPZ water solution exposed to 266 nm laser beam to treat pseudotumor tissues produced on rabbit eyes showed that treatment results, in terms of recuperating the qualities of healthy corneal tissues, depend on the CPZ concentration at the beginning of laser irradiation and the exposure time length.

At the same time, one knows that the exposed solutions contain around 200 new photoproducts [**2-4**]. In the experiments on rabbits, solutions exposed to UV laser beam were applied on pseudotumor tissues produced on eyes, i.e the mixtures in water of the 200 photoproducts obtained by irradiation. At that stage, it was not possible to specify which out of the generated photoproducts, individual or as a group with synergistic effects was efficient in pseudotumor cure, but the overall effects may be clearly outlined. Since the exposed CPZ solutions are stable in time, they may be applied on the eye at any time. The application of CPZ irradiated solutions on rabbit eyes pseudotumors seems to produce a faster recovery of tissues with respect to the control eyes, but it remains to quantify the

differences. The principle and methods described in this paper could further serve for fighting MDR or tumoral process.

Acknowledgement
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