

## ENHANCING OF METHOTREXATE ACTIVITY DURING OPTICAL BEAM IRRADIATION IN PHOTODYNAMIC THERAPY OF CANCER AND NON-CANCER DISEASES

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Photodynamic therapy (PDT) as a method used in the treatment of some types of cancer may also be used to treat some non-cancerous diseases of the skin or eye. PDT uses laser or other light sources, combined with a light sensitized drug (sometimes called a photosensitizing agent) to destroy cancer cells. Once the drug is accumulated in the tumour cells, if photons of ultraviolet and visible light (UV-VIS) are incident to them, the drug is activated and the cancer cells are destroyed. In the present work, we studied the photosensitizer properties of the cytostatic drug (methotrexate), used as photosensitizing agents, by its exposure to UV-VIS radiations. An experiment model for a possible clinical application was performed, also. Its effects were analyzed on the optical microscopic examination of the biological material used. As a result, we could observe a cytostatic activity enhancement.

(Received April 26, 2004; accepted after revision November 29, 2004)

*Keywords:* Methotrexate, Photodynamic therapy, Photosensitisers, UV-vis irradiation

### 1. Introduction

PDT combines the preferential accumulation of the photosensitizer in the target tissue with precise illumination of this tissue, to provide the selectivity of the treatment. The light penetrates the tissue [12] and causes excitation of the photosensitizer [1,2]. The excited photosensitizer transfers his energy to the molecular oxygen from the cell which is transformed into oxygen singlet and destroys cancer cell. In this study, methotrexate (MTX) is used as a photosensitizer.

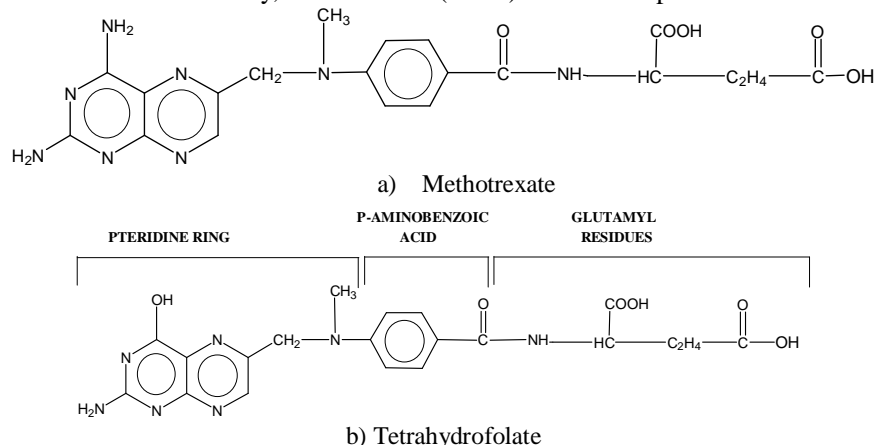


Fig. 1. The chemical structures of a) methotrexate and b) tetrahydrofolate.

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MTX is an antifolate (Fig. 1a), widely used in cancer chemotherapy, in psoriasis and rheumatoid arthritis treatment [6,7]. It stops the DNA synthesis by inhibiting the action of dihydrofolate reductase (DHFR) which is responsible (Fig. 1b) for the tetrahydrofolate concentration level within the cell [3,4]. DHFR plays an important role in purine nucleoside and DNA synthesis. MTX belongs to a pterine group, which photo-dissociates in 6-formylpterin and p-aminobenzoylglutamic acid, at UV-vis light exposure [5]. Fig. 1 presents the chemical structures of MTX and tetrahydrofolate molecules, which are almost identical. [13,14]

The clinic studies use the coupling of the UV-vis light irradiation with MTX chemotherapy, as a new procedure of cancer treatment [10,16].

Studies on the photochemistry of the folic acid reported by Thomas et al. [11] showed the photodissociation effects of the light on the absorption and fluorescence characteristics of *folic acid* as a function of the exposure time. MTX belongs to the pterine group with the folic acid and aminopterin, which undergo photodissociation under UV-vis exposure forming 6-formylpterine derivatives and p-aminobenzoylglutamic acid (Fig. 2) [9]. Recently, we have proposed the combination of the UV-vis irradiation and MTX as a possible new treatment for cancer [8,14,15].

## 2. Materials and methods

ANTIFOLAN (MTX) was supplied by Sindan S.R.L., Bucharest, Romania. MTX solutions in natural saline (0.9% NaCl) at concentrations of  $5 \times 10^{-5}$  M and adjusted to pH = 8.4 by addition of the NaOH were used. The MTX solutions were exposed to UV-VIS using an uncoherent Hg lamp (power density =  $110 \text{ W/m}^2$ ), for 1 minute to 5 minutes. The Hg lamp emission was in continuous wave (cw) mode; the covered spectral range was between 300 nm and 600 nm with high intensity lines at 365 nm, 406 nm and 435 nm.

In order to apply optimally the radiation on the tumour cells, an optical fiber was used.

The absorption spectra of the samples were recorded with a Perkin Elmer Lambda 2S spectrophotometer in the UV-vis range. The excitation and emission fluorescence spectra were recorded with an Aminco-Bowman spectrophotofluorometer. Both spectrometers were assisted by computers.

## 3. Results and discussions

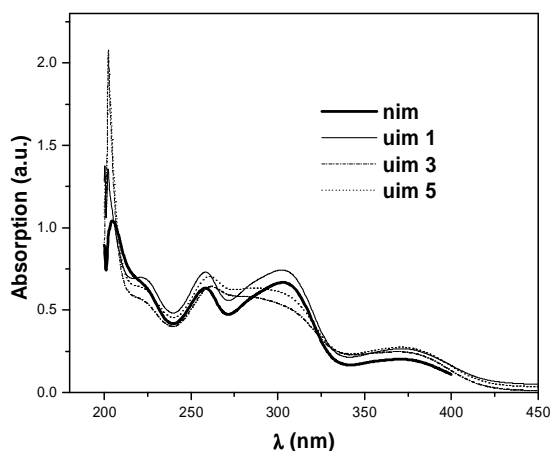


Fig. 2. The absorption spectra of the non-irradiated MTX solution (nim) and the irradiated MTX solutions (uim).

The Fig. 2 shows the changes in the absorption spectrum of the MTX solution, as a function of the irradiated time. The terms “uim X” and “nim” mean the spectrum obtained after X minutes of irradiation and non-irradiated MTX solution, respectively.

The main absorption bands are situated at the wavelengths of 270 nm, 320 nm and 375 nm. The largest UV absorption was detected at 320 nm wavelength. All the absorption bands present a slight hypsochromic effect with the increasing of the irradiation time.

The intensity of the absorption band situated at 320 nm decreases with the increasing of the irradiation time while the intensity of the other absorption bands increases slightly. The half-band widths and the presence of three isosbestic points for all absorption spectra suggest the homogeneity and purity of the samples. Changes in the spectra indicate slight modifications of physical properties of the MTX, after irradiation with Hg lamp beam.

The fluorescence excitation spectra were recorded at 470 nm emission wavelength. They are presented in the Fig. 3, where one can see the relative intensity of the fluorescence excitation as a function of irradiation time. The terms “uim X” and “nim” have the same significance like in the Fig. 2.  $I_{ex}$  represents the fluorescence intensity, measured in arbitrary units.

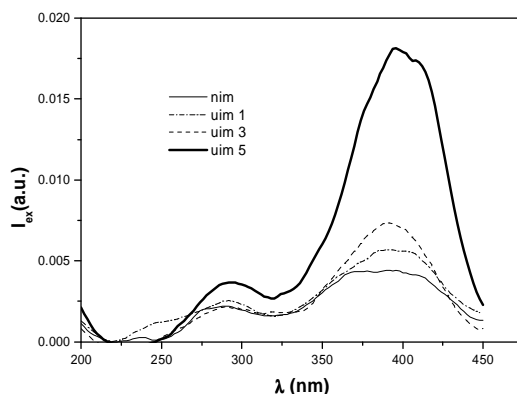


Fig. 3. The fluorescence excitation spectra of the non-irradiated MTX solution (nim) and the irradiated MTX solutions (uim).

The highest fluorescence excitation intensities were detected at 390 nm wavelength. For this reason the wavelength was selected as the excitation wavelength for the fluorescence emission spectra.

The Figure 4 shows the fluorescence emission spectrum of the non-irradiated MTX solution and the fluorescence emission spectra of the irradiated MTX solutions as a function of the exposure time. This analysis was performed in the 450 nm - 700 nm range.

The terms “uim X” and “nim” have the same significance like previous figures.  $I_{em}$  represents the fluorescence emission intensity, presented in arbitrary units.

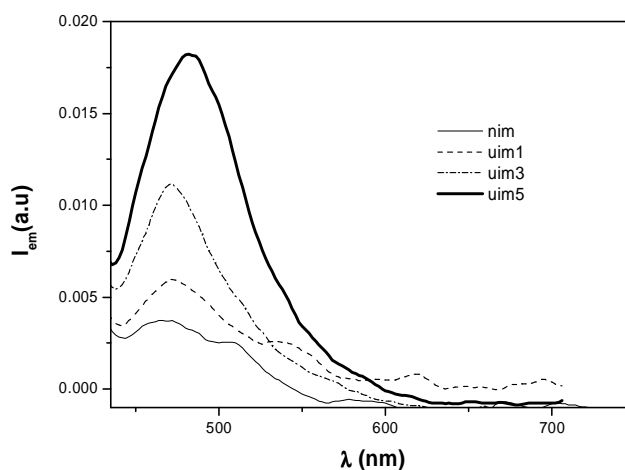


Fig. 4. The emission fluorescence spectra of the non-irradiated MTX solution (nim) and the irradiated MTX solutions (uim).

The excitation wavelength was of 390 nm and the maximum fluorescence emission intensity was situated at 470 nm.

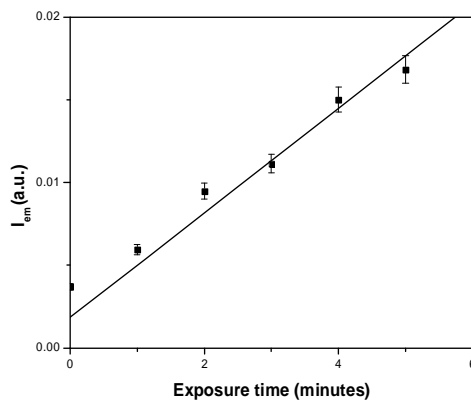


Fig. 5. The relative intensity of the 470 nm fluorescence emission maximum ( $I_{fm}$ ) versus irradiation time.

The maximum intensity of the fluorescence emission spectra of the UV-vis irradiated samples represented as a function of irradiation time (Fig. 5) seems to be a linear function, showing that the process is unsaturated in the irradiation range used in our experiments

### 3.1. Experiment model for a possible clinical application

An experiment mode for a possible clinical application on rabbit eyes was performed. The pseudo-tumors with new vascularization were induced by sewing a catgut stitch (diameter = 0.88 mm) at rabbit eye cornea. The experiment was performed on six rabbits.

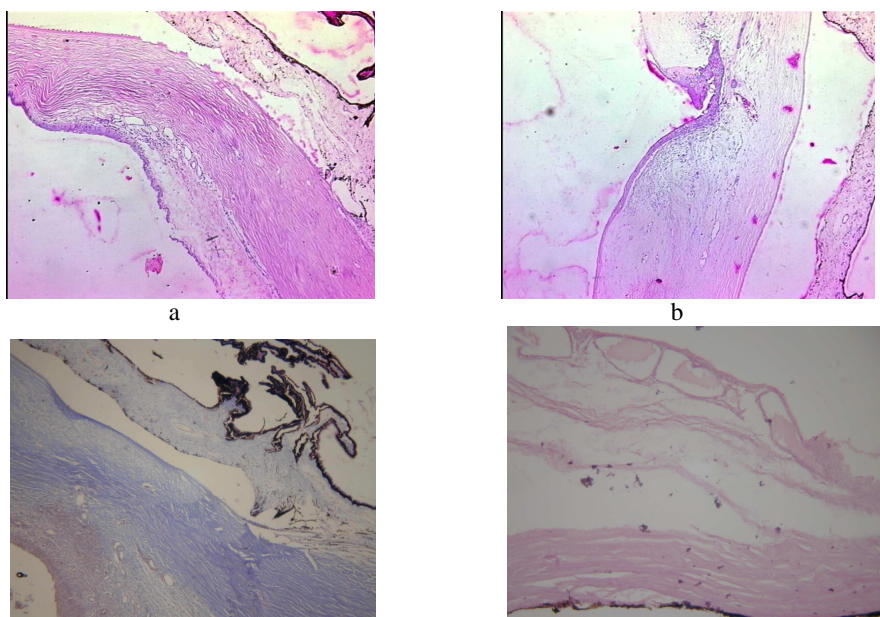


Fig. 6. Microscopically images of the rabbit cornea in different experimental conditions. a) The microscopic aspect of the rabbit eye cornea injected with MTX. One can see that it presents a diffusing inflammatory part; b) The rabbit eye cornea irradiated with Hg lamp for 2 minute after injection with MTX. The image presents dye blemishes that represent the neoformation vessels without own walls.; c) The diffusing inflammatory part of the rabbit eye cornea irradiated with Hg lamp for 3 minute after injection with FU; d) The tumour regress of the rabbit eye cornea irradiated with Hg lamp for 5 minute after injection with MTX.

The rabbit eyes were previously injected with  $5 \times 10^{-5}$  M MTX solutions in saline water solution (0.9% NaCl). Three rabbit eyes injected with MTX and non-irradiated were used as witnesses. The next three eyes were irradiated with Hg lamp, for 1 minute. Other three eyes were irradiated for 3 minutes, and the last three eyes were irradiated for 5 minutes.

All the eyes exposed to the irradiations were irradiated 3 times per week. The duration of the treatment was 4 weeks. After these 4 weeks, the pathological examination of the conjunctive tissue was made using an electronic microscope Nikon 6 with 10x zoom.

The images obtained by the electronic microscope are presented in the Fig. 6.

The non-irradiated rabbit eye cornea and the rabbit eye cornea irradiated for 1 minute and 3 minutes present an inflammatory part, instead the rabbit eye cornea irradiated for 5 minutes, present a significant tumour regress, proving that the dose of the irradiation must be greater than a threshold one.

#### 4. Conclusions

The analysis of the fluorescence emission spectra of the MTX solutions shows an increased intensity of the fluorescence with irradiation dose. The increase of the fluorescence emission intensity with the irradiation time is linear, leading to the conclusion that the MTX transformation is not saturated. A longer irradiation time may be used to increase the photo-reaction product concentration.

The fluorescence emission spectra of the irradiated MTX solutions correlated with the pathological status of the pseudo-tumoural tissue, injected with MTX and after the irradiation with different doses show that the photo activity, which is responsible on the tumour regress, is correlated with the emission fluorescence enhancement.

The present studies are useful for clinical applications of the cytostatics. It is pointed out that the exposure of the tumour to light, at an optimal dose, after the injection of the cytostatic, enhances the clinical effects in destroying tumour tissues.

#### Acknowledgements

We are grateful for technical advice and helpful discussions, to Professor Aurel Popescu from Faculty of Physics, University of Bucharest.

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