Biochemistry & Pharmacology: Open Access

Pascu, Biochem & Pharmacol 2012, 1:2
DOI: 10.4172/2167-0501.1000e111

Editorial Open Access

# The Laser Use in Generating New Species of Medicines with Antitumor and Antibacterial Properties: New Processes in Drug Discovery

**Mihail Lucian Pascu***

*National Institute for Laser, Plasma and Radiation Physics, Atomistilor 409, 077125 Magurele/Ilfov, Romania*

## Editorial

Reports made during the last decades, mention the development of drug resistance mechanisms acquired by tumor cells and bacteria. To-date, the multiple drug resistance (MDR) identified for the malignant tumor cells [1-3] and bacteria (*Mycobacterium tuberculosis, Staphylococcus aureus, Escherichia coli* etc.) [4] is well documented. With respect to this challenge the science community is asked to find solutions in order to fight MDR and the research efforts may be included in two main categories: (a) development of new medicines and (b) identifying new ways to make efficient again the existing ones.

The promotion of new medicines is costly, needs sustained efforts and takes long time. Approaching new methods to make the existing medicines efficient in fighting MDR is more convenient, cheaper, faster and simpler, the more so because the medicines utilized to treat particular maladies may become, once modified, efficient in the treatment of completely different illnesses than targeted by the parent compounds.

A promising procedure applied to produce modifications of medicines at molecular level consists in exposing them in solutions at laser radiation that has particular characteristics in terms of wavelength, energy/power, time distribution of the emission (i.e. continuous wave-cw or pulsed), pulse duration and repetition rates; according to the present knowledge, the polarization state of the beams and their time and spatial coherence do not have critical influence on the processes of interest.

Out of the reports about cytostatics modified by exposure to laser radiation in order to be applied efficiently on tumor tissues in MDR cases we selected two widely utilized chemotherapeutic substances: Methotrexate (MTX) and 5 - Fluorouracil (5 - FU).

Methotrexate (MTX) is an antifolate used in the chemotherapy of cancer, as well as in the treatment of psoriasis and rheumatoid arthritis. MTX stops DNA synthesis by inhibiting the action of the dihydrofolate reductase and thus reducing the tetrahydrofolate level in the cell. Studies on the photochemistry of folic acid showed the photodissociation effects of the light on the absorption and fluorescence characteristics of folic acid function of the exposure time [5,6]. MTX belongs to the pterine group, together with folic acid and aminopterine, which form under UV-VIS exposure 6-formylpterine derivates and p-aminobenzylglutamic acid [7].

The combination of UV-VIS radiation and MTX as a possible new treatment for cancer was described in [1] and [8] as well as the application of UV laser radiation emitted at 337.1 nm by a nitrogen pulsed laser [9,10]. The examined MTX spectra showed that major modifications occur in the absorption and fluorescence characteristics of the MTX solution in natural saline following exposure and that MTX photodissociation starts if the irradiation fluence is greater than 0.56 J/cm$^2$; most probably, the appearance of the reaction products takes place at a low rate. These studies were coupled with tests about MTX photosensitizing properties measured on rabbit eyes conjunctive pseudotumors produced by the Schmidt-Erfurth method [11]. Comparative data were obtained on the evolution of neovascularisation of the conjunctive between untreated rabbit eyes and eyes impregnated with cytostatics at 5x10$^{-5}$ M in natural saline which are exposed to coherent or noncoherent light beams. The model simulates the tumor and the tests have shown that conjunctive and neovascularisations disappear after 1-2 treatments. 5-FU is a pyrimidine analog that belongs to the family of antimetabolites. It is weakly fluorescent, but it emits intense fluorescence after exposure to UV-VIS light. The UV-VIS absorption spectra of 5-FU did not show any structural modification after exposure to UV laser beams, but the FTIR spectra showed that the molecular geometry of 5-FU was changed after irradiation. The exposure to UV laser radiation transformed the 5-FU molecule into a tautomeric form. The increase of the fluorescence is attributed to a higher conversion to the keto-enol (lactim form) fluorescing tautomer due to monochromatic and high intensity laser radiation, which allows an effective and selective excitation. The radicals formed by the deprotonation of 5-FU could initiate the tumoricidal effect. The spectroscopic studies were coupled with preliminary tests on 5-FU photosensitizing properties measured on rabbit eyes conjunctive tissues [2]. Comparative data were obtained about the evolution of neovascularisations in the conjunctive tissue between untreated rabbit eyes and eyes impregnated with 5-FU 10$^{-4}$ M in natural saline and exposed to a UV-light beam emitted by a nitrogen pulsed laser. These experiments showed that the eye conjunctive inflammation and neovascularisation disappear faster after the treatment, as well.

The development of bacteria resistance to antibiotic treatment is considered a natural evolution process. The reversal of the resistance to antibiotics includes not only the treatment against pathogens which directly affect the patient's health but also the fight against the microbes developed in the hospital environment due to repetitive disinfection or against the resistant bacteria developed accidentally. The resistance to antibiotic treatment is also signaled as MDR at simultaneous treatment with different drugs.

An important number of drugs employed currently in medicine have their origins in the chemical processing of phenothiazines [12,13]. Chemical manipulation of a compound for the creation of new derivatives is limited by the existing science of organic chemistry, is time consuming and the percent yield of an active product is quite small. Exposure of a phenothiazine to UV - VIS yields a product that is more biologically active than the un-exposed parent [14,15].

***Corresponding author:** Mihail Lucian Pascu, National Institute for Laser, Plasma and Radiation Physics, Atomistilor 409, 077125 Magurele/Ilfov, Romania, Tel: 0040 21 4575739; Fax: 0040 21 4575739; E-mail: mihai.pascu@inflpr.ro

**Received** March 04, 2012; **Accepted** March 09, 2012; **Published** March 12, 2012

**Citation:** Pascu ML (2012) The Laser Use in Generating New Species of Medicines with Antitumor and Antibacterial Properties: New Processes in Drug Discovery. Biochem & Pharmacol 1:e111. doi:10.4172/2167-0501.1000e111

Lasers have been used in recent years for localized effects on tissues when the tissue and the phenothiazine are simultaneously irradiated [16]. The difference between exposures of a phenothiazine to a UV laser versus that to incoherent UV is that a laser emits radiation of a specific wavelength that may be as narrow as $10^{-3}$ nm, with an energy level at the given wavelength $10^{3}$-$10^{5}$ fold greater, at the same wavelength, than that produced by the UV incoherent source employed in biological studies [17]. We therefore considered the possibility that lasers could be used to rapidly modify molecules with prolongation of exposure to hours in order to produce reactions not possible with routine techniques used in organic chemistry.

A series of studies was started to evaluate the effect of a high energy laser beam on the structure of CPZ and correlate these with its bioactivity against bacteria. To standardize the method, more variables were controlled. Firstly, deionised water was used as solvent in order to minimize reactions that are solvent dependent. Secondly, we selected a 266 nm laser beam because the peak absorbance of CPZ is at 254-256 nm, and the modification of the CPZ was thereby assured. Thirdly, a 6.5 mJ energy level was chosen because it was the lowest energy level that would completely transform a minimum concentration of CPZ within a period of time of the order of minutes. Under these conditions the sequential development of products from exposure to the 266 nm could be studied. With prolonged exposure to the laser beam, at any given concentration of CPZ, the amounts of products identified via thin layer chromatography (TLC) increased. With no exceptions, all of the products formed were more polar than the control un-irradiated CPZ. Irradiation of the smallest concentration of CPZ (2 mg/mL) resulted in the complete conversion of CPZ into products that fluoresced under the 366 nm UV lamp whereas CPZ did not emit fluorescence. Increasing the concentration of CPZ exposed to the laser afforded the identification of TLC products that could not be detected with the smallest concentration of CPZ (2 mg/mL) . Prolongation of exposure increased the presence of these products so that by the end of 16 hrs, the major product was one that migrated behind CPZ in the TLC system. This product could be seen to decrease with further prolongation of exposure to 24 hrs and was identified as promazine. The examination of the 4 hrs TLC of the 2 mg/mL of CPZ shows that a product that migrates closely behind CPZ begins to appear concomitantly with the transformation (disappearance) of CPZ. This product emits fluorescence and is not seen with exposures of higher concentrations of CPZ nor with prolonged exposures of concentrations higher than 2 mg/mL. We interpreted these findings to suggest that the rapid formation of products from CPZ at its lowest concentration yields a radical that is short lived and slightly more polar than CPZ and that it may represent a species that contains the sulfoxide. Monitoring the pH of the 2 mg/mL of CPZ during exposure indicated a rapid drop of 3 to 4 pH units within 5 minutes that was maintained for the duration of the two hour exposure. This suggests that the protons were generated from the reaction that leads to sulfoxides and that because de-oxygenated water still gives rise to sulfoxides formed from exposure of CPZ to UV, the protons generated from exposure of CPZ to the laser have their source in water.

Our results indicated that exposure of 2 mg/mL of CPZ to a 266 nm laser yielded a product with greater biological activity against the test organism ATTC 25923 Staphylococcus aureus. Although the mechanism of action accounting for the increased antibacterial activity produced by laser exposure is not yet studied, phenothiazines such as CPZ have been shown to inhibit efflux pumps of bacteria [18] and of significant importance, promote the killing of intracellular mycobacteria [19-22].

Because phenothiazines that affect the viability of Staphylococcus aureus in general also affect that of Mtb and a number of important genes are similar in both species, one could anticipate that the irradiated CPZ would also have bioactivity against Mtb.

The evaluation of the products from exposure to the 266 nm laser beam by HPLC-DAD and HPLC-MS/MS indicated the presence of 4 major species produced. Their amounts increased with duration of exposure such that by the end of 24 hrs, the parental compound CPZ is no longer present and PZ, hydroxypromazine or PZ sulfoxide, hydroxypromazine sulfoxide and CPZ sulfoxide account for the major products present. However, around two hundred other products are present which due to inadequate amount of sample, could not at this time be identified. The identification of many, if not all of the products formed from prolonged irradiation with the 266 nm laser beam, may afford their detection in other systems that produce derivatives such as that enzymatic system present in the liver which can yield as many as 40 compounds when the patient is treated with a single dose of CPZ or that are not easily noted with pyrogenic systems in that their lives are short lived since unlike the irradiation process, the stopping of any pyrogenic based chemistry is difficult whereas that of the laser based method, the process is immediately stopped when the beam is removed. Lastly, it should be noted that the spectrophotometric and TLC properties of the products formed from laser exposure are stable for at least three months.

The mentioned results allow defining a new and very promising field that of the modification of medicines at molecular level in order to produce new compounds with possible applications in personalized biomedicine and technology.

## References

1. Pascu ML, Staicu A, Voicu L, Brezeanu M, Carstocea B, et al. (2004) Methotrexate as a photosensitiser. Anticancer Res 24: 2925-2930.
2. Pascu ML, Brezeanu M, Voicu L, Staicu A, Carstocea B, et al. (2005) 5-fluorouracil as a phosensitiser. *In Vivo* 19: 215-220.
3. Ochsner M (1997) Photophysical and photobiological processes in the photodynamic therapy of tumours. J Photochem Photobiol B 39: 1-18.
4. Pascu ML, Nastasa V, Smarandache A, Militaru A, Martins A, et al. (2011) Direct modification of bioactive phenothiazines by exposure to laser radiation. Recent Pat Antiinfect Drug Discov 6: 147-157.
5. Thomas AH, Suarez G, Cabrezio FM, Martino R, Capparelli AL (2000) Study of the photolysis of folic acid and 6-formylpterin in acid aqueous solutions. J Photochem Photobiol A: Chem 135: 147-154.
6. Thomas A, Einschlag FG, Feliz MR, Capparelli AL (2000) First steps in the photochemistry of the folate in alkaline medium. J Photochem Photobiol A: Chem 116: 187-154.
7. Robinson K, Pilot TF, Meany JE (1990) Time dependent inhibition of xanthine oxidase in irradiated solutions of folic acid, aminopterin and methotrexate. Physiol Chem Phys Med NMR 22: 95-103.
8. Staicu A, Pascu ML, Mogos I, Voicu L, Truica S, et al. (2001) Contribution to the spectroscopic study of the cytostatic molecules. Proc SPIE 4430: 680-687.
9. Pascu ML, Carstocea B, Staicu A, Truica S, Ionita M, et al. (2001) Spectroscopic studies of drugs in the treatment of malignant tumors in ophthalmology. Proc SPIE 4606: 52-58.
10. Pascu RA (2009) Studies on the efficiency of some medicines molecules used for ophthalmological treatment following exposure to optical radiation, PhD Thesis, UMF Carol Davila, Bucharest.
11. Schmidt-Erfurth U, Birngruber R, Hasan T (1996) Photodynamic therapy ocular vascular diseases. IEEE J Select topics Quantum Elect 2: 988-996.

12. Amaral L, Martins M, Viveiros M (2007) Phenothiazines as anti-multi-drug resistant tubercular agents. Infect Disord Drug Targets 7: 257-265.

13. Mosnaim AD, Ranade VV, Wolf ME, Puente J, Antonieta Valenzuela M (2006) Phenothiazine molecule provides the basic chemical structure for various classes of pharmacotherapeutic agents. Am J Ther 13: 261-273.

14. Wainwright M (2008) Photodynamic therapy: the development of new photosensitisers. Anticancer Agents Med Chem 8: 280-291.

15. Wainwright M, Amaral L (2005) The phenothiazinium chromophore and the evolution of antimalarial drugs. Trop Med Int Health 10: 501-511.

16. Ficheux H (2009) [Photodynamic therapy: principles and therapeutic indications]. Ann Pharm Fr 67: 32-40.

17. Danaila L, Pascu ML (2001) Lasers in Neurosurgery. Ed Acad Rom.

18. Amaral L, Martins A, Molnar J, Kristiansen JE, Martins M, et al. (2010) Phenothiazines, bacterial efflux pumps and targeting the macrophage for enhanced killing of intracellular XDRTB. *In Vivo* 24: 409-424.

19. Amaral L, Martins M, Viveiros M, Molnar J, Kristiansen JE (2008) Promising therapy of XDR-TB/MDR-TB with thioridazine an inhibitor of bacterial efflux pumps. Curr Drug Targets 9: 816-819.

20. Martins M, Viveiros M, Kristiansen JE, Molnar J, Amaral L (2007) The curative activity of thioridazine on mice infected with Mycobacterium tuberculosis. *In Vivo* 21: 771-775.

21. Ordway D, Viveiros M, Leandro C, Bettencourt R, Almeida J, et al. (2003) Clinical concentrations of thioridazine kill intracellular multidrug-resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother 47: 917-922.

22. Amaral L, Viveiros M, Kristiansen JE (2001) Phenothiazines: potential alternatives for the management of antibiotic resistant infections of tuberculosis and malaria in developing countries. Trop Med Int Health 6: 1016-1022.