ДОПОВІДІ
НАЦІОНАЛЬНОЇ
АКАДЕМІЇ НАУК
УКРАЇНИ

МЕДИЦИНА

doi: https://doi.org/10.15407/dopovidi2017.11.090
UDC 616.438 : 615.849.19 : 612.017.1

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The effect of laser (384 nm) irradiation of a Lewis lung carcinoma on the tumor volume and the formation of metastases

Presented by Corresponding Member of the NAS of Ukraine A.P. Soldatkin

We study the effect of laser irradiation upon implanted Lewis carcinoma. The irradiation of mice with Lewis carcinoma by a femtosecond pulsed laser with a wavelength of 384 nm was performed. Four groups of animals with different irradiation schemes were used in the experiments. The tumor volume and the number of lung metastases were detetermined. The activity of matrix metalloproteinases MMP-2 and MMP-9 in the primary tumor tissue was studied using zymography in polyacrylamide gel. The significant inhibition of the primary tumor growth and a decrease in the number of lung metastases were showed. A significant decrease of the MMP-9 level in the tumor was also observed. The most promising is the irradiation of mice with Lewis carcinoma on the 18th day after cells inoculation. There was a significant (more than 2-fold) reduction in the tumor volume, number of metastases in lungs, and MMP-9 level. Laser irradiation with a wavelength of 384 nm may be promising for the treatment of superficial tumors.

Keywords: *Lewis carcinoma, femtosecond laser with tunable wavelength, lung metastases, matrix metalloproteinases 2/9.*

Lasers are widely used in medicine, including the treatment of cancer. The important direction is the applying of low-intensity (low-level) laser technologies [1—3]. It is known that laser light with low power density affects the proliferation of stem cells, remodeling cardiomyocytes and endotelial cells [3, 4], is able to enhance the synthesis of collagen, and stimulates the expression of proinflammatory cytokines and apoptosis in irradiated cells [5, 6]. The aim of this work was to study the effect of low-intensity femtosecond laser radiation in the near ultraviolet spectral band on the rate of tumor growth in mice with transplanted Lewis lung carcinoma, the number of metastases, and the level of collagenases MMP-2 and MMP-9 in tumors, which play a key role in the metastasis formation.

Materials and methods. Experiments were carried out with female mice C57BL/6 bred in the vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine (40 animals weighing 17—18 g). Inoculation of Lewis lung carcinoma (3LL) — 500 thousand cells/mouse by intramuscular injection in the right thigh — was carried

out in conventional manner. We received the institutional Ethics Committee approval on carrying out the experiments with animals.

Irradiation was carried out using a femtosecond pulsed tunable laser complex ("Coherent", USA). Laser type — chameleon ultra II, with the second harmonic generator. Laser parameters in the experiment: wavelength — 384 nm; bandwidth — 2 nm; average power density per unit area — 10 mW/cm^2; pulse duration — 140 fs; pulse rate — 80 MHz; the diameter of a laser spot on the surface of the animal body — 1 cm; exposure time — 10 min. Before irradiation, the depilation of the right hind limb of mice with the transplanted tumors was performed. Before the procedure, mice were administered with the light anesthesia: thiopental sodium (1 mg/ml, 0.2 ml intraperitoneally).

In preliminary experiments, it was found that, due to the pulsed nature of the irradiation, the laser beam penetrates to a depth of 0.8 mm with a loss of approximately 70—80 % of the power.

Tumor volume was calculated in three orthogonal dimensions (a, b, c) according to the standard formula: $a \times b \times c \times \pi/6$.

To study the effect of laser irradiation on the activity of matrix metalloproteinases MMP-2 and MMP-9 in the primary tumor tissue, zymography in polyacrylamide gel was used [7].

The animals were sacrificed on the 25th day after the carcinoma cells inoculation, both lungs were placed into Bowen liquid (75 ml of a saturated picric acid solution, 25 ml of neutral 40% formalin, and 5 ml of glacial acetic acid) for the fixing and further counting of the number of metastases. The percentage of primary tumor growth inhibition was also calculated.

Statistical data processing was carried out by Student's test. *P* values <0.05 were considered as significant.

Table 1. **Effect of laser irradiation on tumor tissue volume and number of metastases in mice with Lewis carcinoma on the 25th day after transplantation**

Group (5 mice)	Tumor volume, %	The number of metastases in each group	Reduction in the number of metastases, %
Control	100	53	0
I	62.8 ± 13.98*	16	69.81
II	73.2 ± 17.39	15	71.70
III	53.9 ± 6.62*	5	90.57

Notes. Group I — one-time exposure on the 10th day after transplantation; II — double exposure on the 10th and 18th days; III — one-time exposure on the 18th day. * Difference from the control significant, $P < 0.05$; $M \pm m$ ($n = 5$).

Table 2. **Effect of laser irradiation on the activity of MMP-2/9 in tumor tissue of mice with Lewis carcinoma(25 days after transplantation)**

Laser exposure scheme	MMP-2, μg/g of the tissue	MMP-9, μg/g of the tissue
Irradiation on the 10th day after cells transplantation ($n = 4$)	11.5 ± 5.3*	6.3 ± 2.9
Irradiation on the 10th and 18th days after cells transplantation ($n = 3$)	6.0 ± 2.4	6.0 ± 2.7
Laser exposure on 18th day after cells transplantation ($n = 4$)	3.0 ± 3.8	4.6 ± 4.0*
Control ($n = 3$)	6.5 ± 0.7	9.4 ± 3.1

Notes: n — number of animals, * $P < 0.05$; $M \pm m$.

Results and discussion. All animals were distributed into groups of 5 mice each: Group I — single irradiation of the primary tumor on the 10th day after the Lewis lung carcinoma cells transplantation; Group II — double irradiation of the primary tumor on the 10th and 18th days after the inoculation; Group III — single irradiation of the primary tumor on the 18th day after the transplantation; Group IV — control (tumor-bearing mice without laser exposure) (Table 1).

It was found that all variants of laser irradiation resulted in the inhibition of primary tumor growth. The most significant reduction in the tumor volume occurred as a result of the single irradiation on the 18th day after the carcinoma cells inoculation (see Table 1). The number of lung metastases was also markedly decreased, particularly in the animals of Group III (>90 %).

Laser irradiation on the 10th day after the transplantation causes a considerable increase in the MMP-2 (matrix metalloproteinases) activity (Table 2). A significant decrease in the MMP-9 activity in the tumor (more than 2 times) on the 25th day after the inoculation was noted only in Group III (single irradiation on the 18th day).

The biochemical mechanisms underlying the effects of a laser on a tumor have not been studied sufficiently. The laser wavelength, 384 nm, coincides with the main lines of the absorption of magnesium and iron ions that can influence the activity of enzymes containing these metals in the active sites.

As it was shown, the irradiation with low-energy laser affected the collagen synthesis, the expression of MMPs and their tissue inhibitors — TIMP (tissue inhibitor of metalloproteinases) [8, 9] that may be associated with the formation of metastases. The effect of a laser on the proliferation of stem cells was described [3]. Moreover, irradiation of the thymus projection area with a laser at the 384-nm wavelength resulted in changes of the expression profile of cytokines and chemokines in the thymus gland, responsible for the maturation, proliferation, and migration of myeloid cells involved in the formation of innate immunity [5].

Conclusion. The most promising is the irradiation of mice with Lewis carcinoma on the 18th day after the cells inoculation. In such scheme of treatment, there is a significant (more than 2-fold) reduction in the tumor volume, number of metastases in lungs, and MMP-9 activity in animal tumor tissue, which may contribute to the suppression of metastasis. The described method of the laser irradiation with the specified parameters can be recommended for preclinical trials as an adjuvant at chemotherapy of superficial tumors.

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Received 03.07.2017

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ВПЛИВ ЛАЗЕРНОГО ОПРОМІНЕННЯ (384 НМ) КАРЦИНОМИ ЛЕГЕНІВ ЛЬЮЇС НА РОЗМІР ПУХЛИНИ І УТВОРЕННЯ МЕТАСТАЗІВ

Вивчено вплив лазерного опромінення на імплантовану карциному Льюїс. Мишей з карциномою Льюїс опромінювали фемтосекундним, імпульсним, перебудовуваним лазером з довжиною хвилі 384 нм. Визначали об'єм пухлини, кількість метастазів у легенях і рівень матриксних металопротеїназ ММР-2 і ММР-9 у тканині первинної пухлини. Показано значне пригнічення росту первинної пухлини і зменшення кількості метастазів у легенях. Також відзначено достовірне зниження рівня ММР-9 у пухлині. Встановлено, що найбільш перспективним є опромінення мишей з карциномою Льюїс на 18-й день після трансплантації пухлини. При цьому відбувається значне (більш ніж удвічі) зменшення розміру пухлини, кількості метастазів у легенях і рівня ММР-9.

***Ключові слова*:** *карцинома Льюїс, фемтосекундний лазер зі змінною частотою, метастази в легені, матриксні металопротеїнази 2/9.*

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ВЛИЯНИЕ ЛАЗЕРНОГО ОБЛУЧЕНИЯ (384 НМ) КАРЦИНОМЫ ЛЕГКИХ ЛЬЮИС НА ОБЪЕМ ОПУХОЛИ И ОБРАЗОВАНИЕ МЕТАСТАЗОВ

Изучено влияние лазерного облучения на имплантированную карциному Льюис. Мышей с карциномой Льюис облучали фемтосекундным, импульсным, перестраиваемым лазером с длиной волны 384 нм. Определяли объем опухоли, количество метастазов в легких и уровень матриксных металлопротеиназ ММР-2 и ММР-9 в ткани первичной опухоли. Показано значительное ингибирование роста первичной опухоли и уменьшение количества метастазов в легких. Также отмечено достоверное снижение уровня ММР-9 в опухоли. Установлено, что наиболее перспективным является облучение мышей с карциномой Льюис на 18-й день после трансплантации опухоли. При этом происходит значительное (более чем в 2 раза) уменьшение объема опухоли, количества метастазов в легких и уровня ММР-9.

***Ключевые слова*:** *карцинома Льюис, фемтосекундный лазер с перестраиваемой частотой, метастазы в легкие, матриксные металлопротеиназы 2/9.*