ВЛИЯНИЕ ПЛЮРОНИКА F127 НА ФОТОДИНАМИЧЕСКУЮ АКТИВНОСТЬ КРАСИТЕЛЯ МЕТИЛЕНОВОГО СИНЕГО В ОПЫТАХ *IN VITRO*

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Метиленовый синий – краситель фенотиазиновой группы. В последнее время активно исследуют его применение в качестве фотосенсибилизатора при фотодинамической терапии локальных инфекционных поражений. Фотофизические и фотохимические свойства метиленового синего дают основания полагать, что этот краситель может быть хорошим фотосенсибилизатором и для фотодинамической терапии опухолей.

Задачей нашего исследования было определить темновую цитотоксичность метиленового синего, его фотодинамическую активность и возможность усилить последнюю с помощью полоксамера плюроник F127.

Работа выполнена *in vitro* на Т-клеточной линии злокачественно трансформированных лимфоцитов человека Jurkat. Облучение клеток осуществляли полупроводниковым лазером производства ЧМПП «Фотоника-Плюс» (г. Черкассы) с длиной волны излучения 658 нм. Темновую цитотоксичность метиленового синего определяли методом последовательных разведений. Гибель клеток определяли тестом исключения трипанового синего.

Установлено, что концентрации красителя 0,5-5 мкг/мл не являются токсичными для клеток линии Jurkat. Метиленовый синий в концентрациях 10 мкг/мл и 50 мкг/мл вызывает гибель соответственно 20% и 100% клеток.

При исследовании фотодинамической активности метиленового синего были применены два режима облучения клеток (плотность мощности 10 мВт/см^2, дозы – 12 Дж/см^2; или 20 мВт/см^2, 24 Дж/см^2) и краситель в нетоксичных концентрациях (0,5-5 мкг/мл). В результате показано, что уровень смертности облученных клеток зависит как от концентрации фотосенсибилизатора, так и от дозы облучения. Гибель 50% клеток наблюдалась как при концентрации метиленового синего 2 мкг/мл и дозе облучения 24 Дж/см^2, так и при концентрации красителя 4 мкг/мл и дозе облучения 12 Дж/см^2. Отметим, что гибель 15% облученных клеток была зарегистрирована уже при концентрации метиленового синего 0,5 мкг/мл и дозе облучения 12 Дж/см^2.

Были проведены опыты с целью выяснить, влияет ли на фотодинамическую активность метиленового синего его комплексирование с полоксамером плюроник F127. Полоксамеры – это амфифильные триблоковые полимеры, молекула которых состоит из двух гидрофильных полиэтиленоксидных блоков и одного гидрофобного полипропиленоксидного блока.

Благодаря такой структуре плюроник F127 присоединяет к себе гидрофобные, гидрофильные и амфифильные молекулы, и, встраиваясь в поверхностную мембрану клеток (преимущественно опухолевых – благодаря наличию в их мембране липидов низкой плотности), переносит в клетку молекулы, которые к нему присоединились, в том числе и фотосенсибилизаторы.

Нами показано, что композит метиленового синего с плюроником F127 при молярном соотношении краситель:плюроник F127 = 2,5:1 обладает в два раза более высокой фотодинамической активностью по сравнению со свободным метиленовым синим в той же концентрации. Данный эффект сохраняется после изменения концентрации фотосенсибилизатора при условии соблюдения соотношения компонентов композита.

Таким образом, применение плюроника F127 может быть перспективным для повышения эффективности фотодинамической терапии опухолей.

USING OF OPTIC-ELECTRONIC SYSTEM FOR ANALYSIS OF THE VASCULAR TONE AND CHARACTER OF THE LOCAL BLOOD FLOW TO ASSESS THE VIABILITY OF THE BODY

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Introduction**.** Assessment of the viability of a patient's body part is often presented as a complex practical problem of clinical surgery. Especially when there are no obvious signs of necrosis, which is important when jamming bowel loops, postoperative wounds and critical lower limb ischemia [1]. In practice, a survey of diseased organ carried out by evaluation of its appearance, tactile definition of regional temperature and pulsation of the arteries or evaluation of response to external stimuli. During research are studying of microhemodynamics by laser Doppler flowmetry, transcutaneous oximetry and other methods are using with stress tests, including a test of reactive hyperemia [1, 2]. These methods image the state of compensatory mechanisms circulation of the lower limbs. However, it is insufficient attention is paid to the investigation of vascular tone and character of the local flow as prognostic keeping organ factors.

The aim of this work is to examine the ability of evaluation of vascular tone and character of the local blood flow to determine the viability of the organ and the prospects for its keeping on the example of the foot.

Materials and methods**.** Stand investigations were carried out on the layout, which included tubes with hard and soft walls. Sodium chloride solution 0,9% and diluted canned erythrocytic mass of sodium chloride 0,9% at a ratio of

1:1 were passed through the tubes in continuous and discontinuous (pulsing) mode at the same pressure and rate (60 ml/min). A photoplethysmographic sensor has been set on the surface of the tubes with different properties.

Subsequent Recognition**.** There were examined 108 patients aged 18 to 82 years in total. They were divided into 4 groups: I - 31 persons without evidence of ischemia of the lower limbs, II - 27 patients with 2nd stage of ischemia, III - 29 patients with 3rd stage ischemia, IV - 21 patients with 4th stage ischemia. In the group III were selected two subgroups: IIIA - no swelling of the foot (11 patients) and IIIB - swollen foot (18 patients). Physical examination, ultrasound scan, arteriography and determining of the level of regional systolic blood pressure were conducted.

Discussion of results**.** In bench researches, when it was continuous flow of liquid in tubes with solid and soft walls, low-amplitude, irregular, chaotic, approximate to the background, signal was registered. It form was like a signal obtained in patients with 4th stage of ischemia. When it was the pulsatile liquid flow, regular intermittent signal was obtained from the surface of tubes with soft walls. It shape was as shape of the signal with the absence of pathology of major arteries. And on the surface of the tubes with solid walls the same regular signal was obtained, but it had smaller amplitude.

The patients of group I without signs of ischemia and pathology of the main arteries of the lower limbs at all levels were registered with a high-amplitude regular intermittent (30 people) or low-amplitude (1 person) signal. The high-amplitude, regular signal was registered in the projection of the arteries, when were determined their pulsation using palpation. The amplitude of the signal at the first toe accounted for 0,81±0,08 of the amplitude of the signal thumb. 3 patients had swelling and some structural features on the ankle area thus palpable pulsation in the artery and posterior tibial artery rear foot not determined. After LPPH blood flow of foot was defined as high-amplitude, regular signal, and after ultrasound scan was defined the main flow.

In patients with 2nd stage of ischemia has been dominated (92,6%) peer occlusion. Ultrasound scanning in the main arteries below the occlusion showed collateral compensated or main modified blood flow. After LPPH, in 19 patients was recorded the intermittent, high-amplitude signal, in 8 patients – low-amplitude signal. The amplitude of the signal at the first toe was 0,26±0,06 and the amplitude of the signal thumb (P<0,05). During the reactive hyperemia the rapid (within 21,8±1,9 p) reaction was noted with increasing amplitude of the signal at 34,7±3,9%. In 4 patients pulsation in popliteal, posterior tibial artery and the artery rear foot not determined palpable. After LPPH blood flow of studied arteries was defined as low-amplitude, regular signal, and after ultrasound scan was defined compensated collateral flow.

Among patients with 3rd stage of ischemia predominated multi-occlusion (79,3%). Ultrasound scanning of the main arteries below the level of the occlusion showed decompensated collateral blood flow. After LPPH 5 patients of IIIA subgroup was detected low-amplitude regular intermittent signal, 6 – low-amplitude irregular, chaotic signal. The amplitude of the signal at the first toe was 0,12±0,04 of the amplitude of the signal thumb (P<0,05). The reactive hyperemia test showed the slow (over 68,7±1,6 p) reaction with increasing amplitude of the signal at 16,7±4,3%. Among patients of IIIB subgroup 4 was recorded low-amplitude regular intermittent signal, 14 – low-amplitude irregular, chaotic signal. At the shin was recorded low-amplitude regular intermittent signal with a ratio shin / forearm 0,23±0,07. After the reactive hyperemia test noted slaw (85,4±2,1 p) reaction with increasing amplitude of the signal at 13,4±2,3%.

During 12 months in the cases of registration of high-amplitude regular intermittent signal, disease was stable and critical ischemia was not developed. Among patients, who was detected low-amplitude regular intermittent signal (17 persons), it was occurred need for amputation in 2 patients who had practically no reaction on the reactive hyperemia test. After detecting low-amplitude irregular chaotic signal (41 patients) the need for amputation occurred in 36 cases.

During the reactive hyperemia test significantly increases the rate of blood flow, to which the endothelial cells are very sensitive, that significantly increase the production of nitric oxide [3]. In patients with preserved reserve of collateral circulation recorded a strong reaction. With the progression of arterial disease and decompensation of regional circulation on the basis of endothelial dysfunction, reaction progressively weakens and disappears. The ability to increase the amplitude of the signal and appearance of the pulsatile flow regarded as a feature of preservation of functional reserve and relatively good prognostic sign.

The ability to estimate the pulsatile is the most valuable property of LPPH. Even significantly reduced pulsatile is evidence of sufficient supply of tissues at the resting state and provides perspectives for healing surgical wounds, including amputations.

Conclusion**.** As a result of studies identified that low-amplitude irregular, chaotic signal, corresponding to the flow nonpulsatile signal, is the predictor of critical limb ischemia, and organ could be lost for 12 months. A limb segment below the level of losing pulsatile character of blood flow is wasted for it saving.

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