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THE INHIBITING EFFECT OF FS-1 DRUG ON THE ANTIOXIDANT PROTECTION SYSTEM OF MYCOBACTERIA TUBERCULOSIS

Abstract. Results of inhibitory action of FS-1 drug on antioxidant system of pathogenic mycobacteria tuberculosis, including resistant MDR strain, are presented. The study of the effect of FS-1 drug on the activity of the antioxidant system was carried out on the reference strain *Mycobacterium tuberculosis* H37Rv and MDR (rifampicin, isoniazid, streptomycin, ethambutol, ethionamide, kanamycin, cycloserine and pyrazinamide resistant) strain *Mycobacterium tuberculosis* 320. FS-1 drug under experimental conditions *in vitro* showed a new mechanism of action on mycobacteria tuberculosis - suppression of functional activity of the enzyme superoxide dismutase, which protects the microorganism from oxidative stress. The loss of resistance to oxidative stress by a bacterial cell, i.e. the ability to neutralize highly toxic oxygen radicals, leads to the destruction of cellular structures, metabolic and energy processes, disruption of the respiratory system and, as a result, its death. Antioxidant activity of *Mycobacterium tuberculosis* H37Rv after exposure with FS-1 preparation at concentrations of 4μg/ml is inhibited by 90.64 %, while at concentration of 2 μg/ml on bacterial culture of this strain - by 89.07 %. The obtained results show significant suppression of functional activity of superoxide dismutase enzyme in bacterial culture of *Mycobacterium tuberculosis* H37Rv under the influence of FS-1 in these concentrations, showing pronounced inhibitory effect. Similar studies of the effect of iodine-containing FS-1 drug on the antioxidant system were carried out on the bacterial culture of *M. tuberculosis* multidrug resistant strain 320. It was found that antioxidant activity of FS-1 preparation in concentration 4 μg/ml is inhibited by 99 %, while in concentrations 2 μg/ml FS-1preparation suppresses antioxidant activity of strain 320 by 98 %.

Thus, the studies showed that the FS-1 preparation at the test concentrations of 4 μg/ml and 2 μg/ml has a mechanism for pronounced inhibition of the functional activity of the enzyme superoxide dismutase in *Mycobacterium tuberculosis* of both the reference sensitive strain H37Rv and the multidrug resistant strain 320. This leads to disruption of the redox transformations of various chemical compounds that form the respiratory process in the bacterial culture, providing the energy demand of the microorganism.

Key words. Iodine-containing FS-1 drug, mycobacterium tuberculosis, enzymes of the antioxidant system of bacterial cells, superoxide dismutase (SOD), UV spectrometry.

Introduction. Tuberculosis is one of the most common infectious diseases that occurs in all countries of the world. The causative agent of tuberculosis is the bacterium of a closely related complex (MTBC) *Mycobacterium tuberculosis*, which most often affects the lungs and is prone to genetic changes with the development of new forms. One of the reasons for the decrease in the effectiveness of treatment is the increased level of spread of the multidrug-resistant (MDR) infectious agent to anti-tuberculosis drugs.

Currently, an important role in the spread of tuberculosis is played not only by the pathogen drug resistance, but also by defense mechanisms factors of both immune system of macroorganism and of the bacterial cell itself [1-3]. Over recent years, according to the literature, the search for intracellular targets has expanded to create new anti-infective drugs [4-8]. Thus, there is evidence that antibiotics can cause an increase in the production of ROS (reactive oxygen species), which leads to oxidative stress. In the works of Kohanski M.A. et. al.; Kohanski M. A., Dwyer et. al.; Belenky, P. et al. [5,6,9] oxidative stress in the bacterial cell itself, as exemplified in *E. coli*, is considered to be one of the components of the antimicrobial activity of antibiotics leading to their death. Oxidative stress is a condition of cells characterized by an excess content of free oxygen radicals. Kurbanov A.I., Zenkov N.K. et al. [7,8] in their works state that the course of disease and the nature of treatment of many infections are influenced by free radical oxidation processes. Free radicals are produced as a result of respiratory function and the use of oxygen received by cells for energy production. Molecular oxygen is the main source of free radicals in the body. Oxygen is not an essential component of metabolic processes in the body.

Superoxide is the active form of oxygen is. Its change is catalyzed by the antioxidant enzyme superoxide dismutase (SOD), which is produced during aerobic respiration, a chemical reaction, and transfers energy to cells. SOD catalyzes dismutation of O_2 - radicals and prevents transformation of the superoxide radical anion into the OH hydroxyl radical, which is highly toxic. Hence, SOD is a key enzyme that directly ensures termination of free radical reactions chains in the cells of aerobic organisms [10-12]. This enzyme accelerates biochemical process in the cell from constantly generated highly toxic oxygen radicals and always "works" in tandem with catalase, which quickly and efficiently breaks down hydrogen peroxide into neutral compounds.

Thus, enzyme superoxide dismutase, which is considered to be a universal mechanism of pathogenesis in infections, plays an important part in the antioxidase defense system of the microbe organism against oxidative stress. This prompted us to study the effect of FS-1 drug developed at our Scientific Center for Anti-Infectious Drugs JSC [13,18] on the activity of SOD in mycobacterium tuberculosis.

Initial research carried out by us in experiments on *M. smegmatis* saprophyte culture selected a method for measuring SOD activity and established the ability of FS-1 drug to suppress SOD activity [14]. This work presents the results of studying the effect of FS-1 drug on the activity of antioxidant system of mycobacterium tuberculosis as a reference sensitive strain and a highly pathogenic multidrug-resistant strain of this pathogen type.

Materials and Methods. The study of the effect of the FS-1 drug on the activity of antioxidant system was carried out on the reference strain *Mycobacterium tuberculosis* $H_{37}Rv$, as well as MDR strain *Mycobacterium tuberculosis 320*, resistant to rifampicin, isoniazid, streptomycin, ethambutol and pyrazinamide [15].

The investigated concentrations of the FS-1 drug of 2 μg/ml and 4 μg/ml proceeded from the MBC of the taken mycobacterium tuberculosis cultures. The suspension of the studied *M. tuberculosis* cultures was prepared at a concentration of 1.5x108 CFU/ml in sterile saline. Tests of samples of different mycobacterium tuberculosis strains were carried out simultaneously on the same day under the same settings.

Determination of the effect of the test substance on the antioxidant system of bacteria was carried out by adrenaline autooxidation method *in vitro* [16]. We used 0,1% solution of adrenaline hydrochloride; 0.2 M bicarbonate buffer solution (pH = 10.65).

For the research, 2 ml of bicarbonate buffer was poured into test tubes, a 2 ml suspension of the test culture of Mycobacterium tuberculosis at a concentration of 1.5x108 CFU/ml prepared in physiological saline was added, the investigated concentrations of the FS-1 drug were also added, then 0.2 ml 0, 1% solution of adrenaline hydrochloride. The test tubes were incubated for 15 min at room temperature, then the supernatant was separated by centrifugal settling at 5000 rpm for 5 min.

The control was a sample without the addition of test substances, i.e. containing 2 ml of bicarbonate buffer, 2 ml of saline and 0.2 ml of 0.1% adrenaline hydrochloride solution.

The optical density of the test samples of the supernatant was measured every minute for 30 min (30 cycles) in the spectral range from 200 to 500nm on a Lambda 35 double-beam spectrophotometer (Perkin-Elmer, USA). The operation principle of this device is based on measuring the ratio of two light fluxes that had passed through the reference channel (blank - 2ml bicarbotate buffer, 2ml saline solution) and the sample channel in the cell holder, which allows cutting off baseline values.

The degree of impact of the test substance on the antioxidant system of bacteria was calculated using the formula described in the method [16]:

inhibition % (units) = [1 - ($OD_{control}$/$OD_{experiment}$)] x 100% (1) where $OD_{control}$ is the mean value (n = 30) of control sample optical density; $OD_{experiment}$ is the mean value (n = 30) of the optical density of the test sample.

The results of measuring the kinetics of adrenaline autooxidation process in an alkaline medium and in the presence of the investigated concentrations of the FS-1 drug are presented as mean values (n=30) from 2 independent experiments.

According to the method, values above 30% were taken as a significant suppression of activity of the bacterial antioxidant system exposed to the test substance.

Processing and visualization of experimental data was carried out using the Origin package. URSS [17].

Obtained Results. The effect of iodine-containing FS-1 drug on the antioxidant system of *M. tuberculosis* bacterial cell of the H_{37}Rv reference strain and multidrug resistant strain 320 was studied by adrenaline autooxidation method *in vitro*. Figure 1 shows the results of a spectral study of a control sample (0.1% adrenaline solution) in the range from 200 to 500 nm. Three absorption maxima were found at 242, 292, and 347 nm.

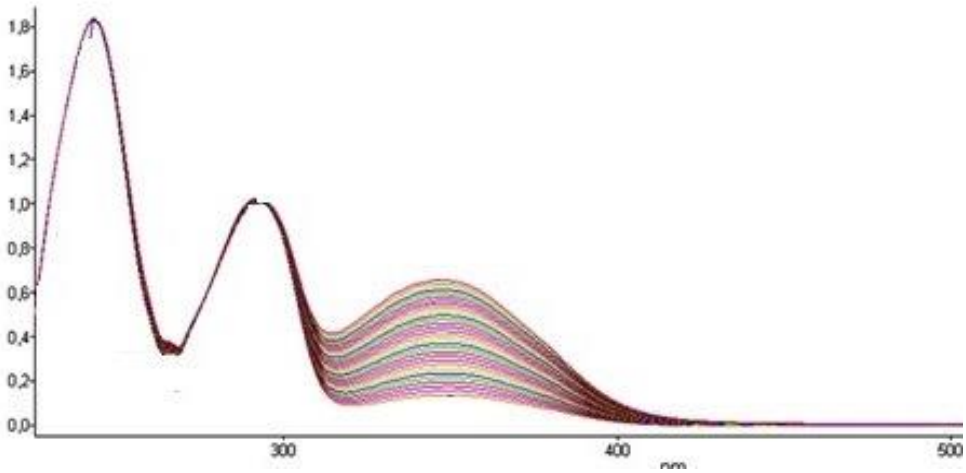

Figure 1 - UV absorption spectrum of the control sample

It should be noted that when measuring an aqueous adrenaline solution with pH=7.0, its maximum absorption at a wavelength of 280 nm was established. When measuring the kinetics of the autooxidation of adrenaline in a bicarbonate buffer pH=10.65, the maximum absorption in the UV region was determined at a wavelength of 292 nm. Observation of the entire spectrum at a length of 347 nm for 30 min showed the dynamics of spectral changes increasing in direct proportion to the measurement time. The increase in the optical density of the primary adrenaline oxidation product accumulation was 0.52 op. units/min. When measured immediately, optical density was 0.139A, and after 30 minutes, optical density was 0.658A. However, the optical density in the spectrum typical of adrenaline in an alkaline medium - 292 nm decreased only by 0.01A within 30 minutes (from 1.03A to 1.02A).

Figure 2 shows the results of UV spectroscopy of an experimental sample of *M. tuberculosis* H_{37}Rv supernatant after exposure to FS-1 at a concentration of 2 μg/ml. Three absorption maxima were found at the spectra of 242 nm, 292 nm, and 347 nm.

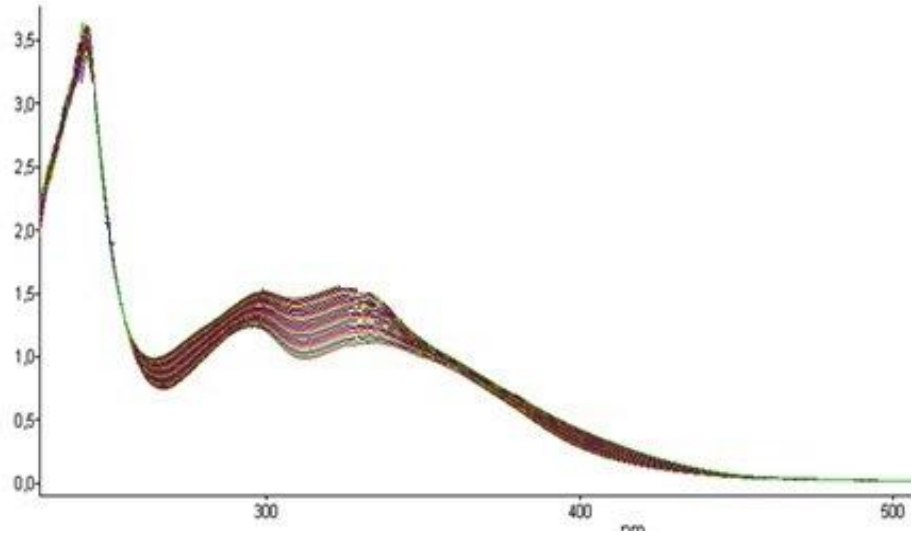

Figure 2 - UV absorption spectrum of a test specimen of *M. tuberculosis* $H_{37}Rv$ supernatanta after exposure to FS-1 at a concentration of 2 μg/ml

The increase in the optical density of the adrenaline autooxidation primary product accumulation at a spectrum of 292 nm exposed to FS-1 drug at a concentration of 2 μg/ml on *M. tuberculosis* $H_{37}Rv$ culture was 0.28 op.u/min. In immediate measurement, the optical density was 1.24A, and after 30 minutes it was 1.52A.

In immediate measurement in the spectrum of 347 nm, the optical density of the test sample after exposure to FS-1 drug on the cell culture of *M. tuberculosis* $H_{37}Rv$ was 1.03A, and after 30 minutes it increased by 0.16A and amounted to 1.19A.

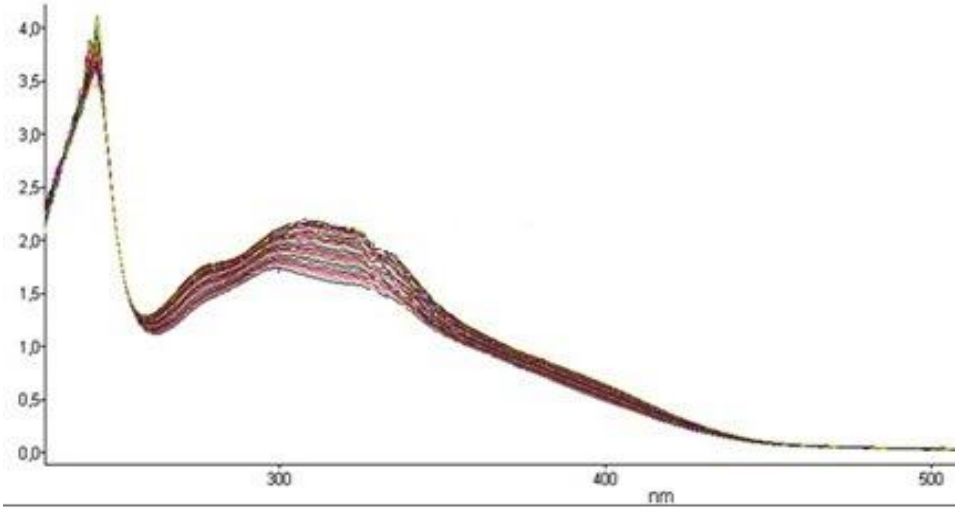

Figure 3 - UV absorption spectrum of a test specimen of M. tuberculosis $H_{37}Rv$ supernatanta after exposure to FS-1 in concentration of 4 μg/ml

Figure 3 shows the UV absorption spectrum of a test sample of *M. tuberculosis* $H_{37}Rv$ supernatant after exposure to FS-1 at a concentration of 4 μg/ml.

Increase in the optical density of adrenaline autooxidation primary product accumulation at a spectrum of 292 nm when exposed to FS-1 drug at a concentration of 4 μg/ml on *M. tuberculosis* $H_{37}Rv$

cell culture averaged 0.46 op.u/min. When measured immediately, the optical density was 1.74A, and after 30 minutes it was 2.20A.

The optical density of the test sample after exposure to FS-1 drug at a concentration of 4 μg/ml on *M. tuberculosis* $H_{37}Rv$ cell culture with immediate measurement in the spectrum of 347 nm was 1.18A, and after 30 minutes it increased by 0.28A and amounted to 1.46A.

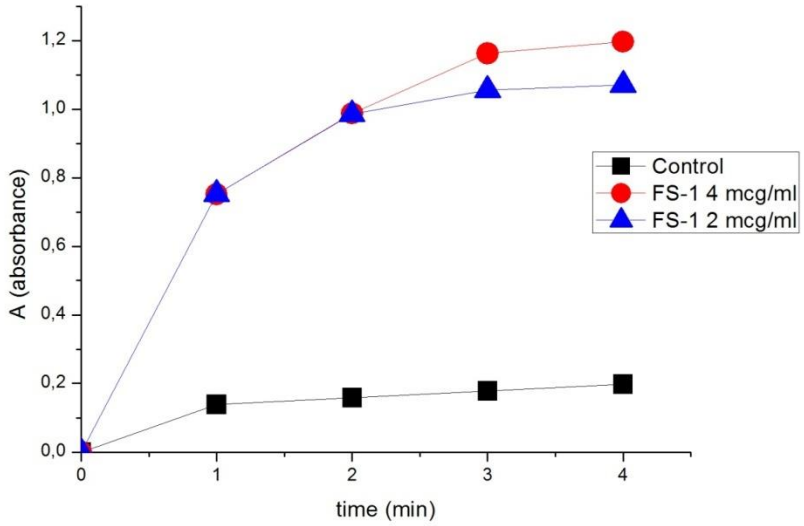

Figure 4 - Dynamics of changes in the optical density of the control and experimental samples supernatanta of *M. tuberculosis* $H_{37}Rv$ at a wavelength of 347 nm

Figure 4 shows the summary data of UV spectroscopy with the dynamics of changes in the optical density of the supernatant of experimental samples of *M. tuberculosis* $H_{37}Rv$ after exposure to FS-1 at concentrations of 2 μg/ml and 4 μg/ml at a wavelength of 347 nm for 30 minutes versus control sample without adding the test substance. Observation of the entire spectrum at a length of 347 nm for 30 min showed spectral changes dynamics in the optical density of the culture liquid of *M. tuberculosis* $H_{37}Rv$ exposed to FS-1 drug in the tested doses. Figure 4 shows a graph constructed using the OriginPro software (17). As can be seen from Figure 4, the addition of FS-1 drug to the test samples at concentrations of 4 and 2 μg/ml with *M. tuberculosis* $H_{37}Rv$ culture versus control sample (without the FS-1 drug) 10.7 ($p<0.0001$) and 9.1 ($p<0.0001$) times, increase accumulation of the toxic product of adrenaline autooxidation in the supernatant.

The degree of impact of FS-1 drug at concentrations of 2 μg/ml and 4 μg/ml on the antioxidant system of bacteria, calculated according to the selected research methodology, showed an inhibitory antioxidant activity of the effect on pathogenic mycobacterium tuberculosis. It was found that the antioxidant activity of mycobacterium tuberculosis $H_{37}Rv$ strain after exposure to FS-1 at a concentration of 4 μg/ml is inhibited by 90.64%. This indicates a significant suppression of the functional activity of the superoxide dismutase enzyme in the Mycobacterium tuberculosis bacterial culture exposed to FS-1 drug at a given concentration. The degree of impact of FS-1 drug at a concentration of 2 μg/ml on the bacterial culture of this strain was also determined. It was found that the drug inhibits antioxidant activity by 89.07%. The obtained results indicate a significant suppression of the functional activity of the superoxide dismutase enzyme in the bacterial culture of Mycobacterium tuberculosis $H_{37}Rv$ strain exposed to FS-1 at these concentrations, demonstrating a pronounced inhibitory effect.

We have also simultaneously carried out similar studies of the effect of iodine-containing FS-1 drug on the antioxidant system of *M. tuberculosis* bacterial cell of multidrug-resistant strain 320 by adrenaline autooxidation method *in vitro*.

Figures 5-7 show the spectral studies data of the supernatant of the test samples after exposure to FS-1 drug on MDR *M. tuberculosis* strain No. 320 in the range from 200 to 500 nm. There is also a shift in wavelength observed from 242 nm to 292 nm and up to 347 nm.

In the studied ranges, the time-dependent dynamics of spectral changes are also shown. Figure 5 shows the data of spectral absorption of the experimental sample of the supernatant of *M. tuberculosis* strain No. 320 after exposure to FS-1 drug at a concentration of 4 μg/ml. Three absorption maxima were revealed at the spectra of 242 nm, 292 nm, and 347 nm.

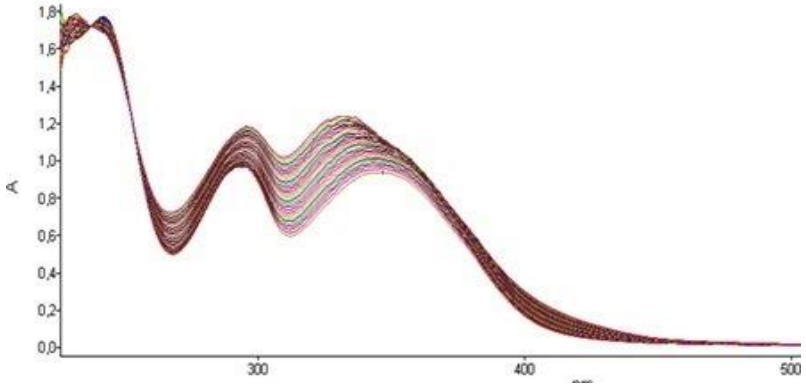

Figure 5 – UV absorption spectrum of a test specimen of supernatant of *M. tuberculosis* strain 320 after exposure to FS-1 in concentration of 4 μg/ml

The increase in the optical density of adrenaline autooxidation primary product accumulation (292 nm) under the impact of FS-1 drug at a concentration of 4 μg/ml in the culture of MDR *M. tuberculosis* strain 320 was 0.11 op.u/min, with immediate measurement the optical density was 1,70A and after 30 minutes it amounted to 1.81A. The optical density of the supernatant of the test sample after exposure to FS-1 drug with immediate measurement in the spectrum of 347 nm was 0.84A, and after 30 minutes - 1.02A increased by 0.18A.

Figure 6 shows the data of spectral absorption of the experimental sample supernatant of the test culture of MDR *Mycobacterium tuberculosis* strain 320 after exposure to FS-1 drug at a concentration of 2 μg/ml. Three absorption maxima were revealed at the spectra of 242 nm, 292 nm, and 347 nm.

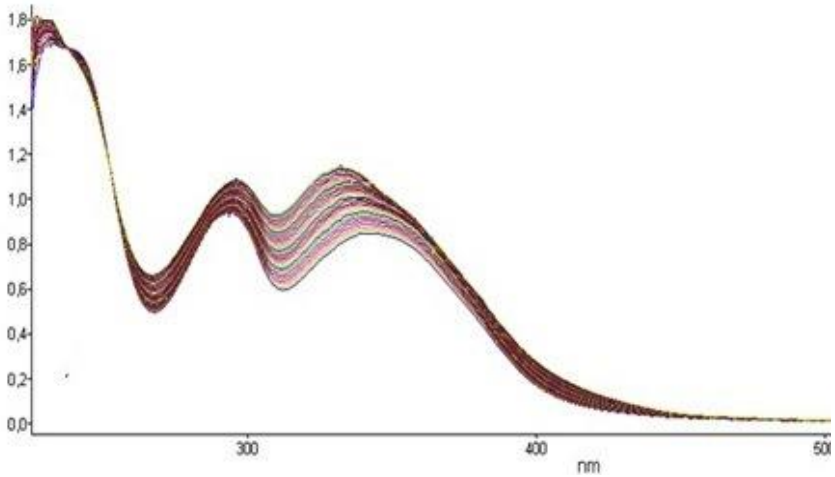

Figure 6 – UV absorption spectrum of a test specimen of supernatant of the MDR *M. tuberculosis* after exposure to FS-1 in concentration of 2 μg/ml

The increase in optical density with the accumulation of the primary adrenaline autooxidation product in the spectrum of 292 nm when exposed to FS-1 drug at a concentration of 2 μg/ml in the MDR *M. tuberculosis* strain 320 of was 0.21 op.u/min, with immediate measurement the optical density was

0.97A, and after 30 minutes it amounted to 1.18A. The optical density of the supernatant after exposure with immediate measurement in the spectrum of 347 nm was 0.94A, and after 30 minutes - 1.11A, which shows an increase of 0.17A.

Figure 7 shows the summary data of UV spectroscopy with the dynamics of changes in the optical density of the control and experimental samples of the supernatant of the MDR of strain 320 of *M. tuberculosis* after exposure to FS-1 on bacterial cells at concentrations of 4 μg/ml and 2 μg/ml at a wavelength of 347 nm within 30 minutes.

As can be seen from Figure 7, the addition of FS-1 drug at concentrations of 4 and 2 μg/ml into experimental samples of the culture liquid of the MDR of strain 320 *M. tuberculosis* in comparison with the control sample without the drug, by 9.3 times ($p<0.0001$) and 8.5 times ($p<0.0001$), respectively, increases accumulation of the adrenaline autooxidation toxic product in the studied culture liquid.

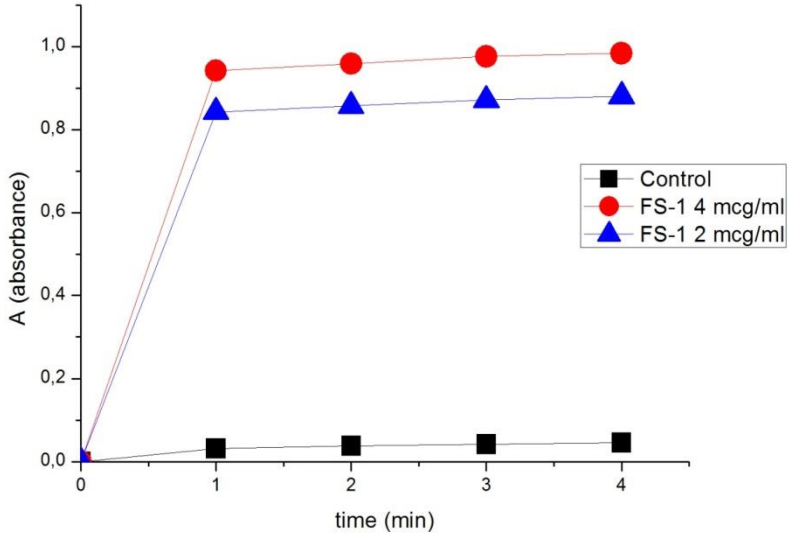

Figure 7 - Dynamics of changes in the optical density of the control and Experimental samples supernatanta of the MDR *M. tuberculosis* at a wavelength of 347 nm

The degree of impact of FS-1 drug on the antioxidant system of bacteria in the studied concentrations was calculated using formula (1) according to the procedure. It was found that the antioxidant activity of FS-1 at a concentration of 4 μg/ml is inhibited by 99%. This indicates a significant suppression of the functional activity of the superoxide dismutase enzyme in the bacterial culture of mycobacterium tuberculosis MDR strain 320 when exposed to FS-1 drug at a given concentration.

FS-1 drug at a concentration of 2 μg/ml inhibits antioxidant activity of the bacterial culture of strain 320 by 98%.

Thus, the conducted studies have shown that FS-1 drug in the studied concentrations has a mechanism for suppressing functional activity of the superoxide dismutase enzyme in bacterial cultures of Mycobacterium tuberculosis, both the reference sensitive strain and the multidrug-resistant strain 320.

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ФС- 1 ДӘРІЛІК ЗАТЫ МИКОБАКТЕРИЯ ТУБЕРКУЛЕЗІНІҢ АНТИОКСИДАТТЫ ҚОРҒАНЫС ЖҮЙЕСІНЕ ИНГИБИРЛЕУШІ ӘСЕРІ

Аннотация. ФС-1 дәрілік затының туберкулездің патогенді микобактерияларының антиоксидантты жүйесіне, оның ішінде көптеген резистентті дәрінің төзімділік штамына тежеуші әсерінің нәтижелері ұсынылған. *Mycobacterium tuberculosis* $H_{37}Rv$ эталондық штамында және *Mycobacterium tuberculosis* 320 штамында(рифампицинге, изониазидке, стрептомицинге, этамбутолға, пиразинамидке, этионамидке, ПАСК, канамицинге және циклосеринге) жүргізілетін антиоксидантты жүйенің белсенділігіне ФС-1 дәрілік затының ену жолдарын зерттеу. ФС-1 дәрілік заты in vitro эксперименттік жағдайында туберкулез микобактерияларына әсер етудің жаңа механизмін, яғни микроағзаны тотығу күйзелісінен қорғайтын супероксиддисмутаза ферментінің функционалдық белсенділігін басуды көрсетті. Бактериялық жасушаның тотығу стресіне төзімділігін жоғалтуы, яғни жоғары уытты оттегі радикалын бейтараптандыру қабілеті жасушалық құрылымды, метаболикалық және энергетикалық процестер мен тыныс алу жүйесін бұзады және нәтижесінде өлім тудырады. ФС-1 препаратымен 4 мкг/мл концентрациясында әсер еткеннен кейін $H_{37}Rv$ штамы туберкулез микобактерияларының антиоксидантты белсенділігі 90,64%-ға, ал осы штамның бактериялық культурасына 2 мкг/мл концентрациясында 89,07%-ға тежелетіні анықталды. Алынған нәтижелер айқын ингибиторлық әсерін байқатып, концентрацияда ФС-1 әсерінен $H_{37}Rv$ штамы туберкулез микобактерияларының бактериялық культурасындағы супероксиддисмутаза ферментінің функционалдық белсенділігінің айтарлықтай басылғанын көрсетеді. Құрамында иоды бар ФС-1 дәрілік затының антиоксидантты жүйеге әсерін ұқсас зерттеулер *M.tuberculosis* көп дәріге төзімді штамм 320 бактериялық культурасына жүргізілді. Бұл ретте 4 мкг/мл концентрациядағы ФС-1 препаратының антиоксиданттық белсенділігі 99%-ға тежелетіні анықталды, ал 2 мкг/мл концентрациясында ФС-1 препараты 320 штамының антиоксиданттық белсенділігін 98%-ға бәсеңдетеді.

Осылайша жүргізілген зерттеулер 4 мкг/мл және 2 мкг/мл зерттелетін концентрациядағы ФС-1 препаратының н37rv референттік сезімтал штамының да, көп дәріге төзімді 320 штамының да туберкулез микобактериясында супероксиддисмутаза ферментінің функционалдық белсенділігін айқын тежеу тетігіне ие екендігін көрсетті. Бұл микроорганизмнің энергетикалық қажеттілігін қамтамасыз ететін бактериялық культурада тыныс алу процесін құрайтын түрлі химиялық қосылыстардың тотығу-тотықсыздану өзгерістерін бұзады.

Түйін сөздер: құрамында йод бар ФС-1 препараты, туберкулез микобактериялары, бактерия жасушасының антиоксидантты жүйе ферменттері, супероксиддисмутаза (СОД), УК-спектрометрия

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ИНГИБИРУЮЩЕЕ ДЕЙСТВИЕ ЛЕКАРСТВЕННОГО СРЕДСТВА ФС-1 НА СИСТЕМУ АНТИОКСИДАНТНОЙ ЗАЩИТЫ МИКОБАКТЕРИЙ ТУБЕРКУЛЕЗА

Аннотация. Представлены результаты ингибирующего действия лекарственного средства ФС-1 на антиоксидантную систему патогенных микобактерий туберкулеза, в т.ч. резистентного МЛУ- штамма. Изучение влияния лекарственного средства ФС-1 на активность антиоксидантной системы проведены на референтном штамме *Mycobacterium tuberculosis* $H_{37}Rv$ и МЛУ (к рифампицину, изониазиду, стрептомицину, этамбутолу, пиразинамиду, этионамиду, ПАСК, канамицину и циклосерину) штамме *Mycobacterium tuberculosis* 320. Лекарственное средство ФС-1 в экспериментальных условиях *in vitro* показал новый механизм действия на микобактерии туберкулеза – подавление функциональной активности фермента супероксиддисмутазы, защищающего микроорганизм от окислительного стресса. Потеря бактериальной клеткой устойчивости к окислительному стрессу, т.е. способности нейтрализовать высокотоксичные кислородные радикалы ведет к разрушению клеточных структур, метаболических и энергетических процессов, нарушению дыхательной системы и , как следствие, к ее гибели.

Установлено, что антиоксидантная активность микобактерий туберкулеза штамма $H_{37}Rv$ после воздействия препаратом ФС-1 в концентрациях 4 мкг/мл ингибируется на 90,64 %, тогда как в концентрации 2 мкг/мл на бактериальную культуру данного штамма – на 89,07 %. Полученные результаты свидетельствуют о существенном подавлении функциональной активности фермента супероксиддисмутазы у бактериальной культуры микобактерий туберкулеза штамма $H_{37}Rv$ под воздействием ФС-1 в данных концентрациях, проявляя выраженное ингибирующее действие. Аналогичные исследования влияния иодсодержащего лекарственного средства ФС-1 на антиоксидантную систему проведены на бактериальную культуру *M.tuberculosis* множественно лекарственно устойчивого штамма 320. При этом установлено, что антиоксидантная активность препарата ФС-1 в концентрации 4 мкг/мл ингибируется на 99 %, тогда как в концентрациях 2 мкг/мл препарат ФС-1 подавляет антиоксидантную активность штамма 320 на 98%.

Таким образом, проведенные исследования показали, что препарат ФС-1 в исследуемых концентрациях 4 мкг/мл и 2 мкг/мл обладает механизмом выраженного ингибирования функциональной активности фермента супероксиддисмутазы у микобактерий туберкулеза как референтного чувствительного штамма $H_{37}Rv$, так и множественно лекарственно устойчивого штамма 320. Это приводит к нарушению окислительно-восстановительных преобразований различных химических соединений, образующих дыхательный процесс у бактериальной культуры, обеспечивающих энергетическую потребность микроорганизма.

Ключевые слова: иодсодержащий препарат ФС-1, микобактерии туберкулеза, ферменты антиоксидантной системы бактериальной клетки, супероксиддисмутаза (СОД), УФ-спектрометрия.

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