

PROSPECTS FOR USING EXOMETABOLITES OF MESENCHYMAL STEM CELLS AS AN ANTIMICROBIAL AGENT

© Moskalov V. B.

V.N. Karazin Kharkiv National University

vimoskalov93@gmail.com

<https://doi.org/10.34142/2708-5848.2023.25.1.03>

The article presents the influence of living mesenchymal stem cells (MSCs) and biologically active substances secreted by them (exometabolites, or secretome) on bacterial cells and their communities, as well as on immunocompetent cells of the host's body. It is advisable to study the effect of living MSCs on bacterial cells *in vitro* in co-cultivation or co-incubation systems. Such systems made it possible to establish that, under the conditions of interaction, bacterial cells attach and grow worse, and MSCs increase the expression of proteins of the main histocompatibility complex of class II and costimulatory factors, cytokines and growth factors. It was also found that MSCs are able to accumulate antibiotics and release them during contact with microbial cells. The three-component system "MSC-microbe-host" is modeled *ex vivo* and *in vivo*. The most common *ex vivo* model that is used for studying the antimicrobial and concomitant activities of MSCs is pneumonia in perfused human lung. It allows researchers to reproduce the fluid balance in the body, the inflammatory process and bacterial clearance. The improvement of the listed indicators under the influence of MSCs was demonstrated and, probably, mediated through the growth factor of keratinocytes. Similar dynamics of the infectious process can be observed in *in vivo* models, in which, using RNA interference, it was established that the decrease in the concentration of inflammatory mediators is probably caused by MSC secretion of beta-defensin-2, which activates the signaling pathway associated with Toll-like receptor type 4 (TLR-4). Although live MSCs may exhibit greater antimicrobial activity compared to their exometabolites, this may be more probable due to changes in the expression pattern of biologically active substances than to contact mechanisms. The study of the effects of exometabolites of MSCs revealed both direct inhibition of bacterial growth and suppression of signaling of the "quorum sensing" system and biofilm formation. The key element of the antimicrobial activity of the secretome of MSCs is the LL-37 peptide, the expression of which may be enhanced with the help of 1,25-dihydroxyvitamin D3. However, the repertoire of antimicrobial peptides and/or other molecules in the secretome of MSCs is probably much wider and needs to be studied in detail. In addition, MSCs exometabolites are able to modulate the work, proliferation and apoptosis of immunocompetent cells. The described properties of MSC exometabolites make the development of antimicrobial agents based on them promising.

Key words: antibiotic resistance, exometabolites secretion, biotechnology, biological activity

Bacterial infections are one of the most important factors leading to death worldwide. For example, according to the WHO, 1.3–4.0 million cases of cholera and 21,000–143,000 deaths occur annually worldwide [2]. In African countries, the main problem of bacteremia is the difficult access of rural residents to antibiotics. This leads to the fact that 26% of childhood hospital deaths are associated with bacteremia [25]. In India, there are outbreaks of plague (1966, 1994, 2002), leptospirosis, leading to kidney failure in the southern regions of the

country, brucellosis, rickettsiosis and other dangerous bacterial infections [6]. The main reasons for the prevalence and high mortality of bacteriosis in Asian countries are similar to African countries: violation of sanitary standards, low involvement in vaccination, insufficient access to antibiotics [16].

At the same time, the bacterioses death problem is common in relatively prosperous countries of the world. The reason of these deaths in developed countries is the occurrence of "superinfections", which are caused by

antibiotic-resistant strains of pathogens. The Review on Antimicrobial Resistance, prepared by the UK Government, supposed that bacterial antimicrobial resistance (AMR) could kill 10 million people per year by 2050 [27]. Although these forecasts are criticized by the WHO and other organizations and researchers, the problem of the spread of AMR among pathogens is acute [26]. Murray et al. [посилання] note that in 2019, methicillin resistance of the bacterium *Staphylococcus aureus* alone resulted in 100,000 deaths, and six other antibiotic – resistant pathogen combinations caused between 50,000 and 100,000 deaths. There is evidence that about 5 million people now die every year from causes associated with a silent pandemic of bacterial resistance to antimicrobial drugs. This situation is exacerbated by the limited efforts being made to develop new antimicrobials and the inappropriate use of existing antibiotics, which is setting the stage for the spread of AMR [4].

There are two main approaches to improve the effectiveness of antimicrobials: point (targeted) delivery of relatively high concentrations of existing drugs and the development of new drugs. Means of targeted drug delivery can be rather unusual, for example, using bacteria with a modified genome. Mazzolini et al. [21] proposed a transgenic strain of *Mycoplasma pneumoniae* for delivering antibiotics for the treatment of pneumonia caused by *Pseudomonas aeruginosa*. The administration of living non-pathogenic mycoplasma makes it possible to overcome the protective biofilm of the pathogenic bacterium and kill it with an antimicrobial agent.

A search for new bactericidal and bacteriostatic agents is being carried out: the activity of quaternary ammonium compounds, N-halamines, chitosans, polybiguanides, triclosans, nanoparticles of noble metals and metal oxides, as well as plant-based bioactive products is being studied [30]. This approach has two limitations: the potentially high toxicity of the compounds to the patient and the emergence of resistance of target microbes to them. Alternative means can be reactive oxygen species (ROS), which have a high antimicrobial activity against gram-positive and gram-negative bacteria, fungi. There is evidence of their

effectiveness in chronic inflammatory conditions [7]. However, the use of free radicals in some cases can lead to damage of the patient's tissues, necrotic manifestations and the launch of autoimmune processes. Innovative approaches to the development of new antimicrobial agents can be based on microbial genomics, which makes it possible to identify new targets and vaccine epitopes, as well as on the creation of antimicrobial peptides based on host defense mechanisms [18]. Such approaches make it possible to obtain low-toxic agents to which microorganisms are less likely to develop resistance, however these agents are very specific, and their development requires significant resources.

In the paper the antimicrobial and immunomodulatory properties of exometabolites of mesenchymal stem cells (MSCs) are analyzed in order to evaluate the possibility of their use as antimicrobial agents.

The aim of this study is to analyze the antimicrobial and immunomodulatory properties of exometabolites of mesenchymal stem cells (MSCs) in order to evaluate the possibility of their use as antimicrobial agents.

EFFECT OF LIVING MSCs ON THE SURFACE OF MICROBE CELLS

The study of the antimicrobial properties of mesenchymal stem cells *in vitro* requires the development of suitable models. K. Kriebel et al. [15] proposed a model co-cultivation system that includes a pathogenic bacterium (*Fusobacterium nucleatum* or *Porphyromonas gingivalis*, or *Aggregatibacter actinomycetemcomitans*) from the oral microflora and human mesenchymal stem cells (hMSCs) or a primary culture of gingival epithelial cells, or a permanent cell line of the same origin Ca9-22, in DMEM medium during 24 hours. It was shown that hMSCs survive well despite anoxic conditions (40% of live cells against 20% for the Ca9-22 culture after 72 h of growth [15]; the viability of hMSCs on days 1 and 2 was even higher and amounted to 80%), which indicates higher adaptability and plasticity of hMSCs compared to differentiated cells. All studied bacterial species were adhered to hMSCs 6 times less frequently than to

epithelial cells. *Fusobacterium nucleatum* was 10 times more intensively internalized in cells of epithelial origin than in hMSCs [15]. This circumstance indicates a high affinity of fusobacteria to epithelial cells during co-cultivation *in vitro*. A time-dependent increase in IL-8 secretion by gingival epithelial cells was observed when co-cultured with *Fusobacterium nucleatum*, but hMSCs had not a similar effect, probably due to their immunomodulatory effects. Bacteria induce IL-10 secretion by both hMSCs and gingival epithelial cells [15].

Another approach to modeling the interaction between MSCs and microbial cells *in vitro* is a short-term co-incubation of these cells from 30 minutes up to several hours. In this case, bacterial cells were pre-washed from the nutrient medium by centrifugation and placed in the DMEM medium. So, A. Kol et al. [12] applied this method to several serotypes of *Salmonella enterica* subsp. *enterica*, *Escherichia coli*, *Listeria monocytogenes* (as pathogenic organisms), *Lactococcus lactis* subsp. *lactis*, *Bifidobacterium infantis*, *Bifidobacterium longum* and *Lactobacillus acidophilus* (as commensal). The use of this model made it possible to find out, for example, that the invasion of MSCs by bacteria occurs in a different way than the invasion of epithelialocytes [12]. Thus, mutant strains of *Salmonella* lacking the type III secretion system (injectosome, molecular syringe) cannot interact with epithelial cells, but successfully penetrate into the MSC layer. Also, unlike epithelial cells, MSCs had no signs of intoxication and did not die after short-term contact with bacteria. There were no differences in the ability to form associations with MSCs between pathogenic strains and commensals, however, in the first case, invasion was observed, and in the second case, there was adhesion [12]. Stimulation of MSCs by bacteria *in vitro* leads to their activation not associated with their expression of MHC II or costimulatory factors, i.e., they do not acquire the typical antigen-presenting cell phenotype. Both the typical pathogen (*Salmonella enterica* subsp. *enterica* serotype typhimurium LT2) and the typical commensal (*Lactobacillus acidophilus*) induced increased expression of peroxisome proliferation activator receptor gamma (*PPARγ*), *IL6* and *IL8*, while

transcription of cyclooxygenase 2 (*COX2*) and hepatocyte growth factor (*HGF*) was induced only by the pathogenic bacterium. LPS treatment was similar to salmonella co-incubation except for the absence of a marked increase in *COX2* expression. Secreted factors followed gene transcription trends, and IL6, IL8 and PGE2 concentrations were higher when MSCs were treated with a pathogenic strain. It should also be noted that mesenchymal stem cells activated by bacteria in this model showed a higher ability to inhibit T-cell proliferation when MSCs were co-cultivated with peripheral blood mononuclear cells *in vitro* against the background of the T-cell mitogen concanavalin A, and interaction with pathogenic bacteria (salmonella) had a more significant inhibition; blocking the work of cyclooxygenases by indomethacin in MSCs (an anti-inflammatory mechanism) leads to a decrease in their inhibitory effect on the proliferation of T lymphocytes, but does not completely cancel this action [12].

Among the *in vitro* models, those are of greater interest than study the direct antimicrobial activity of MSCs directly and MSCs loaded with antibiotics (possibility of targeted drug delivery). The first type models are practically not used for living cells, they are used for MSC exometabolites and they will be considered later. V. Johnson et al. [10] studied the antimicrobial properties of living mesenchymal stem cells, both alone and with the antibiotic cefazolin. Cocultivation of MSCs with *Staphylococcus aureus* cells more effectively inhibited bacterial growth compared to the *in vitro* effect of cefazolin, but the combined use of MSCs and an antibiotic inhibited the growth of staphylococci even more effectively [10]. As for the targeted delivery of antibiotics, it was shown that MSCs are able to adsorb and store ciprofloxacin, providing an antimicrobial effect against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [31].

Despite high repeatability and relatively simple standardization approaches, *in vitro* models are rarely used in such studies. This is partly due to the difficulty in selecting the optimal conditions for co-cultivation and co-incubation (composition of the medium, temperature regime, observation period, etc.), but more restrictions are imposed by the inability

to take into account all the factors affecting MSCs and bacteria in the human or animal body. The transitional type of model from *in vitro* to animal experiments are *ex vivo* models, which will be considered using the perfused human lung pneumonia model as an example. This type of model allows obtaining clinically valuable data on the functioning of tissues of the studied organ affected by bacteremia under the influence of mesenchymal stem cells, for example, lung fluid balance, acute inflammation, and bacterial clearance, under controlled conditions. It has been shown that MSCs are able to normalize alveolar fluid clearance, reduce inflammation, and increase the efficiency of killing *Escherichia coli* [17], probably due to increased phagocytosis by alveolar macrophages and secretion of antimicrobial factors. It has also been noted that keratinocyte growth factor plays an important role in the antimicrobial activity of MSCs, apparently by reducing monocyte apoptosis [17].

More common models for studying the antimicrobial properties of mesenchymal stem cells are bacteremia *in vivo*. The development of infections resistant to standard antimicrobial therapy is a factor that increases the relevance of such studies. Infectious diseases of the respiratory system are modeled most often, due to their high prevalence and pathogenicity. Lung damage in this case is usually caused by intratracheal administration of high concentrations of bacteria: mice are injected with 10^6 – 10^7 CFU of *Escherichia coli* in a suitable solvent, sheep are injected with 10^{11} CFU of *Pseudomonas aeruginosa* in a suitable solvent [3, 8, 32]. The administration of MSCs in animals with modeled lung pathology increases their survival, reduces the severity of lung damage, determined by excess fluid in them and by histological analysis, enhances bacterial clearance from the alveolar space during 4 hours after the administration of MSCs, reduces the early pro-inflammatory response, which is expressed in a decrease in the concentration of macrophage inflammatory protein 2 (MIP-2), IL-1 α , IL-1 β , IL-6 and TNF- α in the bronchoalveolar lavage fluid, as well as a decrease in neutrophil degranulation in the alveolar space, as assessed by the concentration of myeloperoxidase in the bronchoalveolar

lavage fluid [3, 8, 32]. The observed changes are significant not only in comparison with the control group (saline, phosphate-buffered saline), but also in comparison with the administration of fibroblasts. Using the described models and RNA interference method, it was found that beta-defensin-2 secreted by MSCs plays a key role in the antimicrobial activity of mesenchymal stem cells via toll-like receptor 4 signaling [3, 8, 32].

One problematic type of bacteremia is an infection caused by antibiotic-resistant strains of microorganisms causing, in particular, nosocomial, postoperative infections. The use of innovative approaches in the treatment of this type of pathology is extremely important. Microbial collections contain antibiotic-resistant bacterial strains, such as methicillin-resistant *Staphylococcus aureus*. This strain forms a biofilm, which prevents its contact with drugs, therefore, when modeling this pathology, it is necessary to reproduce the conditions that ensure formation of a biofilm. Modeling in rats is performed as follows: the creation of air pockets by subcutaneous injection of 10 ml of sterile air into the intrascapular region of the back, then the administration of sterilized 1.5% carboxymethyl cellulose in saline into the pocket to form a pouch, and finally inoculation of 2×10^6 CFU of methicillin-resistant *Staphylococcus aureus* together with carboxymethylcellulose in a pouch [34]. Such a three-step protocol allows reproducing the infectious process, accompanied by the formation of a biofilm (carboxymethylcellulose is the substrate to initiate this). Intravenous administration of mesenchymal stem cells at a concentration of 2×10^5 – 2×10^7 /rats effectively reduces the number of bacterial colonies and the expression of many, primarily pro-inflammatory, cytokines and chemokines such as IL-6, IL-1 β , IL-10 and CCL5, promotes the formation of granulation, which is necessary to recovery infected tissue, in contrast to the control groups, including those who were injected with fibroblasts [34].

Sometimes the pathogenesis of diseases is associated not with the vital activity of bacteria, but with decomposition products after their death. To simulate such a situation, experimental animals are injected with bacterial cells killed,

by heating, for example. Generating of killed *Propionibacterium acnes* cells results in liver damage similar to fulminant hepatic failure in humans [35]. The described model made it possible to establish the role of some immunological mechanisms in alleviating the course of liver disease. Thus, in particular, a decrease in infiltration and activation of CD4+ T-cells in the liver, inhibition of T-helper 1 and induction of regulatory T-cells (Treg) were found in case of the use of mesenchymal stem cells [35]. It has also been found out that MSCs-produced prostaglandin E2 induces, in a phosphoinositide-3-kinase-dependent manner, the development of a special population of liver regulatory dendritic cells, which in turn, through the transforming production of growth factor- β , induces the above-mentioned regulatory T cells (Treg) [35].

Sepsis is a dangerous disease that is caused by a generalization of a local infection process and, in the absence of effective therapy, can lead to multiple organ failure and death. Therefore, there is a strong necessity to develop treatment approaches that can cope with this condition and animal models to test them. Sepsis models can be divided into three groups: toxemia models, bacterial infection models, and host barrier disruption models. To simulate toxemia, 10–20 mg/kg of lipopolysaccharide is administered intraperitoneally or intravenously [11, 19]. In models of bacterial infection, peritonitis is most often reproduced and caused by intraperitoneal injection of 1×10^7 CFU of *Pseudomonas aeruginosa* or *Escherichia coli*, but other routes of administration (intravenous, intratracheal) can be also used [13, 29]. Host barrier disruption models are most commonly performed in the form of cecal ligation and puncture (CLP) surgery [22]. Using sepsis models, it was found that the administration of MSCs increases survival (75% against 40% in control), reduces the level of PAI-1 (a marker for the prognosis of severe sepsis), prevents thrombocytopenia associated with the complication of the course of the disease, and increases the clearance of bacteria, which leads to a significant decrease in the number of bacteria and apparently due to an enhancement of phagocytic activity of peripheral blood mononuclear cells [11, 13, 19, 22, 29]. Activation of MSCs by treatment with 1 μ g/mL

of lipopolysaccharide for 24 h increases the efficiency of bacterial clearance both for living cells (the highest activity) and for a conditioned medium containing their exometabolites [29]. It should be noted that MSCs, in addition to having immunomodulatory and anti-inflammatory properties, also have regenerative properties, which ensures the recovery of organ functions even with the onset of multiple organ failure and makes their use even more significant in clinical practice [11, 13, 19, 22, 29].

ANTIMICROBIAL PROPERTIES OF MSCs SECRETOME

A positive effect on the course of the infectious process (antimicrobial, anti-inflammatory and immunomodulatory effects) seems to be provided by both contact and distant mechanisms. This follows from the fact that both living cells and the substances secreted by them (exometabolites, or secretome) have such effect, and living cells are more active. Contact mechanisms are primarily associated with the expression of Toll like receptors (TLR) on the surface of MSCs and the signaling pathways associated with them [10, 23, 32]. Thus, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, and TLR9 were found on their surface, and it was found out that agonists of these receptors, including bacterial origin, have an activating effect on MSCs [23]. However, the secretome has distant effects on bacterial cells and immunocompetent cells, which will be discussed in more detail [10, 23, 32].

One of the most studied mechanisms of the antimicrobial effect of mesenchymal stem cells against gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and gram-positive bacteria *Staphylococcus aureus* is associated with the secretion of the cationic antimicrobial peptide LL-37 (hCAP-18/LL-37) [14, 33]. It is also known that treatment of MSCs with 1,25-dihydroxyvitamin D3 increases the expression of LL-37 and thus enhances the antimicrobial activity of the MSCs secretome [33]. Conversely, vitamin D receptor inhibitors (GW0742), as well as neutralizing antibodies to LL-37, reduce the antimicrobial activity of MSCs [33]. The described effects associated with the activity of the antimicrobial peptide LL-37 manifest themselves both *in vitro* (when MSC

secretome is administrated into bacterial cultures) and *in vivo* (in a mouse model of pneumonia caused by *Escherichia coli*) [14, 33].

However, the repertoire of antimicrobial peptides produced by MSCs is not limited by LL-37. In general, antimicrobial peptides have from 10 to 150 amino acid residues in length and are found in a wide range of living organisms, from prokaryotes to humans [1]. The biological targets of such peptides are congruent with the targets of antibiotics, namely, disruption of membrane integrity, inhibition of protein, DNA or RNA synthesis, etc. Often, antimicrobial peptides can be active against classical antibiotic resistant pathogens, such as multidrug resistant bacteria. In addition to direct microbial activity, this class of substances has indirect effects on bacteria, including chemotactic and antiendotoxin activity, as well as protease inhibition, bacterial opsonization, and angiogenic properties. Thus, cathelicidins have a chemotactic effect on monocytes, neutrophils

and lymphocytes, and LL-37 binds and neutralizes lipopolysaccharide; β -defensins have a chemotactic effect on macrophages, neutrophils, and mast cells, probably through binding to CCR6 [1]. As for hepcidin and Lcn2, they are involved in pathways that regulate the availability of iron, that is a vital element for bacterial growth [1]. There is also evidence that the antimicrobial activity of MSCs is mediated by antimicrobial peptides or proteins belonging to the cathelicidin, defensin, hepcidin, or lipocalin families [1]. Cathelicidins, defensins, and hepcidin are synthesized as pre-propeptides that are cleaved to release mature antimicrobial peptides that interact with the negatively charged surface of bacterial membranes [1]. Lipocalins are able to bind small hydrophobic molecules, bind to specific cell surface receptors, followed by the formation of macromolecular complexes. A generalized scheme for the production of antimicrobial peptides by MSCs is shown in Fig. 1 [1].

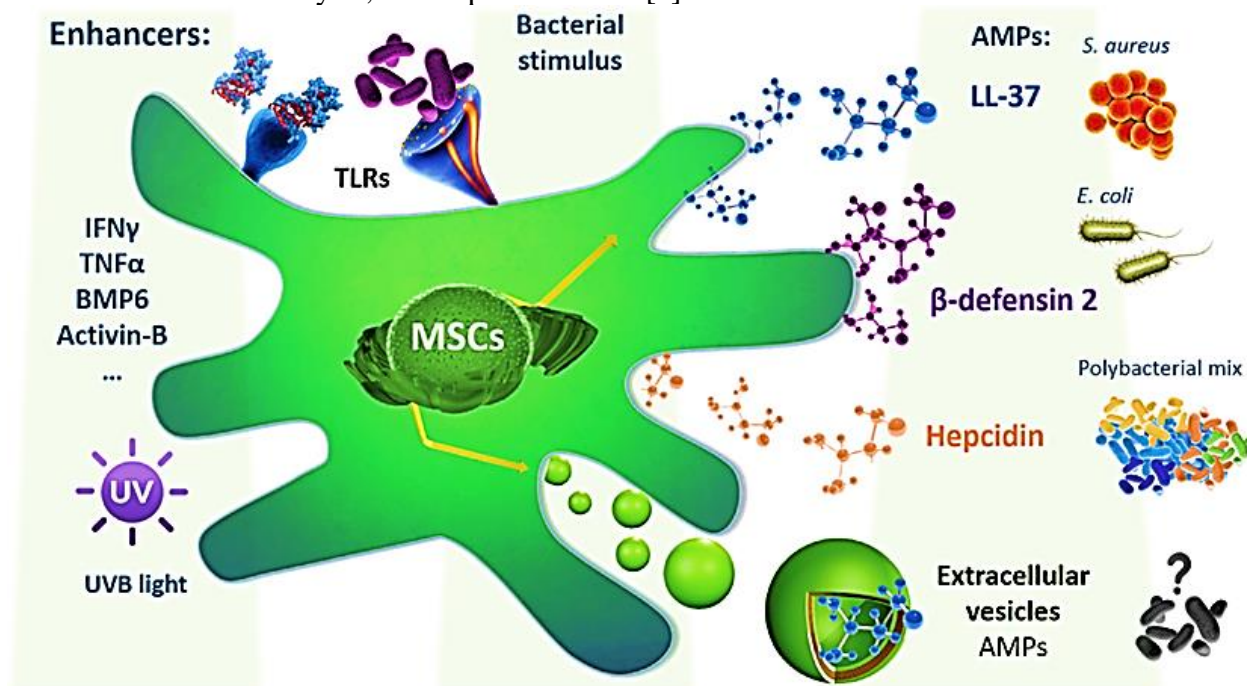


Fig. 1. Scheme of the production of antimicrobial peptides by mesenchymal stem cells, indicating activators and products according to [1]; AMPs – antimicrobial peptides, TLR – Toll-like receptors, UVB – ultraviolet B

Quite often, the antimicrobial properties of therapeutic agents cannot appear due to the protective mechanisms of bacteria. Such mechanisms include the modification of biological targets in bacterial cells or the

production of enzymes that disrupt the activity of antibiotics. One of the well-studied means of achieving antibiotic resistance is the phenomenon of “quorum sensing” (QS), i.e., regulation of microbial gene expression in

response to exposure at the population level. Another protective mechanism, which seems to be little dependent on the nature of the antimicrobial agent, but is related to the action of the “quorum sensing”, is the formation of biofilm. Therefore, an important component of treatment is to reduce the association of bacteria with each other within the population and to reduce the activity of biofilm formation. Using *in vitro* models in the spectrophotometric Crystal Violet Assay and in the hydrocarbon (n-hexadecan) buffer two-phase system, it was found out that MSCs exometabolites weighing up to 30 kDa provide statistically significant biofilm inhibition and a decrease in the hydrophobic properties of the cell membrane in *Staphylococcus aureus* [5]. A decrease in “quorum sensing” activity after using of this MSCs secretome fraction was demonstrated by a significant decrease in bioluminescence in the QS reporter strain *Escherichia coli* JM109 pSB1142 [long-chain (C10–C12) AHL biosensor], which was treated with a cell-free culture of *Pseudomonas aeruginosa* containing long -chain acyl homoserine lactones (C10–C14) [5]. A decrease in the intensity of biofilm formation and inhibition of the *Escherichia coli* and *Staphylococcus aureus* populations growth under the influence of soluble exometabolites of MSCs has been demonstrated [9].

In humans or animals, the action of mesenchymal stem cell exometabolites is not limited to direct effects on microbial populations, but also includes indirect effects on them mediated by the host's immune system. The immunomodulatory properties of MSCs and substances produced by them are quite extensive and are being actively studied; this article will consider only some of them in the context of the formation of antimicrobial immunity.

The first barrier to the development of an infectious process are cells of innate immunity called neutrophils. Neutrophil deficiency, or neutropenia, leads to a decrease in the body's resistance to infections and is especially severe if it is a congenital condition. It has been shown that the addition of MSCs exosomes containing substances secreted by them to neutrophils obtained from patients with severe congenital

neutropenia increases the release of neutrophils into the respiratory tract in patients and increases the lifespan of neutrophils [20]. The effect on the phagocytic ability of neutrophils was exerted only by the whole conditioned medium of MSCs containing both vesicular and soluble components [20].

The study of the effect of MSCs exometabolites on the bacterial infectious process in the body is carried out on various models, in particular, in models of pneumonia which are performed in mice (*in vivo*) or in perfused human lungs (*ex vivo*) by administration of *Escherichia coli*. The *in vivo* model demonstrated an increase in the survival of animals and an alleviation of the course of the disease [24], probably associated with the secretion of keratinocyte growth factor and a decrease in the influx of inflammatory cells, cytokines, proteins and bacteria, increase of monocytic phagocytosis of bacteria, and intracellular ATP concentration in damaged type 2 alveolar epithelial cells. In the *ex vivo* system, there was an increase in alveolar fluid clearance and a decrease in protein permeability, as well as a quantitative decrease in the bacterial load in damaged alveoli [28]. It should be noted that pre-stimulation of MSCs with a toll-like receptor 3 agonist (polyinosinic:polycytidylic acid) enhances the therapeutic effects of the released microvesicles [24, 28].

CONCLUSION

Thus, the study of the action of living MSCs on bacteria *in vitro* has shown the inhibition of the adhesion and growth of microbial cells, probably associated with an increase in the expression of MHC II proteins and costimulatory factors on the membrane and the secretion of cytokines and growth factors. It was established that MSCs are able to accumulate cephalosporins and release them during contact with microbial cells. Due to these properties, live MSCs can be used for targeted delivery of antibiotics to the site of the infectious process. Both MSCs and the exometabolites produced by them have antimicrobial properties, that include such effects as improving the fluid balance in the infected organ, reducing the inflammatory

process, and increasing bacterial clearance. Among the mechanisms of antimicrobial activity, the following can be considered: the secretion of keratinocyte growth factor, which has a pleiotropic effect on damaged tissues and immunocompetent cells, beta-defensin-2, which activates the signaling pathway associated with TLR-4, antimicrobial peptide LL-37 and its analogues, blocking signaling in the "quorum sensing" network and biofilm formation by bacteria. Generating of the

secretome of MSCs can activate neutrophils and protect them from apoptosis, as well as modulate the work of lymphocytes. These antimicrobial, anti-inflammatory, immunomodulatory, anti-QS and anti-film-forming properties, together with the possibility of depositing antibiotics inside cells, provide a wide field for the development of antimicrobial agents using MSCs and their secretome.

REFERENCES

1. Alcayaga-Miranda F., Cuenca J., Khoury M. (2017). Antimicrobial activity of mesenchymal stem cells: current status and new perspectives of antimicrobial peptide-based therapies. *Frontiers in immunology*, 8: 339. DOI: 10.3389/fimmu.2017.00339
2. Ali M., Nelson A. R., Lopez A. L., Sack D. A. (2015). Updated global burden of cholera in endemic countries. *PLoS neglected tropical diseases*, 9(6): e0003832. DOI: 10.1371/journal.pntd.0003832
3. Asmussen S., Ito H., Traber D. L., Lee J. W. et al. (2014). Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. *Thorax*, 69(9): 819–825. DOI: 10.1136/thoraxjnl-2013-204980
4. Brasier N., Ates H. C., Sempionatto J. R., Cotta M. O. et al. (2023). A three-level model for therapeutic drug monitoring of antimicrobials at the site of infection. *The Lancet Infectious Diseases*: S1473–3099. DOI: 10.1016/S1473-3099(23)00215-3
5. Bujňáková D., Čuvalová A., Čížek M., Humeník F. et al. (2020). Canine bone marrow mesenchymal stem cell conditioned media affect bacterial growth, biofilm-associated *Staphylococcus aureus* and AHL-dependent quorum sensing. *Microorganisms*, 8(10): 1478. DOI: 10.3390/microorganisms8101478
6. Chugh T. D. (2008). Emerging and re-emerging bacterial diseases in India. *Journal of biosciences*, 33(4): 549–555. DOI: 10.1007/s12038-008-0073-0
7. Dryden M. (2018). Reactive oxygen species: a novel antimicrobial. *International journal of antimicrobial agents*, 51(3): 299–303. DOI: 10.1016/j.ijantimicag.2017.08.029
8. Gupta N., Krasnodembskaya A., Kapetanaki M., Mouded M. et al. (2012). Mesenchymal stem cells enhance survival and bacterial clearance in murine *Escherichia coli* pneumonia. *Thorax*, 67(6): 533–539. DOI: 10.1136/thoraxjnl-2011-201176
9. Harman R. M., Yang S., He M. K., Van de Walle G. R. (2017). Antimicrobial peptides secreted by equine mesenchymal stromal cells inhibit the growth of bacteria commonly found in skin wounds. *Stem cell research & therapy*, 8(1): 1–14. DOI: 10.1186/s13287-017-0610-6
10. Johnson V., Webb T., Norman A., Coy J. et al. (2017). Activated mesenchymal stem cells interact with antibiotics and host innate immune responses to control chronic bacterial infections. *Scientific reports*, 7(1): 9575. DOI: 10.1038/s41598-017-08311-4
11. Khosrojerdi, A., Soudi, S., Hosseini, A. Z., Eshghi, F., Shafiee, A., & Hashemi, S. M. (2021). Immunomodulatory and therapeutic effects of mesenchymal stem cells on organ dysfunction in sepsis. *Shock*, 55(4), 423–440. DOI: 10.1097/SHK.0000000000001644
12. Kol A., Foutouhi S., Walker N. J., Kong N. T. et al. (2014). Gastrointestinal microbes interact with canine adipose-derived mesenchymal stem cells in vitro and enhance immunomodulatory functions. *Stem Cells and Development*, 23(16): 1831–1843. DOI: 10.1089/scd.2014.0128
13. Krasnodembskaya A., Samarani G., Song Y., Zhuo H. et al. (2012). Human mesenchymal stem cells reduce mortality and bacteremia in gram-negative sepsis in mice in part by enhancing the phagocytic activity of blood monocytes. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 302(10): L1003–L1013. DOI: 10.1152/ajplung.00180.2011
14. Krasnodembskaya A., Song Y., Fang X., Gupta N. et al. (2010). Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem cells*, 28(12): 2229–2238. DOI: 10.1002/stem.544

15. Kriebel K., Biedermann A., Kreikemeyer B., Lang, H. (2013). Anaerobic co-culture of mesenchymal stem cells and anaerobic pathogens-a new in vitro model system. *PloS one*, 8(11): e78226. DOI: 10.1371/journal.pone.0078226
16. Kumar Gupta R., Kumar Rai R., Kumar Tiwari P., Kumar Misra A. et al. (2023). A mathematical model for the impact of disinfectants on the control of bacterial diseases. *Journal of Biological Dynamics*, 17(1): 2206859. DOI: 10.1080/17513758.2023.2206859
17. Lee, J. W., Krasnodembskaya, A., McKenna, D. H., Song, Y., Abbott, J., & Matthay, M. A. (2013). Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. *American journal of respiratory and critical care medicine*, 187(7), 751–760. DOI: 10.1164/rccm.201206-0990OC
18. Lohner K. (2001). Development of novel antimicrobial agents: emerging strategies. Wymondham, Norfolk: Horizon Scientific Press.
19. Lombardo E., van der Poll T., DelaRosa O., Dalemans, W. (2015). Mesenchymal stem cells as a therapeutic tool to treat sepsis. *World journal of stem cells*, 7(2): 368. DOI: 10.4252/wjsc.v7.i2.368
20. Mahmoudi M., Taghavi-Farahabadi M., Namaki S., Baghaei K. et al. (2019). Exosomes derived from mesenchymal stem cells improved function and survival of neutrophils from severe congenital neutropenia patients in vitro. *Human immunology*, 80(12): 990–998. DOI: 10.1016/j.humimm.2019.10.006
21. Mazzolini R., Rodríguez-Arce I., Fernández-Barat L., Piñero-Lambea C. et al. (2023). Engineered live bacteria suppress *Pseudomonas aeruginosa* infection in mouse lung and dissolve endotracheal-tube biofilms. *Nature Biotechnology*: 1–10. DOI: 10.1038/s41587-022-01584-9
22. Mei S. H., Haitzma J. J., Dos Santos C. C., Deng Y. et al. (2010). Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. *American journal of respiratory and critical care medicine*, 182(8), 1047–1057. DOI: 10.1164/rccm.201001-0010OC
23. Mezey É., Nemeth K. (2015). Mesenchymal stem cells and infectious diseases: smarter than drugs. *Immunology letters*, 168(2): 208–214. DOI: 10.1016/j.imlet.2015.05.020
24. Monsel A., Zhu Y. G., Gennai S., Hao Q. et al. (2015). Therapeutic effects of human mesenchymal stem cell-derived microvesicles in severe pneumonia in mice. *American journal of respiratory and critical care medicine*, 192(3): 324–336. DOI: 10.1164/rccm.201410-1765OC
25. Mulholland E. K., Adegbola R. A. (2005). Bacterial infections – a major cause of death among children in Africa. *The New England journal of medicine*, 352(1): 75–77. DOI: 10.1056/NEJMe048306
26. Murray C. J., Ikuta K. S., Sharara F., Swetschinski L. et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325): 629–655. DOI: 10.1016/S0140-6736(21)02724-0
27. O'Neill J. (2016). Tackling drug-resistant infections globally: final report and recommendations. London: HM Government.
28. Park J., Kim S., Lim H., Liu A. et al. (2019). Therapeutic effects of human mesenchymal stem cell microvesicles in an ex vivo perfused human lung injured with severe *E. coli* pneumonia. *Thorax*, 74(1), 43-50. DOI: 10.1136/thoraxjnl-2018-211576
29. Saeedi P., Halabian R., Fooladi, A. A. I. (2019). Antimicrobial effects of mesenchymal stem cells primed by modified LPS on bacterial clearance in sepsis. *Journal of cellular physiology*, 234(4): 4970–4986. DOI: 10.1002/jcp.27298
30. Simoncic B., Tomsic B. (2010). Structures of novel antimicrobial agents for textiles – a review. *Textile Research Journal*, 80(16): 1721–1737. DOI: 10.1177/0040517510363193
31. Sisto F., Bonomi A., Cavicchini L., Coccè V. et al. (2014). Human mesenchymal stromal cells can uptake and release ciprofloxacin, acquiring in vitro anti-bacterial activity. *Cytotherapy*, 16(2): 181–190. DOI: 10.1016/j.jcyt.2013.11.009
32. Sung D. K., Chang Y. S., Sung S. I., Yoo H. S. et al. (2016). Antibacterial effect of mesenchymal stem cells against *Escherichia coli* is mediated by secretion of beta-defensin-2 via toll-like receptor 4 signalling. *Cellular Microbiology*, 18(3): 424–436. DOI: 10.1111/cmi.12522
33. Yagi H., Chen A. F., Hirsch D., Rothenberg A. C. et al. (2020). Antimicrobial activity of mesenchymal stem cells against *Staphylococcus aureus*. *Stem cell research & therapy*, 11(1): 1–12. DOI: 10.1186/s13287-020-01807-3
34. Yuan Y., Guo N., Zhao C., Shen S. et al. (2014). Marrow mesenchymal stromal cells reduce methicillin-resistant *Staphylococcus aureus* infection in rat models. *Cytotherapy*, 16(1): 56–63. DOI: 10.1016/j.jcyt.2013.06.002
35. Zhang Y., Cai W., Huang Q., Gu Y. Et al. (2014). Mesenchymal stem cells alleviate bacteria-induced liver injury in mice by inducing regulatory dendritic cells. *Hepatology*, 59(2), 671–682. DOI: 10.1002/hep.26670

УДК 576.5+ 615.28

**ПЕРСПЕКТИВИ ВИКОРИСТАННЯ ЕКЗОМЕТАБОЛІТІВ МЕЗЕНХІМАЛЬНИХ СТОVBУРОВИХ
КЛІТИН ЯК ПРОТИМІКРОБНИХ ЗАСОБІВ**

Москальов В. Б.

У статті розглянуто вплив живих мезенхімальних стовбурових клітин (МСК) та секретованих ними біологічно активних речовин (екзометаболіти, або секретом) на бактеріальні клітини та їх колонії, а також на імунікомпетентні клітини організму хазяїна. Вивчення ефекту живих МСК на бактеріальні клітини доцільно проводити в умовах *in vitro* у системах співкультивування або співінкубування. Подібні системи дозволили встановити, що за умови взаємодії клітини бактерій гірше прикріплюються та ростуть, а МСК посилюють експресію білків головного комплексу гістосумісності II класу та коstimулюючих факторів, цитокінів та ростових факторів. Також було з'ясовано, що МСК здатні накопичувати антибіотики та вивільняти їх під час контакту з мікробними клітинами. Трикомпонентна система "МСК-мікроб-хазяїн" моделюється *ex vivo* та *in vivo*. Найбільш поширеною моделлю *ex vivo*, що використовується для вивчення протимікробної та супутніх активностей МСК є пневмонія перфузованої легені людини. Вона дозволяє відтворити баланс рідини в органі, запальний процес та бактеріальний кліренс. Було продемонстровано покращення перелічених показників під дією МСК, вірогідно, опосередковане через ростовий фактор кератиноцитів. Схожу динаміку інфекційного процесу можна спостерігати у моделях *in vivo*, на яких з використанням РНК-інтерференції було встановлено, що зниження концентрації медіаторів запалення вірогідно викликано секрецією МСК бета-дефензину-2, що активує сигнальний шлях пов'язаний з Toll-подібним рецептором 4 типу (TLR-4). Хоча живі МСК можуть виявляти більшу протимікробну активність порівняно з їх екзометаболітами, це може бути пов'язано більшою мірою зі змінами у патерні експресії біологічно активних речовин, ніж з контактними механізмами. Вивчення ефектів екзометаболітів МСК виявило як безпосереднє інгібування росту бактерій, так і пригнічення сигналіngu системи "відчуття кворуму" та формування біоплівки. Ключовим елементом протимікробної активності секретому МСК є пептид LL-37, експресію якого можна посилити за допомогою 1,25-дигідроксिवітаміну D3. Однак, спектр протимікробних пептидів та/або інших молекул у складі секретому МСК, вірогідно, значно ширший та потребує докладного вивчення. Крім того, екзометаболіти МСК здатні модулювати роботу, проліферацію та апоптоз імунікомпетентних клітин. Описані властивості екзометаболітів МСК роблять перспективною розробку протимікробних засобів на їх основі.

Ключові слова: антибіотикорезистентність, секреція екзометаболітів, біотехнологія, біологічна активність