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SYSTEMIC RETRANSPLANTATION OF AUTOLOGOUS BONE MARROW DERIVED MONONUCLEAR CELLS FOR HEART FAILURE: REPORT OF SAFETY AND EFFICACY

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Regenerative medicine with using autologous bone marrow derived stem cells (BMSC) has emerged a promising approach.

***Purpose of the study.** Heart failure (HF) is the major cause of death worldwide. Despite the available pharmacotherapy, prognosis is usually harsh. Here we assessed the safety and efficacy of autologous bone marrow-derivedmononuclear cell (BMMC) retransplantation in HF (left ventricular ejection fraction, LVEF <45%).*

***Material and methods.** A whole number of 104 patients were included to the study (male=84 and female=20), age 27 untill 65 with Heart failure. All patients underwent standard examination (12-lead ECG, echocardiography, 6-minute walking test and laboratory tests).*

After systemic BMMC retransplantation, EF was shown to rise at 12 months for both DCMP and ISCM (32,1±1,3 vs 41,3±1,6% and 32,2±1,2% vs 39,3±2,6%, respectively; p-value <0,005). ProBNP recognized as an early marker of HF tend to reduce sufficiently by 3 monthsand continued to recover at one-year follow-up for both diseases (3266, ±344,4 vs 361±35,9 ng/ml; p-value <0,0001 and 4618±267 vs 286±35,9 ng/ml; p-value<0,0001 for ICMP and DCMP, respectively).

In conclusion, these results suggest BMMC transplantation is safe and well tolerated. BMMC infusion has improved systolic myocardial function, and a consequence has delayed the progression of HF, which is confirmed by recovering proBNP. Moreover, BMMC transplantation might favor by preliminarycell cultivation due to enhanced mitochondrial function.

***Conclusion.** Taken as a whole, BSMSC retranplantation has proven to be safe and well tolerated. No patient diagnosed with tumour, immune rejection, acute inflammation and worsening cardiac events. BMSC reinfusion was shown to improve systolic myocardial function, and as a result to slow down the progression of HF, which is confirmed by recovering proBNP known as an early marker of HF. What is more, it is now possible to enhance the mitochondrial function by preliminary BMSC cultivation, especially, by using antioxidative agents such as phosphocreatine.*

***Key words:** stem cells, heart failure, bone marrow, ischemic cardiomyopathy. dilated cardiomyopathy.*

Heart failure (HF) is the leading cause of mortality worldwide. Up to date, standard pharmacological treatment is the only available option for these patients, but usually the prognosis for those with end-stage HF is unfavorable. Regenerative medicine with using autologous bone marrow derived stem cells (BMSC) has emerged a promising approach [1, 2, 3, 4]. However, the available data for using BMSCinHF is scare and usually the results are uncertain. In our study, we aimed to evaluate the safety and efficacy of systemic BMMC retransplantation in patients with HF (EF <45%) at one-year follow-up.

Moreover, there is a gap in internationally accepted protocols considering BMSC transplantation (safety, methods of delivery and cultivation). Supporting the concept of cell dysfunction due to chronic stress [5], we attempted to enhancemitochondrial function as the main triggering factor of apoptosis and consequent cell death through preliminary BMMC cultivation on antioxidave cocktails.

Material and methods

Subjects were prospectively recruited at the National Medical Research Centre (Astana, Kazakhstan) between 2012 and 2014. A total of 104 patients aged from 27 to 65 years (male=84, female=20) were considered to the study.

Including criteria were following:

- II-III stages of HF (NYHA)
- LVEF <45%

All patients were categorized into 2 groups:

1. DCMP (n=51).
2. ICMP (n=53).

Each participant provided written informed consent. All subjects underwent 12-lead ECG, echocardiography, 6-minute walking test (6MWT) as well as assessment for hematologic indexes (pro BNP, IL-6, IL-10 and cancer biomarkers). After clinical examination patients were exposed to bone marrow aspiration from posterior iliac crest with local anesthesia (on average volume 400 ml). Then, isolation of

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mononuclear culture was done by means of biotechnological methods, followed by cell cultivation on antioxidative agents for 72 hours (phosphocreatine, "Neotone" and fructose-1,6-diphosphate, ''FDP"). Cell counts and flow cytometry for BMMC were done by suing standard methods (6). Electron microscope Libra-120 (Zeiss) was used for visual examination. BMMC transplantation was carried intravenously for 60-90 minutes (on average $140x10^6$ cells). Following acute adverse effects, blood pressure, heart rate and temperature were measured during infusion. Afterwards, BMMC reinfusion performed once in a 3-month period.

All patients received standard HF treatment (ACE inhibitors, b-blockers, aldosterone antagonists and diuretics). Quality of life was evaluated through Minnesota Living With Heart Failure Score (MLHFS) questionnaire [13]. Statistical analysis was done by using t-test.

Results

Prior to cultivation, BMSC were presented by a heterogeneous cell population such as stem cells (SC), endothelial progenitor cells (EPC) and other differentiated morphological subtypes. Under electron microscope mitochondria were basically characterized by dense matrix and a large number of cristae with translucent spaces. However, it was noticed most mitochondrial cristae are enlarged and destructed, signifying cell dysfunction [picture 1] and as a result, affected production of ATP that is the main source of cell energy. Today, secondary impaired mitochondrial function is considered to be a general cause ofmajor chronic diseases [7, 8]. It is interesting mitochondrial failuremight trigger the process of lysosome growth. Moreover, mitochondrial dysfunction leads to enlargement and degranulation of endoplasmic reticulum (ER), as well as immaturity of secretory granulesthrough affected protein synthesis. At the same time, some BMMC were highly predisposed to abnormal processes such as swelling, necrosis and apoptosis. Commonly, cell signs – impaired membranes and cytoskeletons being typical for mitochondrial energy depletion indicate the onset of apoptosis.

Though, after BMMC cultivation there were found 2 cell populations: large BMMC containing light nucleus, and small BMMC with hyperchromatic horseshoe-shaped nucleus and abundant organelle inclusions (vacuoles and dense granules). Both types demonstrated specific microscopic features; so, cell cytoplasm of larger BMMC included free ribosomes, immature organelles and decreased mitochondrial dense matrix along with delineated cristae, and these all reflect the enhanced phosphorylation and reduced macroenergy compounds [picture 2]. While horseshoe-shaped BMMC tend to have a more complex structure. Two types of ER represented the cytoplasm: a non-granular comprised small and hypertrophic cavities, and granular included protein, but dense secretory granules were typical for both. There were no signs of mitochondrial dysfunction, and what is more; its structure was noted to be polymorphic.

After BMMC cultivation on FDP, inclusions of glycogen were found in the cytoplasm. There is a body of evidence that FDP provides anaerobic glycolysis by preserving-glycogen stores [10, 11, 12]. However, there is no effect of FDP on mitochondrial function. Whereas appearance of the following cell features such as minor dense matrix and delineated cristae after BMMC cultivation on "Neotone", known as antioxidant drug, is the evidence of enhanced mitochondrial function.

Taken together, BMMC cultivation prior to transplantation was demonstrated to improve mitochondrial function. Furthermore, the procedure has been associated with reduced adverse effects. Although some hematologic indexes such as leukocytes were raised after infusion, they have a trend to recover in next 48 hours. According to MLHFS questionnaire, improvement of the quality of life found in 97,1% (101) patients.

For ischemic cardiomyopathy (ICMP), there was a positive correlation between EF and BMSC retransplantationat 12 months (32,2±1,2% vs 39,3±2,6%, respectively; p-value <0,05) [table 1]. Despite the tendency to decreasing end-diastolic volume (EDV) and end-systolic volume (ESV) by 12 months, the associations were not statistically significant.

ProBNP tend to drop sufficiently at 3 months (3266±344,4 ng/ml vs 1622±315,7 ng/ml, respectively; p-value <0,005), and it continued to fall down to 361±35,9 ng/ml by 12 months (p-value <0,0001).

After 4 BMSC reinfusions, anti-inflammatory marker, IL-10 increased from 3,98±2,7 to 13,8±3,8 (p-value <0,001), whereas inflammatory indicator, IL-6 reduced from 9,51±3,1 ti 2,58±1,01, correspondingly (p-value <0,001).

For dilated cardiomyopathy, association between EF and BMSC reinfusion was also positive (32,1±1,3% vs 41,3±1,6% at the baseline and at 12 months respectively, p-value <0,001) [table 2]. EDV reduced from 212,6±10,1 ml to 155,5±24,9 ml by 12 months (p-value<0,005), while ECV changes were not significant.

After first BMSC infusion, proBNP have a tendency to decrease, and what is more 10-fold reduction has found by 12 months (4618±267 ng/ml and 286±35,9 ng/ml, respectively; p-value <0,0001). Significant raise of IL-10 from 5,84±4,7 to 19,8±4,8 (p-value <0,001) and drop of IL-6 from 6,6±2,1 to 1,76±0,63 (p-value <0,001) at 12 months were also determined.

Taken together, BMMC transplantation has been demonstrated to improve systolic myocardial function, and therefore delaying the progression of HF that is confirmed by decreasing proBNP known as early indicator of HF.

Discussion

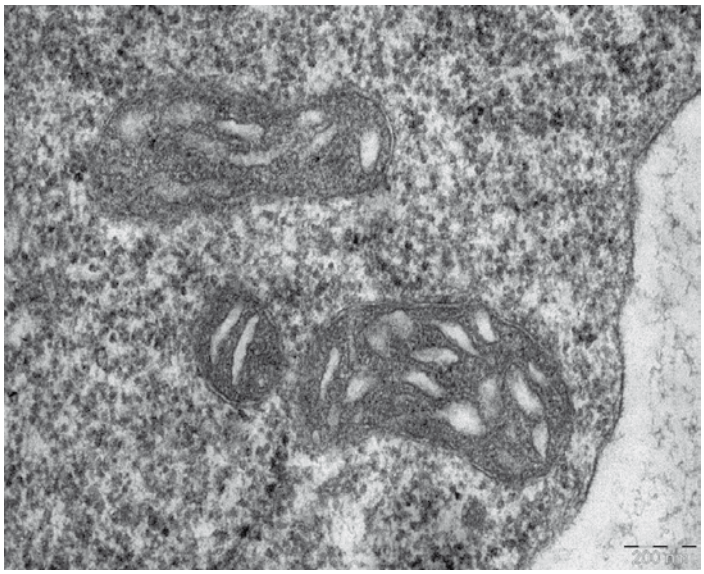
It has been proposed that BM suspension iscomposed of a heterogeneous cell population, for example, stem cells (SC), mesenchymal SC (MSC), hematopoietic SC (HSC) and EPC. These cells secrete a large number of growth factors, which might be responsible not only for cellular survival, but also for regeneration of damaged tissues. In this process, angiogenesis may play a major role. In current study, we observed the positive transformation of cellular organelles as a result of preliminary BMMC cultivation onantioxidative cocktails; particularly, these cells found to comprise a large number of dense and translucent secretory vacuoles as well as developed complexes of Goldie. Overall, these findings have been revealed the importance of diminished oxidative stress in order to inhibit apoptosis and subsequent cell death, and hence favoring cell transplantation.

Table 1 – **Dynamics of echocardiography and serum indexes at one-year follow-up in patients with ICMP**

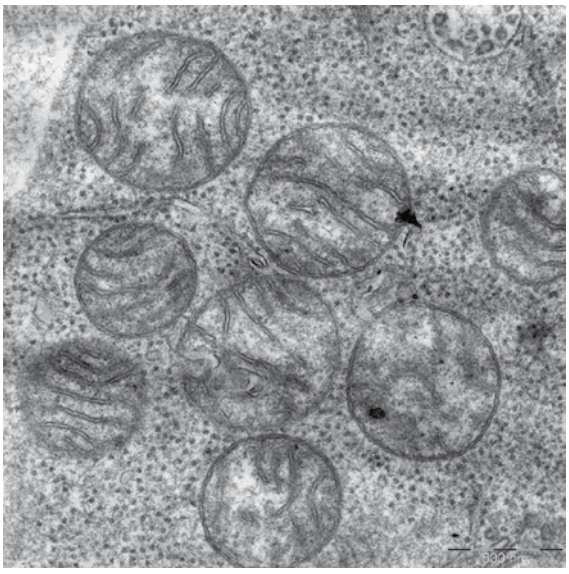
	1 month	3 months	6 months	9 months	12 months
EF, %	32,2±1,2	33,1±1,3	34,2±1,24	35,9±1,3	39,3±2,6*
EDV, ml	218,6±8,1	215,3±9,9	214,6±12,6	215,1±14,1	201,5±24,9
ECV, ml	148,3±7,1	150,6±9,3	154,1±11,6	147,1±14,1	141,3±20,5
ProBNP, ng/ml	3266±344,4	1622±315,7	1505±461,2	1005±54,6	361±35,9*
IL-6	9,51±3,1	6,04±4,1	2,05±1,41	2,51±3,1	2,58±1,01*
IL-10	3,98±2,7	4,34±3,5	6,38±2,1	10,9±3,6	13,8±3,8*
*p-value<0.05					

Table 2 – **Dynamics of echocardiography and serum indexes at one-year follow-up in patients with DCMP**

	1 month	3 months	6 months	9 months	12 months
EF, %	32,1±1,3	34,1±1,5	32,8±2,08	30,9±1,3	41,3±1,6*
EDV, ml	212,6±10,1	208,3±10,9	204±9,8	192,1±14,1	155,5±24,9*
ESV, ml	145,9±8,5	140,6±9,4	143,1±9,6	147,1±14,1	141,3±20,5
ProBNP, ng/ml	4618±267	1876±392	1154±365	1005±54,6	286±35,9*
IL-6	6,6±2,1	3,2±6,7	2,4±0,95	2,51±3,1	1,76±0,6*
IL-10	5,84±4,7	8,3±1,2	19,6±4,1	19,9±3,6	19,8±4,8*
*p-value<0.05					



Picture 1 – BMMC before cultivation.
Mitochondria are characterized by dense matrix and enlarged cristae.
Electronography



Picture 2 – BMMC after cultivation on phosphocreatine.
Cytoplasm is presented by a large number
of mitochondria and free ribosomes. Electronography

Conclusion

Taken as a whole, BSMSC retranplantation has proven to be safe and well tolerated. No patient diagnosed with tumour, immune rejection, acute inflammation and worsening cardiac events. BMSC reinfusion was shown to improve systolic myocardial function, and as a result to slow down the progression of HF, which is confirmed by recovering proBNP known as an early marker of HF. What is more, it is now possible to enhance the mitochondrial function by preliminary BMSC cultivation, especially, by using antioxidative agents such as phosphocreatine.

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Р Е З Ю М Е

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СИСТЕМНОЕ ВВЕДЕНИЕ АУТОЛОГИЧНЫХ МОНОНУКЛЕАРНЫХ ПРЕКУЛЬТИВИРОВАННЫХ КЛЕТОК КОСТНОГО МОЗГА ПРИ СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТИ

Идея регенеративной терапии с использованием стволовых клеток костного мозга уже стала реальностью.

Цель исследования. Изучить безопасность и эффективность системного и повторного введения аутологичных мононуклеарных клеток костного мозга (МККМ) у пациентов с хронической сердечной недостаточностью (EF<45%).

Материал и методы. Обследовано 104 пациента (84 мужчины и 20 женщин) в возрасте от 27 до 65 лет с синдромом хронической сердечной недостаточности (ХСН) II-III ФК (NYHA). Выполнены клинические, биохимические анализы крови, исследование на онкомаркеры (РЭА, NSE, CYFRA, CA72-4, PCA, α-фетопротеин), электрокардиография (ЭКГ), эхокардиография (ЭхоКГ). Всем пациентам после отбора по критериям включения проведена миелоэксфузия из подвздошной кости с последующей биотехнологией получения гемопоэтических стволовых клеток и аутогенной трансплантацией внутривенно из кондиционированной среды – клетки в количестве 140x10⁶ в течение 60-90 минут в локтевую вену под контролем измерений артериального давления, частоты сердечных сокращений и термометрии. Повторное введение клеток проводилось через 3-6-9-12 месяцев. Все пациенты получали базовую терапию ХСН (ингибиторы АПФ, β-блокаторы, антагонисты альдостерона, мочегонные). Качество жизни больных с ХСН определяли по Миннесотскому опроснику (MLHFO). Для электронно-микроскопического исследования культуральные клетки фиксировали в 2,5% растворе глютаральдегида с постфиксацией в 1% растворе четырехокиси осмия, проводили по общепринятой методике и заключали в эпон. Ультратонкие срезы изучали в электронном микроскопе Libra-120 (C. Zeiss). Результаты обрабатывали с помощью t-критерия Стьюдента.

Результаты и обсуждение. Установлено, что методика повторного и внутривенного введения аутологичных мононуклеарных клеток костного мозга безопасна и хорошо переносится пациентами, уменьшаются полости левого желудочка, улучшается систолическая функция миокарда, снижается функциональный класс хронической сердечной недостаточности.

Выводы. Трансплантация аутологичных прекультивированных мононуклеарных клеток костного мозга при ДКМП является безопасной процедурой. Повторное введением прекультивированных МККМ в сочетании с оптимальной медикаментозной терапией ХСН приводит к улучшению общей и локальной сократительной функции миокарда, а также нормализации систолической и диастолической функций левого желудочка.

Путем прекультивирования и введения ряда энерготропных фармакологических препаратов выявлено улучшение морфофункционального состояния деэнергезированных стволовых клеток, включая усиление мощности митохондриального аппарата и синтеза защитных адаптивных белков.

Ключевые слова: *стволовые клетки, костный мозг, ИБС, ДКМП, интерлейкины.*

Т Ұ Ж Ы Р Ы М

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ЖҮРЕК ЖЕТКІЛІКСІЗДІГІ КЕЗІНДЕГІ АУТОЛОГИЯЛЫҚ МОНОНУКЛЕАРЛЫ ҚОРЕКТІК ОРТАДА ӨСІРІЛГЕН СҮЙЕК МИЫНЫҢ КЛЕТКАСЫН ЖҮЙЕЛІ ТҮРДЕ ЕНГІЗУ

Сүйек миының бағаналы жасушаларын пайдалану регенеративті терапия екендігі шындыққа айналды.

Зерттеу мақсаты. Созылмалы жүрек жеткіліксіздігі (EF<45%) бар науқастарға сүйек миының аутологиялық мононуклеарлы клеткасын (МККМ) жүйелі және қайталамалы түрде енгізудің қауіпсіздігі мен тиімділігін оқып үйрену.

Материал және әдістері. Созылмалы жүрек жеткіліксіздік (СЖЖ) II-IIIФК (NYHA) синдромы бар 27-65 жас шамасындағы 104 науқас зерттелді (оның ішінде ер – 84, әйел – 20). Клиникалық, қанның биохимиялық анализдері, онкомаркерге зерттеу, электрокардиография (ЭКГ), эхокардиография (ЭхоКГ) жасалынды. Талапқа сәйкес келген барлық науқастарға сүйек мықынынан алынған миелоэксфузия жүргізілді, әрі қарай биотехнология арқылы алынған гемопоэтикалық бағаналы клеткалар алынып, қайта аутогенді трансплантация арқылы көктамыр ішіне кондиционирленген ортадан алынған клеткалар 60-90

мин көлемінде шынтақ венасына 140x10⁶ көлемінде енгізілді. Енгізу барысында науқастардың артериальды қан қысымы, ЖЖЖ және дене температурасы бақыланды. Клетканы қайта енгізу 3-6-9-12 айларда жүргізілді. Зерттеуге қатысқан барлық науқастар ЖСЖ-ның базалық емін қабылдады(ААФ ингибиторлары, β-блокаторлары, альдостерон антагонистері, зәр айдайтын препараттар). ЖСЖ бар науқастың өмір сүру сапасы Миннесотский (MLHFO) сауалнамасы арқылы анықталды. Электронды микроскопиялық зерттеу үшін қоректік ортада өсірілген клетка 2,5% глютаральдегид ерітіндісімен бекітілді, бекітілген клетка 1% төртокисті осмий ерітіндісімен өңделді. Ультражіңішке кесінділер электронды микроскоп Libra-120 (C.Zeiss)арқылы зерттелінді.Нәтижелер Стьюденттің t-критериімен талқыланды.

Нәтижелері және талқылауы. Сүйек миынан алынған аутологиялық жасуша көк тамыр ішіне және қайта енгізу тәсілі науқастарға қауіпсіз және оны жақсы көтереді, сонымен қатар сол жақ қарынша қуысы кішірейіп миокардтың систолиялық жұмысы жақсарды, созылмалы жүрек жеткіліксіздігінің функциональдық класы төмендеді.

Қорытынды. ДКМП кезінде сүйек миының бағаналы аутологиялық мононуклеарлы жасушаларын трансплантациялау қауіпсіз процедура болып табылады.

Созылмалы жүрек жетіспеушілігінде оңтайлы медикаментозды терапиямен бірге сүйек миының бағаналы аутологиялық жасушаларының қайталамалы енгізілуі миокардтың жалпы және жергілікті жиырылу қызметінің жақсаруына және де сол жақ қарыншаның систолалық және диастоланың функциясының қалпына келуіне әкеледі.

Қайта өсіру әдісі арқылы және энерготропты фармакологиялық препараттарды қабылдау дәэнергияланған бағаналы жасушалардың морфофункционалды жағдайын жақсартуға, митохондриялық аппараттың қуаттылығын және қорғаушы адаптивті ақуыздық синтезді күшейтуге әкеледі.

Негізгі сөздер: *бағаналы жасушалар, сүйек миы, ЖИА, ДКМП, интерлейкиндер.*

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