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Evaluation of the antioxidant properties of ascorbic acid at cryopreservation of cord blood nucleated cells with DMSO

Cord blood (*CB*) is a generally accepted in the world source of hematopoietic stem cells for transplantation in the treatment of many diseases. In this regard, there was organized a large number of cryobanks for storage of *CB* preparations. At present there is a considerable diversity of technologies for *CB* nucleated cells cryopreservation. All of them are designed to maximize the preservation of the structural and functional cell full value that plays an important role in the success of transplantation. Despite this, there are many factors that could reduce the success of cryopreservation. During cryopreservation there is an increase the of reactive oxygen species (*ROS*) content. Their number depends on the efficiency of cryopreservation technology. One possible way of avoiding this effect is the use of antioxidants. Ascorbic acid (*AA*) is a powerful antioxidant that can «trap» free radicals, thus providing protection against oxidative stress and, as a consequence, of cell death.

In this regard, the **purpose** of the work was to evaluate the effect of different concentrations of *AA* on *ROS* content and integrity of *CB* nucleated cells after cryopreservation with D*MSO*.

In our research we used *CB* nucleated cells concentrate isolated with polyglucin and cryopreserved under the protection of *DMSO* at a final concentration of 5 %. The study was carried out using the flow cytometry with a cytometer *FACS Calibur* (*BD*, USA) using a fluorescent dye *DCFH2-DA* and monoclonal antibodies *CD45 FITC, CD34 PE*.

Experiments to evaluate the antioxidant activity of different concentrations of *AA* (25, 50, 100, 150, 200, 500 μM) demonstrated that low concentrations of *AA* (25 and 50 μM) did not provide any significant effect on the studied parameters. Solutions of *AA* 200 and 500 μM resulted in increased numbers of cells with excessive content of *ROS* by 2.6 ± 0.8 % and 12.4 ± 3.2 % respectively with *DMSO*. In this case there was a decrease in cell preservation by 4.5 ± 1.8 % and 12.2 ± 3.7 % respectively. The results indicate pronounced prooxidant effect of *AA*. This may be due to the fact that the *AA* is presented in the liquid as *AA* and ascorbic radical A. If such *AA* accumulates excessively it can induce the formation of free radicals. Therefore, higher concentrations of *AA* may lead to an accumulation of the intermediate product, an ascorbic radical A; and to induce oxidative stress. Comparing the effect of concentrations *AA* 100 μM and 150 μM, the best results have been obtained using 100 μM antioxidant solutions. This concentration can reduce the accumulation of *ROS* in 6.3 ± 2.3 % of cells. At the same time there is an increase of nucleated cells safekeeping by 4.5 ± 1.7 % in relation to the same samples cryopreserved without *AA*.

Thus, the *AA* input into the cryopreservation medium, depending on the concentration, can exhibit both antioxidant and prooxidant effect on nucleated cells. It was shown that 100 μM *AA* concentrations reduce the content of *ROS* in the cells and increase their safekeeping.

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Effect of mesenchymal stem cells on experimental allergic encephalomyelitis in rats

Transplantation of mesenchymal stem cells (*MSCs*), including the use of immunosuppressive properties and ability to stimulate repair processes in the central nervous system, is associated with the development of multiple sclerosis (*MS*) pathogenetic therapy. Recently, there appeared a certain amount of works that demonstrate the impact of *MSCs* on clinical development and course of experimental autoimmune encephalomyelitis (*EAE*), which is a model of *MS*. Nevertheless the effect of *MSCs* derived from adipose tissue has not been studied in this pathology yet.

The **purpose** is to study the effects of rat adipose tissue *MSCs* on the development of experimental autoimmune encephalomyelitis and the level of autoantibodies to myelin basic protein (*MBP*).

Materials and methods. *EAE* in the rats was induced with spinal cord homogenate in complete Freund's adjuvant. Obtaining and culturing *MSCs* of adipose tissue were carried out according to the methods (*Sharifulina S.*, 2004). *MSCs* were injected intraperitoneally on 5th, 8th day after induction of *EAE*. The animals of the control group were administered with saline. Clinical observations of the animals were carried out every day for 1 month. For each experimental animal we determined the disease severity, taking into account developed criteria (*Belskaya L.*, 2003). The level of antibodies to *MBP* was determined by *ELISA*.

Results and discussion. In most animals the first neurological symptoms developed on 6-8th days after the induction of *EAE*; the peak of clinical manifestations was observed on 12-15th day; restoration of muscle tone and motor activity was noted on 28-30th day. We determined statistically significant ($p < 0.05$) increase in the level of antibodies to *MBP* during the clinical manifestation of the disease, which is kept for 25 days after induction of *EAE*. Double intraperitoneal administration of adipose tissue *MSCs* provided positive protective effect on the clinical course of disease, and was accompanied by a decrease in the level of antibodies to *MBP*, mostly in animals with mild *EAE*.

Conclusion. Thus, the studies suggest that *MSCs*, derived from adipose tissue, exhibit a therapeutic effect in rats with *EAE* and can be used to develop methods of cell and tissue therapy of autoimmune diseases.

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Clinical features of renal transplant dysfunction in the early postoperative period

In recent years, the indications for renal transplantation were significantly enhanced. The **purpose** of research was to analyze the features of the restoration of the renal graft function in the early postoperative period in patients considering the trend.

Materials and methods. We have studied the results of 231 kidney transplants performed in patients with end-stage renal failure. Recipients were divided into two groups:

I group – 125 patients operated on in 1999-2004;

II group – 106 patients operated on in 2008-2013.

Between patients in both groups there were the following significant differences: their age in the first group ranged from 12 to 62 years, and in the second group – from 7 to 71 years. The first group had mainly transplantation from cadaveric donors (76.8 % of cases), in the second group – from related donors (68.8 % of cases). In the first group transplantation was performed in patients with glomerulonephritis and pyelonephritis. In the second group, 18 patients had additional risk factors: diabetes in 11 patients, systemic lupus erythematosus in 5 cases, amyloidosis in one case, and the replacement of the aortic and mitral valves, due to bacterial endocarditis 4 months before transplantation in one case. In the first group we used Neoral, Azathioprine or CellCept and Prednisone (Metipred) for immunosuppression after transplantation. At rejection, immunosuppression was supplemented with antibody anti-lymphocyte preparations. In the second group, in all cases at the stage of induction we used *anti-CD25* monoclonal antibody (Zenapax, Simulect); basic immunosuppression was performed using Neoral, CellCept or Myfortic and Medrol.

Results. The primary function of the renal transplant occur in 89 (71.2 %) patients in the first group and 83 (78.3 %) patients of the second group. The study of daily diuresis, creatinine concentration in blood and urine allowed to distinguish four variants of the clinical course of delayed renal graft function in the form of: 1) anuria; 2) oliguria; 3) normuria; 4) secondary delayed function, when, after several days of polyuria, daily diuresis decreased up to anuria. We have established a leading role of initially delayed renal graft function in the development of ischemia. Therefore, anuria at renal transplantation from a related donor is very probable feature of vascular thrombosis. At secondary delayed oligoanuria the main reason was the rejection. Survival of patients with delayed function in the first group was 80.6 %, and in the second group, largely due to the absence of septic complications, 100 %.

Conclusion. During the last decade under the influence of divergent trends there was an increase in the number of primary functioning kidney transplant, which stipulates the best start for a long-term rehabilitation of the recipients.

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Epidemiology of hepatitis B in the renal transplantation

Viral hepatitis B complicates renal transplants. In recent years its preventability has increased significantly. At the same time number of patients in the queue for transplantation has increased and indications for its performance have expanded.

The **purpose** of research was to evaluate the effect of these divergent trends in the incidence and prevalence of hepatitis B at renal replacement therapy.

Materials and methods. In the period from 1998 to 2013 at Donetsk transplantation center 543 patients received renal replacement therapy, including 239 – in 1998-2004 and 304 – in 2005-2013. Viral hepatitis B was diagnosed in 228 patients (42 %). Hemodialysis was performed in all patients. Kidney transplantation was performed in 385 (71 %) patients.

Results and discussion. The prevalence of hepatitis B among patients receiving renal replacement therapy in the 2005-2013 is much lower – 25 % (78 patients) compared to 1998-2004 – 65 % (150 patients). Prior to 1999, the fact of patients' infection was monitored only be the Australian antigen.

Since 1999, patients of transplant center were gradually examined for the entire spectrum of hepatitis B markers. Almost a 4-fold increase (from 19.3 % to 71.8 %) of patients with hepatitis-B before dialysis in 2005-2013 in comparison with the 1998-2004 period reflects an improvement in its detection. Due to vaccination, the incidence of acute hepatitis B decreased from 31.4 % in 1998 to 3.4 % in 2004. Throughout the 2005-2013 period cases of acute hepatitis B were not registered. That allowed a more efficient implementation of transplantation programs. Amount of transplants (114) performed in patients infected with hepatitis B in 1998-2004 1.7 times outnumbered the transplantations (65) in uninfected recipients. In the 2005-2013 period the number of patients (180), without hepatitis B virus, was 6.9 times higher than the number of infected recipients (26). Among the causes of patients death in 1998-2004 in eight cases was hepatopathy because of viral hepatitis B. In 2005-2013 mortality related to the complications of hepatitis B was absent.

Conclusion. The high prevalence of hepatitis B among patients, receiving renal replacement therapy, is largely due to the high incidence of this infection in the general population. Immunoprophylaxis of hepatitis B is the leading element in reducing of HBV-infection. Optimizing the selection and preparing candidates for renal transplantation with the clinical course and the possible outcome of HBV-infection has significantly reduced its prevalence among kidney transplant recipients and improved the results of renal replacement therapy in general.

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Indications for renal transplantation with simultaneous ipsilateral nephrectomy

Renal transplantation is the most effective method of renal replacement therapy. Techniques of this operation continue to improve, due to expansion of indications and the desire to increase its security. The choice of surgical approach when it is necessary to extract kidneys in transplant candidates remains a challenge. This study was carried out to assess the current capabilities of kidney transplantation with ipsilateral nephrectomy in patients with urinary tract infection or microcysts.

Materials and methods. Renal transplantation with simultaneous ipsilateral nephrectomy was performed in 12 patients. Five patients had megaureter, hydronephrosis (children from 7 to 14 years); in one case – polycystic (dimensions of kidney 15x29 cm); in four cases – glomerulonephritis; in one case – lupus nephritis, microcysts; and in one case – diabetes, microcysts. Duration of anuria with nephritis ranged from 6 to 13 years. Related donor kidneys were transplanted in 8 patients, cadaveric kidneys – in 4.

Results and discussion. Due to the improving of sutures quality, coagulation, tools, lighting, use of optics, and stents, the technical opportunities for performing operations has increased significantly. To prevent sepsis in 6 patients with urinary tract infection, and in 6 patients with no conditions to form uretero-cystic anastomosis kidney transplantations with ipsilateral nephrectomy were performed through the ordinary pararectal approach (in 8 cases – left, in 4 – right) with its cranial increase by 5-8 cm. Thus, the duration of an operation increased by 30-45 minutes. During the formation of the uretero-ureteral anastomosis in 5-patients we used stents, which were removed after 4-5 weeks. The key to success of simultaneous operations was a reliable lymphostasis and hemostasis. Difficulty of its providing was due to the uremia and heparinization during hemodialysis. Loss of time, associated with the achievement of hemostasis, was compensated by suturing of the surgical wound tightly, thus avoiding infectious complications that can occur when using the drainages. We injected prophylactic antibiotics during and after operation. Correction of immunosuppression was not performed.

There were not any complications requiring repeated surgical intervention. Postoperative survival rate of patients and kidney transplants was 100 %.

Conclusion. Kidney transplantation with simultaneous ipsilateral nephrectomy is an acceptable solution, when it is necessary to remove the patient's own kidneys or impossible to form uretero-vesical anastomosis.

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Long-term results of renal transplantation in recipients with high operative risk

The maximum term of satisfactory function of the kidney transplant, transplanted at the Center in standard conditions into 44-year-old patient with chronic glomerulonephritis, is more than 24 years. Monitoring is continued. For further evaluation of the therapeutic potential of kidney transplantation we studied the maximum duration of satisfactory function of the transplanted kidney in different groups of patients with high operative risk.

Materials and methods. Among 686 kidney transplants performed at the Center from April 1986 to June 2014, we conditionally classified 94 as a high surgical risk: 35 transplants performed for children, 27 – patients with diabetic nephropathy, 11 – with systemic diseases, 6 – on the background of arenal state. Five patients were elderly (to 71 years), two had complex cardiac surgery. In one case there were microcysts after a 13-year anuria. Nine transplantations were performed again, for the third or fourth time.

Results. One-year survival of these recipients was 95.7 % with a good quality of life. The maximum duration of satisfactory function of the transplanted kidney after transplantation in children was 16 years. Monitoring continues. Despite a significant increase in weight and height during puberty, kidney transplants function satisfactorily. The maximum duration of satisfactory function of the kidney transplant from a cadaveric donor in patients with diabetes was 7 years old. The patient's condition is satisfactory, monitoring continues. The maximum duration of satisfactory function of the transplanted kidney after bilateral nephrectomy with polycystic kidney disease was 13 years, monitoring continues. The maximum duration of satisfactory function of the transplanted kidney among elderly patients was 7 years old. The most elderly patient within 5 years after transplantation is currently 73 years old. She leads an active lifestyle, does sports (swimming). The maximum duration of satisfactory function of the transplanted kidney in patients with systemic diseases was 7 years with the Goodpasture syndrome, monitoring continues. In a patient with microcysts on the background of 13-year anuria transplant is satisfactory for 5 years after transplantation, the function of the bladder has also recovered fully. The maximum duration of satisfactory function after repeated transplantation was 10 years after the fourth transplantation.

Conclusions. The results of renal transplantation in patients with high operative risk indicate the possibility of long-term effectiveness of rehabilitation and help to reduce the list of contraindications for this operation.

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Optimization of immunosuppression after renal transplantation

Management of renal transplant recipients includes permanent immunosuppression, which complications have a negative impact on the quality and duration of their lives. This study was performed to analyze the ways to optimize immunosuppressive therapy.

Materials and methods. In 2013, at the Centre 124 renal transplant recipients were under the supervision, 22 of them were operated by us throughout the year. The list of used immunosuppressants included Simulect, anti-thymocyte globulin, Neoral, Prograf, Advagraf, CellCept, Myfortic, Sertican, Imuran, Medrol and others. Blood concentration of cyclosporine, tacrolimus, and, if necessary, mycophenolate and everolimus was determined with analyzers *CDX 90* (*ThermoFisher Scientific,* Germany), and *ARCHITECT i1000* (*Abbott Diagnostics*, USA).

Results. The survival rate of patients, operated at the Centre in 2013, and renal transplants (14 – from related and 8 – from cadaveric donors) was 100 %. At the of induction phase of immunosuppression we used Simulect in all cases.

Among 124 observed patients, 17 (13.7 %) people did not get prednisolone or its analogs because of diabetes (4), osteodystrophy (3), and infection (10). Terms of observation after stop of steroid treatment ranged from 6 to 72 months. Tasks to stop the infection, decompensate diabetes, osteodystrophy progression have been resolved. The remaining patients, who were treated with antibody inductive immunosuppression, got a daily dose of 4 mg of Medrol at discharge.

Prescribing Neoral, we focused not only on C_0 and C_2 cyclosporine concentration, but also on the clinical peculiarities of the patient. Due to the difficulties of individual selection of Neoral in 12 (9.8 %) patients, it was replaced by Prograf or Advagraf. At severe graft dysfunction and severe infections Neoral application was stopped. Using Sertican at poor response to mycophenolate also allows reducing the recommended concentration of cyclosporine in blood 'by half'.

In recent years a serious problem has become the appearance of generic immunosuppressants in the pharmaceutical market. They are cheaper than the original drug, but less studied regarding their efficacy and safety profile. With increasing pool of recipients the more important problem has become non-compliance and lack of related specialists' training in matters of immunosuppression in transplant recipients.

Conclusions. Modern selective immunosuppressants allow providing minimal but adequate immunosuppression in individual protocols. However, reliable criteria of safety minimization of immunosuppression are not provided. This requires further studies on their search and appliance instead of the empirical approach.

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Epidemiology of hepatitis B in the renal transplantation

Objective: to analyze the problems of brain death declaring in intensive care units, which are the bases of organ removal, and to determine their possible solution.

Materials and methods. During the period from January 2012 to June 2014, 119 patients aged 19 to 62 years with severe dominant brain lesions due to injury or disease were examined in the intensive care departments of the Donetsk region. All patients underwent generally accepted clinical, laboratory and instrumental investigations.

Results. 29 patients had clinical signs of brain death, which could be considered as grounds for the apneic oxygenation test. And, after obtaining data, confirming the brain death with maintained blood flow, it was possible to make the appropriate protocol and confirm the death. However, this was not done. Only at the one organ removal institution anesthesiologist could collect arterial blood for determination of carbon dioxide tension within the apneic oxygenation test.

Despite the presence of the gas analyzers (*NPT 7, «Radiometer»*, Denmark), the vast majority of anesthesiologists do not have the appropriate skills to work and do not tend to acquire them, although this is not difficult. They do not consider this their duty. According to the official requirements this analyzer is automatic, and it is only necessary to have a medical degree and study the instructions for its use. Thus, rejection of the diagnosis of brain death leads to the fact that the dead patients continue for indefinitely long period and unreasonably get an expensive intensive care due to budgetary and extra-budgetary funds; and donor process is blocked.

Direct or indirect refusal of anesthesiologists to diagnose brain death with all required indications can be explained by busyness, a lack of considering of brain death as the criterion of a person's death, lack of motivation. In all developed countries there are accepted universal rules determining the moment a person's death. Such moment is considered the end of the brain death diagnosis. In Ukraine, at the state level there are no current rules determining the moment a person's death, but the diagnosis of brain death is mentioned only in connection with the post-mortem donation, which is actually a narrow matter of determining the moment of a person's death.

Despite the considerable organizational difficulties the team of Donetsk transplant center removed kidneys in 10 cadaveric donors with asystolia; 17 kidneys were transplanted; although the actual number of donor organs could be higher.

Conclusion. Currently in Ukraine there is an urgent need for the edition of modern rules for determining a person's death and rules for determining the moment of resuscitation measures termination at the national level. Diagnosis of brain death, including maintained blood flow, should be fixed in the qualifying characteristics and functional responsibilities of anesthesiologists, neurologists and other specialists involved in intensive care.

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The contribution of Professor V. Ponomarenko in the development of transplantology

Working as the Deputy Minister of Health of Ukraine Victor Ponomarenko, made a significant contribution to the scientific basis and the definition of the state policy in the field of transplantology. On his initiative in 1994 with the purpose of organizational and procedural management, providing interoperability of institutions carrying out transplantology activities, and their informational support and control, the Ministry of Health has established a Coordinating Center (Director – Professor V. N. Bugaev). On its basis there was established Coordinating Council for Transplantation of organs, tissues and cells, which includes the heads of almost all research teams and department staffs engaged or ready to engage in transplantation issues in Ukraine. These were urologists, surgeons, cardiac surgeons, ophthalmologists, hematologists, traumatologists, endocrinologists, cryobiologists, immunologists, and healthcare managers – a total of 23 specialists. Victor Ponomarenko headed the Council, outlined the work plan and submitted it to the first meeting held in the Ministry of Health. The plan included:

1. Determination of the institutions list in Ukraine, working towards transplantation of organs and tissues.
2. Establishment of working groups to study the work of the organs and tissues transplantation centers. Preparation of proposals on their further development and specialization (personnel, technical support, and the need for additional funding).
3. Preparation of a unified service development program in Ukraine (training system, the priority development of the domestic industry of medical technology, the program of foreign technology purchases).
4. Development of prices for various types of medical care.
5. The working-out of the situation and the organization of Health Insurance Society.
6. Establishment of the Voluntary Society of donors and recipients.
7. Organization of transplantologists society in Ukraine.

Thus, these were major tasks, which would provide the impetus for further development of transplantology in Ukraine. Over the years, members of the Council on a regular basis (3-4 times a year) got together and discussed problematic issues, current problems, process of their implementation and the order of collective interaction. In the late 90s and early 2000s there was made a legal and personnel base of transplant surgery in Ukraine.

On the basis of these developments, there was passed the Law of Ukraine «On the transplantation of organs and other anatomical materials in human» in 1999. Cabinet of Ministers and the Ministry of Health published more than 20 legal documents for its implementation. An important step in the work of the Coordinating Council was the substantiation of necessity of government support in this field, followed by the adoption of government funded programs for the development of transplantation for 2002-2005 and for 2008-2012 by the Cabinet of Ministers. This allowed buying new equipment for the transplant centers and performing modern organ transplantations. There was created a specialty of transplantologist. There was approved a new specialty 14.01.08 – «Transplantology and artificial bodies»; optimized the work of Research Institute of Surgery and Transplantation of NAMS of Ukraine and transplant centers in Donetsk, Lviv, Zaporizhzhya, Odessa, Kharkiv, Dnipropetrovs'k; created the Association of Transplantologists; published journal «Transplantology»; and held on 5th Congresses of Ukrainian transplantologists. The results of Ukrainian transplantologists' studies were presented at scientific meetings abroad. Some medical universities started teaching transplantology.

The contribution of Professor V. Ponomarenko in development of transplantation was significant. To date, in great demand is a support for transplant surgery at the level of top managers who were willing and able to work as effectively as V. Ponomarenko.

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The reasons for stagnation of transplantology in Ukraine

Unsatisfactory state of Ukrainian transplantology demands comprehension of current reasons and understanding the problems by professionals.

Problems of organizational, financial, human resources and legal maintenance of organs, tissues and cells transplantation have been the subject of much discussion throughout the modern history of Ukraine. Despite an adequate assessment of the situation, grounded recommendations of professionals and fundamental possibility of solving the existing problems in a relatively short time, the positive dynamics in transplantology development has not been reached. But the most global problem of transplantology is the dominant role of money in our society.

The results of the Ukrainian Institute of Social Research survey are indicative in this regard. The participants were informed that more than 1 million lives were saved all over the world thanks to the transplantation of organs. Despite this, 42 % of respondents do not see the need for this industry development in Ukraine, mainly due to the high costs. That is, the life-saving ceases to be a priority of modern Ukrainian society in connection with certain costly process. The development of health care in general and transplantology in particular are not the priority of the authorities. The current outlook and the established order of social relations leaves no space for «trust» as a fundamental category of the decision, including the problem of transplant donation.

In this regard, transplantology experts should be aware that the current negative situation in Ukraine is unique and long enough. Due to the favorable global trends characterized by large demand and scientific progress, the results of transplantations in Ukraine, despite the low transplant activity, meet modern standards. Therefore, professionals need to continue working within the law and achieving a correct understanding of the problems in transplantology development from the government.

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Study of the effect of viable cryopreserved human amniotic membrane transplantation on the inflammation in the rabbit cornea

Considering the deficiency of donor corneal transplants, unique anti-inflammatory properties of amniotic membrane (*AM*) as well as the absence of the manufacturer, producing *AM* for ophthalmology in Ukraine, a study of the properties and possible applications of cryopreserved AM in ophthalmic surgery is important.

Purpose. To study experimentally the effect of transplantation of viable cryopreserved amniotic membrane on the clinical course of the inflammatory process in the cornea.

Materials and methods. In the experiment on the developed model of bacterial keratitis (patent № UA87119U from 27/01/2014) we studied the effect of transplantation of *AM*, cryopreserved under the protection of 10 % *DMSO* in the four-stage program of slow freezing with a controlled crystallization. That cryopreservation technology provides the safety of *AM* cell metabolism after thawing, which reduces the loss of properties of *AM* to a minimum. We performed *AM* transplantation in 30 eyes of 30 Chinchilla rabbits 14 days after the modeling of bacterial keratitis, using the technique of biological coatings. Monitoring lasted for a month. The control group included 10 rabbits with modeled bacterial keratitis treated with conventional medical therapy.

Results. Performing a surgery we noted exceptional clarity and high elasticity of cryopreserved *AM*. All animals (30 eyes) had pale pink conjunctiva at the end of the experiment. In 24 rabbits (24 eyes, 80 %) amniotic membrane was intact on the surface of the cornea, without losing its transparency. In 6 cases (6 eyes, 20 %) *AM* was missing, while the surface of the cornea was completely epithelized. In 18 rabbits (18 eyes, 60 %) we observed mild edema in the corneal stroma in the area of former defect, and in12 rabbits (12 eyes, 40 %) corneal edema was absent. Surrounding cornea was intact except 6 cases (6 eyes, 20 %) when a weakly pronounced edema of the cornea outside the defect was marked. In all cases (30 eyes) corneal vascularization was not observed. In the control group, eight rabbits (8 eyes 80 %), a slightly pronounced edema remained in the stroma of the cornea. In 7 rabbits (7 eye 70 %) fluorescein staining was observed in the defect area by type of epitheliopathy. In 4 cases (4 rabbits, 40 %) we observed vascularization of the cornea.

Conclusion. Thus, according to the described method, cryopreserved AM showed good strength and elastic properties, indicating the possibility of its use in ophthalmic surface surgery. Being non-reactive to rabbit's eye tissue, cryopreserved *AM* has a pronounced anti-inflammatory effect, stimulates epithelialization and prevents the development of corneal vascularization.

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Therapeutic keratoplasty in the rehabilitation of patients with severe inflammatory disorders of the cornea

The main causes of sight deterioration and blindness due to inflammatory diseases of the cornea are severe keratitis and corneal ulcers. Over the last decade ulcerous-necrotic processes in the cornea (infectious and neurotrophic ulcers and abscesses, autoimmune keratomalacia) are the most frequent indications for keratoplasty (*KP*) in Ukraine and represent more than 35 % of all keratoplasty. Various kinds of keratoplasty and corneal coating are used in treatment of severe inflammatory corneal diseases (*SICD*).

The **purpose** of the research is to analyze the results of keratoplasty performed with therapeutic aim in patients with severe inflammatory disease of the cornea.

Materials and methods. There were analyzed the results of 113 KP performed in patients aged 52,6 ± 14,8 years with severe inflammatory disease of the cornea. Among them 21 (18.6 %) eyes were diagnosed with severe keratitis; 84 (74.3 %) – corneal ulcers; 8 (7.1 %) – abscesses. The etiology of inflammatory processes of cornea was bacterial – 31 (27.4 %), herpetic – 29 (25.7 %), fungal – 7 (6.2 %), mixed – 11 (9.7 %), neurotrophic – 17 (15.0 %), autoimmune – 18 (16.0 %). Depending on the severity of the initial state we produced various kinds of KP: biological coating (n = 11), endothelial and deep anterior lamellar KP (n = 42), penetrating KP (classical, n = 16), and stepped KP (n = 44). In five cases we performed a partial blepharorrhaphia along with the therapeutic KP.

In 98 cases (86.7 %) ulcers were complicated by descemetocele and perforation of the cornea, in 8 – by endophthalmitis. The evaluation criteria of treatment results were the eye keeping, inflammatory process relief, the intraocular pressure level, visual acuity, and prospects for the KP with an optical purpose.

Results. An eye, as the anatomical organ, was preserved in all patients. Terms of the inflammatory process relief amounted to 20.1 ± 9.9 days. After the surgery, transparent engraftment of penetrating graft was in 33 patients (55 %), semi-transparent – in 18 (30 %), the turbid – in 9 patients (15 %). At lamellar KP the transplants engrafted transparently in 30 patients (71.4 %), semi transparently – in 10 (23.8 %), lacklustre – in 2 (4.8 %).

Intraocular pressure was within the normal range in 42 cases (37.2 %). In 44 cases (38.9 %) it was normalized by antihypertensive drugs. In 27 patients (23.9 %) we performed antiglaucomatous operation. Average corrected visual acuity was 0.2 ± 0.12 after therapeutic KP. As a result, there was a prospect of medical rehabilitation in all patients.

Conclusion. Therapeutic KP is an effective method of rehabilitation of patients with severe inflammatory disease of the cornea. It allows removing of the affected layers of the cornea, excising of inflammatory-destructive focus, relief of the inflammatory process and its further surgical treatment for an optical purpose.

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Remodeling of speech-motor function in children with cerebral palsy after administration of bone marrow multipotent mesenchymal stromal cells

Adverse pregnancy (affecting intrauterine retardation of the fetus), the use of aggressive methods of delivery (including birth trauma, cerebral hemorrhage, cerebral edema, and others) and the impact of immunogenetic factors are on the first place in the etiology of the most disabling disease in children – cerebral palsy (*CP*). As a result of literary analysis and our own research, the authors concluded that the most promising (in terms of the predicted positive effect of cell therapy) should be considered children that were born with low birth weight and spent in a condition of hypoxia less than 2 hours (including children born using assisted reproductive technologies and with *CP* features). By the time of the birth the child's brain is immature, especially in premature infants. The main damaging factor in intra- and postnatal periods is a hypoxia, which leads to ischemic brain lesion. But in newborns effects of hypoxia are not always destructive, because the brain itself has a number of compensatory abilities. Immature (but undamaged) brain is subsequently able to restore neuronal glial cells. The transplantation of multipotent mesenchymal stromal cells (*MMSC*) can accelerate and increase the efficiency of the process.

The **purpose** of this study was to develop criteria for the selection of *CP* patients for the cell therapy and assessment of *MMSC* transplantation effectiveness at this pathology.

Materials and methods. According to our patient selection criteria the study involved 12 children with cerebral palsy with double hemiparetic form (7 males and 5 females aged from 1 to 7 years). Among them 2 children were born after *IVF*. After a thorough examination and exclusion of contraindications of cell therapy we collected 15-50 ml of bone marrow from the iliac bone in aseptic conditions. A primary culture of *MMSC* was isolated by gradient centrifugation and cultured for 2-4 weeks under standard conditions. For the identification and characterization of cell cultures we used criteria *ISCT*, 2006. 12.5-50 million of cells on passage 2-3 was transplanted intravenously. The transplantation was carried out in 1-4 stages.

Results. Catamnesis ranged from six months to two years. State without changes and without deterioration was observed in 5 children. 4 children had a clear improvement in motor skills. Motility and function of speech increased in 3 children. Dynamics was consistent with scales assessing the severity of *CP*: aged 1 to 2 years used scale *INFANIB*, older than 2 years – the scale of *GMFCS*.

Conclusions. Obtained preliminary data suggest a beneficial effect of autologous *MMSC* cell therapy in the treatment of children with cerebral palsy. Thoughtful and pathogenetically reasonable selection of children with cerebral palsy allows optimizing the correct determination of the biological effect of cell therapy with the least possible amount of material application.

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Effect of cord blood stem cells transplantation on the quality of patients' life with reduced contractility of the myocardium. The first experience

To date, the prevalence of heart failure (*HF*) is called epidemic. The most radical treatment method of end stage HF is heart transplantation, but this operation is very limited. Deficiency of donor hearts did palliative measures mandatory. One of the promising but insufficiently researched method is cell therapy. Only a few studies have been devoted to the analysis of quality of life dynamics in patients with heart disease after stem cell transplantation (*SCT*).

Therefore, the **purpose** of this study was to analyze changes in the quality of patients' life with chronic heart failure after transplantation of cord blood stem cells. This purpose led to the following objectives: 1) to analyze the quality of life of patients with heart failure before and after stem cell transplantation by general and disease-specific questionnaires; 2) to determine the effect of stem cell transplantation on quality of life of patients with *HF*.

Materials and methods. The work was based on retrospective analysis of the treatment of 6 patients who underwent cord blood *SCT*. The criterions for inclusion of patients in the observation group were: stage *IIA-IIB HF*, *FC* by *NYHA 3-4*, lack of traditional conservative *HF* therapy effectiveness. Questioning of patients to assess the quality of life was performed using the procedures of the general questionnaire *SF-36* and Minnesota Living with Heart Failure questionnaire (*MLHFQ*).

Results and discussion. Assessing the quality of life of patients with *HF* with a questionnaire *SF-36* we detected changes of both physical and mental health components. Before *SCT* the physical health component in the examined patients was 34.6 ± 4.1 %. A month after *SCT*

we registered improving of physical component to 40.8 ± 6.8 %, and in 3 months improvement was up to 54.1 ± 3.9 %. Differences of the 1st and 3rd observation month after *SCT*, compared with the initial state, were reliable ($p < 0.05$). After *SCT* patients noted the improvement of mental health component. Until *SCT* it was 34.5 ± 10.6 %, 1 month after *SCT* we registered inessential improvements up to 37.4 ± 11.6 % (probability of difference from the initial state was unreliable: $p = 0.15$). In 3 months after *SCT* there was an improvement of mental health component up to 46.8 ± 14.0 % (differences from initial state were reliable: $p < 0.05$).

Similar results were received assessing changes in the quality of life related to the health status in patients with heart failure, using *MLHFQ*. Comparing the results obtained with questionnaires *MLHFQ* and *SF-36* there was a greater possibility of *HF* problem detailing with *MLHFQ*.

Conclusions. The results showed that *SCT* allows improving of the quality of patients' life with reduced contractility of the myocardium in short terms. A month after *SCT* there was registered a tendency to improve the majority of the studied parameters and in 3 months there was fixed a statistically significant difference.

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Effect of transplantation of mesenchymal stem cells on the profile of miRNAs in patients with systemic lupus erythematosus

Systemic lupus erythematosus (*SLE*) is one of the most common autoimmune diseases, difficult to treat by traditional therapy. In recent years, evidences about the applying the methods of cell-based therapies have been accumulating, including transplantation of mesenchymal stem cells (*MSCs*), in patients with *SLE*, resistant to standard treatment protocols. *MSCs* choice is proved by their anti-proliferative, anti-inflammatory and immunosuppressive properties. According to some reports, allogeneic *MSCs* have a more pronounced immunomodulatory effect than autologous *MSCs*. However, in some patients there is no response to the application of *MSCs*.

Micro-RNAs are fundamental agents of post-transcriptional control of gene expression, which play an important role in autoimmune processes and can act as a specific diagnostic marker, including *SLE*.

Aim of this study was to investigate the expression profile of *micro-RNA* panel in patients with *SLE* compared with healthy people, as well as the role of *microRNAs* in the effectiveness of allogeneic MSC transplantation in patients with refractory to standard *SLE* therapy.

In order to identify markers of *SLE* we examined in 18 patients (1 male, 17 females) aged 24-59 years. The control group was 20 healthy people of the same age. All the patients showed the expression level panels of 16 *micro-RNA* peripheral blood leukocytes fraction: *miR-146a / b,* 155, 125b, 203, 369-3p, 16, 17-3p, 99b, 29, 21, 132, 143, 145, 221, and 223.

Selection of *micro-RNA* panel was performed using *miRWalk* database. To select *microRNAs* we used a set *NucleoSpin®miRNA* (*Macherey-Nagel*, Germany), for reverse transcription a set *TaqMan® MicroRNA Reverse Transcription Kit* (*Applied Biosystems*, USA). The expression level of *microRNAs* was determined in relatively small nuclear *RNA* in the sample based on the difference of cycles ΔCt. The study revealed that there are differences between the levels of expression of various *micro-RNA* in patients with *SLE* compared with healthy people. In *SLE* patients we observed significantly increased level of *miR-16* expression, 17-3p, 21, 99b, 132, 145, 146a, 203, 223, 369-3p. These results are consistent with the literature.

To determine the effect of transplantation of bone marrow *MSCs*, there was carried out transplantation of allogeneic *MSCs* (100 million intravenously), derived from a healthy donor, into 3 *SLE* patients (women). Clinical evaluation of the patients included the study of disease activity index *SLEDAI* and index of lesions in major organs and tissues in *SLE* patients (index *SLICC*) before and after 6 months *MSC* transplantation. At the same time 2 patients had positive dynamics of the disease, and 1 patient had no positive dynamics. In the same period, the profile of micro-*RNA* was studied in the patients. There were no significant differences in the profile of *micro-RNA* 6 months before and after *MSC* transplantation.

Thus, we have identified specific differences of *micro-RNA* profile in patients with *SLE* compared with healthy people, which can be considered as a specific epigenetic «molecular handwriting» of the disease. According to preliminary data, transplantation of *MSCs* does not affect the profile of *micro-RNA*, despite the positive trend of *SLE.*

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Cell therapy of liver cirrhosis of different etiologies

According to *WHO*, deaths due to chronic liver failure take the fifth place in the world. At compensation stage of the disease 10-year survival rate is 47 %, but at the stage of decompensation 5-year survival rate is observed only in 16 % of cases. Organ transplantation is performed on

patients with decompensated liver cirrhosis (*LC*), but this method of treatment is available to only a small number of patients because of the shortage of donor organs, high material costs and others. These circumstances are a major cause of finding new, more effective and affordable treatments for patients with *LC* of various etiologies.

Thus, the **purpose** of the study was to investigate the safety and clinical efficacy of transplantation of autologous bone marrow-derived multipotent mesenchymal stromal cells (*MMSC*) in the treatment of *LC*.

Materials and methods. Cell therapy was performed on 20 patients suffering from *LC* with different etiologies. All patients underwent complete clinical, instrumental and laboratory examination before and after treatment. Transplantation was performed in 1-4 stages; cellularity of the graft was 25-50 million. Assessment of results in the form of the objective condition and laboratory and instrumental parameters was performed in 3rd, 6th and 12th months after primary transplantation *MMSC*.

Results. Transplantation of autologous *MMSC* was not accompanied by development of side reactions. 13 patients had clinical stabilization: no growth or regression of edema ascitic syndrome, lower levels of cytolysis, increasing number of platelets. In 7 patients according to ultrasound we observed a decrease in liver size and reduce of the portal hypertension (decrease of plane spleen and portal vein lumen). In all patients we marked the normalization of protein synthesis and detoxification of the liver, coagulation parameters and improvement by Child-Pugh scores criteria (on 4-24th week).

Conclusion. Autologous bone marrow *MMSC* therapy is safe and can be considered as a new approach in the treatment of *LC*. Further research is being planned.

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Efficacy of platelet-rich plasma in experimental and clinical practice

Platelet-rich plasma contains a high concentration of platelets, which are the source of a large number of cytokines: epithelial growth factor from platelets, fibroblast growth factor, insulin-like growth factor, vascular endothelial growth factor and others. This situation contributes to cell growth and differentiation, formation and growth of blood vessels, collagen synthesis, and fibroblast proliferation, i.e. a process of neoangiogenesis and tissue regeneration of liver after toxic injury.

In 2009-2014 we conducted several series of experiments on the creation of liver and reproductive system toxic injury, contact dermatitis, chondrodystrophy and others followed by the use of platelet-reach plasma (*PRP*) as a therapeutic agent in the laboratory of experimental modeling of Human Anatomy Department in Odessa National Medical University. In all cases, we obtained distinct positive effect due to activation of neoangiogenesis and tissue regeneration after experimental injury. At the same time *PRP* were used in a medical institutions in Odessa and Kyiv to correct aging changes of the face, stress incontinence, osteochondrosis, pain syndrome, etc. These results demonstrated a significant clinical efficacy of *PRP*.

Thus, the use of platelet-rich plasma can be considered as an effective method of morpho-functional organs and tissues reparation.

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Prediction of renal allograft function by the dynamics of free water clearance

The excretion of nitrogenous slag, as a standard criterion for the kidneys functional state evaluation, does not reflect the full extent of renal allograft (*RAG*) function recovery. Due to the lack of nitrogen metabolism indicators there is proposed a method, which characterizes the concentration of renal function based on the evaluation of osmotic homeostasis and determination of free water clearance (*FWC*).

The **purpose** of the study is the development of an effective method of *RAG* function predicting.

Materials and methods. The functional state of *RAG* in 26 patients, who underwent donor kidney allotransplantation, was estimated by the dynamics of nitrogen metabolism indicators (urea and creatinine levels of blood plasma) and *FWC*. Assessment of graft function was carried out in the early postoperative period, as well as in the follow-up period up to 5 years.

Results and discussion. The forecast of *RAG* function is positive when FWC is less than (-1.2) ml/min, doubt – *FWC* is from (-1.2) to (0.3) ml/min, negative – *FWC* is more than (-0.3) ml/min. Terms of *FWC* normalization in 20 (76.9 %) patients ranged from 2 to 72 days, on average (23,2 ± 17,8) days. There were not any clinical and laboratory signs of dysfunction in 6 patients; their *FWC* after a period of reduction or without it started to rise. Terms between *FWC* increase and increase of urea and creatinine levels ranged from 8 to 22 days, on average (14.6 ± 2.5) days. Assessing the dynamics of *FWC* in the remote period we allocated options of *RAG* functioning: positive in 13 (50 %) patients, negative

in 6 (23.1 %) patients, doubtful in 7 (26.9 %) patients. To determine the causes of renal disorders and chose the treatment method we carried out *RAG* biopsy.

Conclusion. Thus, the proposed method allowed to predict the possibility of *RAG* tubules dysfunction 15 days earlier compared to traditional laboratory methods (based on the changes in the nitrogen metabolism parameters). It is expedient to assess *FWC* dynamics not only for early diagnosis, but also to monitor the usefulness of renal concentration recovery, as well as specifying the type of dysfunction (acute tubular necrosis, rejection crisis, cyclosporine nephropathy). In addition, this non-invasive method can determine the indications for *RAG* biopsy for clarifying the real dysfunction cause and its prompt focused correction.

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The problem of graft's short renal vein: solutions

The standard technique of vascular anastomosis blending in kidney transplantation is properly processed and provides reliable results. However, graft with «short» vein, there is often a problem in venous anastomosis formation, particularly in kidney transplant from a living donor or autotransplantation with extracorporeal reconstruction of vessels.

The **purpose** of the study is to evaluate the effectiveness and safety of using the recipient's superficial femoral and internal iliac veins to form the venous anastomosis in kidney transplantation surgery.

Materials and methods. Since November 2008 at the Centre of organ and other anatomical materials transplants of Lviv Regional Clinical Hospital 54 kidney transplant from a living donor and 9 renal autotransplants with extracorporeal reconstruction of vessels were performed. The need for lengthening and reconstruction of «short» graft vein of the right kidney was in 6 (9.5 %) cases.

To lengthen the short graft's vein at 2 transplantations from a living donor in recipients we took 5 cm segment of superficial femoral vein just below the confluence of the deep femoral veins from lateral access in the upper third of the femur. In 2 other patients we mobilized and resected internal iliac vein before the branching. The anastomosis of the graft's vein was performed on the back-table. In all cases, the graft was placed in the contralateral iliac fossa, cold preservation was performed with Custodiol. Venous anastomosis was formed with the external iliac vein, and arterial one was imposed at the end of the internal iliac artery.

Autografting right kidney with extracorporeal reconstruction of arteries in 2 patients we performed resection of the aneurysm and plastic of critical stenosis of the arteries in the kidney gate (1) and resection of the bifurcation of the renal artery aneurysm (1). For lengthening of short renal vein we used a segment of superficial femoral vein, taken by the method described above.

Results and discussion. We obtained immediate results in all described cases of reconstruction. At further observations during 18-66 months graft function was preserved. Duplex ultrasound control did not show any venous outflow disorders. In one case, 10 days after transplantation, despite adequate antithrombotic prophylaxis, there was a phlebothrombosis successfully treated conservatively.

In most cases superficial femoral and internal iliac veins coincide geometrically with renal vein graft, providing venous outflow. Superficial femoral vein sampling or internal iliac vein mobilization is not difficult for an experienced surgeon. The choice of graft's short renal vein lengthening method should be based on intraoperative surgeon's assessment and appropriate comparison of remote results of reconstructions patency depending on the autologous venous material.

Conclusion. Superficial femoral and internal iliac veins of the recipient are effective and safe alternative in reconstruction of graft's short vein in kidney transplantation from a living donor or autologous transplantation with extracorporeal reconstruction of vessels.

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The first experience of HALS living donor nephrectomy

Objective: to analyze the results of hand-assisted laparoscopic (*HALS*) living donor nephrectomy in the first ten transplants performed at Dnipropetrovs'k transplant center.

Materials and methods. During the period from December 2012 to October 2013 there were performed 10 *HALS* living donor nephrectomies (2 of 9 in 2012, and 8 of 9 in 2013). Donors of the recipient were parents in 6 cases, a sister of the patient in 1case, and 3 other donors were married to the recipient. The donors were 6 men and 4 women; mean age was 50.2 ± 5.8 years. In all cases a mono arterial kidney was removed. According to renal dynamic scintigraphy we selected a kidney with worse function. In 8 cases nephrectomy was performed on the left, in 2 ones – on the right. Nephrectomies were performed using a retroperitoneal approach with laparoscopic port Dextrus. Clipping of renal vessels was performed with clips Hem-o-Lok, 2 clips on an artery and a vein. Transplantation was performed by a standard method of forming termino-lateral vascular anastomoses between the renal vascular graft and common iliac vessels with ureteroneocystostomy by Litch.

Results and discussion. All nephrectomies were performed without intraoperative complications, conversions were not performed. The average duration of surgery was 147.7 ± 64.3 minutes. The average anesthetics use in the postoperative period was 3.3 ± 0.92 days, and

hospital stay was 8.1 ± 0.54 days. All donors were discharged in satisfactory condition, postoperative complications were observed in 1 case as a varicocele in the nephrectomy side, which did not require surgical correction. Immediate graft function was in all recipients. Average time of thermal ischemia was 166.5 ± 29.5 seconds, and cold ischemia – 128 ± 18.8 minutes. All recipients were discharged in satisfactory condition within 10-14 days after transplantation. Postoperative complication in the form of lymphocele formation was in 1 recipient; 6 months after transplantation lymphocele fenestration was performed.

Conclusion. The first experience of *HALS* related donor nephrectomy demonstrated the safety and efficacy of this technique, highlighted the positive aspects as a good cosmetic effect and minimization of anesthetics dose. But rather long term of thermal ischemia needs further improvement of surgery techniques.

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In Vivo Survival of Murine Adipose-Derived Stem Cells in Hydrogel Composed of Carbomer 974P

Three-dimensional cell cultures on biocompatible matrixes mimic tissue structure and as a consequence may improve regeneration of damaged organs. These cultures partly simulate niche microenvironment with cell-cell interaction and so facilitate their engraftment *in vivo*.

Materials and methods. Cells from inguinal fat pad of *FVB-Cg-Tg (GFPU) 5Nagy / J* mice were isolated by fermentation with 0.1 % collagenase type *1A*. After washing the cells were seeded into culture flasks in *DMEM-F12* medium supplemented with 15 % *FBS* and cultured under standard conditions. When the cells reached approximately 80 % confluence they were sub-cultured and phenotyped by flow cytometry for *CD34*, *CD44*, *CD45*, *CD73*, *CD90* and *CD117*.

The hydrogel was prepared of carbomer *974P*, glycerol, propylene glycol, triethylamine and agarose. Then the scaffolds 10 mm diameter and 3 mm thickness were formed, lyophilized and sterilized under UV. Adipose-derived stem cells (*ASCs*) after the 2nd passage were seeded in concentration $3\text{-}4 \cdot 10^4$ cells in each hydrogel. The scaffolds were cultured for 2 weeks under standard conditions in *DMEM-F12* medium supplemented with 15 % *FBS*. The medium was refreshed every 3-4 days.

Hydrogels were transplanted subcutaneously by surgery into the back of *FVB* mice. After 4 and 6 weeks the animals were submitted to euthanasia and the transplants were removed. Cultured or excised hydrogel scaffolds were fixed in 4 % *PFA*, then the histological sections were prepared and stained with hematoxylin.

Results. The *ASCs* from murine adipose tissue were isolated and seeded into *3D* cultures of carbomer *974P* based hydrogels. On the early stages of culturing (3 days) the round shaped cells located on the surface of the hydrogels. After 7 days *ASCs* penetrated into the hydrogels, elongated and formed contacts with other cells. On the 14th day of culturing the amount of cell-cell contacts increased and cells formed a network.

28 days after transplantation hydrogels retained their volume. The invasion of recipient's cells on the periphery was observed. The number of vessels on the periphery of the grafts was higher in samples with *ASCs* compared to hydrogels without donor's cells.

Histological study showed the presence of recipient's cells at different depths from the edges in hydrogels without donor's cells. In the grafts that were previously seeded with *ASCs* significant numbers of cells with intercellular matrix production between hydrogel fibers were revealed.

Conclusions. Conditions for culturing murine *ASCs* in hydrogels based on carbomer *974P* were set. The in vivo migration of recipient's cells to transplanted hydrogel was demonstrated. *ASCs*, cultured in hydrogels based on carbomer *974P*, being transplanted in vivo survive, proliferate and produce extracellular matrix.

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Effect of cytokines and hormones on the certain pathways of the neurodegenerative disease as a possible way to improve cell therapy efficiency

Currently, tools and approaches are investigated to improve a neurotransplantation efficiency, an activation of autologous neural stem cells (*NSCs*) and a search for their new sources in a treatment of multiple sclerosis and Parkinson's disease. In this regard, it is important to study the factors of micro- and macroenvironment of *NSCs* that could affect the developmental stages of such diseases as oxidative stress, neuroinflammation with activation of microglia, the damaging effect of peripheral T-lymphocytes.

Purpose. To study on experimental models of neurodegenerative disorders, the possibility of changes in endocrine function of the thymus, as well as in the brain content of the antioxidant enzymes, T-lymphocytes and *NSCs* under the influence of melatonin and leukemia inhibitory factor (*LIF*).

Materials and methods. A model of multiple sclerosis was created in adult mice line 129/Sv by administration of neurotoxin cuprizone with food daily for 4 weeks. A group of mice received intraperitoneally 1 μg recombinant human LIF simultaneously with cuprizone. In the brain we determined fraction of *CD45*$^+$, *CD3*$^+$ *CD4*$^+$ and *CD8*$^+$ cells, and in the blood – the level of thymic serum factor (*TSF*). To create a model of hemiparkinsonism adult rats were stereotaxically injected into the left uplink forebrain bundle with 6-hydroxydopamine (*6 HODA*), which is selective to dopaminergic neurons. A week later, we studied the degree of unilateral degeneration of dopaminergic neurons using apomorphine behavioral tests. A part of experimental rats with behavioral asymmetry in the test and without it (unilateral degeneration degree of dopaminergic neurons of the substantia nigra is approximately 96 % and 44 %, respectively) were injected intraperitoneally with melatonin at 1800 every day for 4 weeks, 10 mg/kg. In the striatum, we determined the levels of superoxide dismutase, catalase, glutathione peroxidase, and in the culture of the olfactory bulb (*OB*) – *NSCs* by share of nestin-positive cells.

Results. 1. Proportion of *CD45*$^+$, *CD3*$^+$ and *CD4*$^+$-cells in the brain of mice treated with cuprizone is higher than in the group without cuprizone (2.4, 1.4 and 1.4 times respectively).

After the *LIF* administration figures approach the control values. Level of *TSF* increases in blood, which lowers the levels of proinflammatory cytokines, regulates the differentiation of T-lymphocytes in the thymus and their migration to the periphery.

2. In the rats treated with *6-HODA* the intensity of antioxidant enzymes reduction in the striatum and *TSF* in the blood correlates with the level of dopaminergic neurons degeneration and the percentage of *NSCs* in *OB* cell culture. Thus, the increase of nestin-positive cells proportion is characteristic to the rats without behavioral asymmetry (up to 99.3 % versus 91.2 % in controls), in which the changes in the of enzymes level in the striatum and the function of the thymus were the lowest. The course of melatonin increases the content of antioxidant enzymes and *TSF*, and retains proportion of nestin-positive cells in rats without behavioral asymmetry.

Conclusion. The results can be useful in the development of new biotechnological approaches to the treatment of multiple sclerosis and Parkinson's disease.

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Effect of endocrine factors on the biological properties of multipotent mesenchymal stromal cells and hematopoietic bone marrow stem cells in animals

Currently multipotent mesenchymal stromal cells (*MMSCs*) and hematopoietic stem cells (*HSCs*) of bone marrow are widely used in regenerative medicine. Realization of their biological properties can take place with the participation and interaction of the pineal gland, the adrenal cortex and the thymus.

Purpose: to investigate the biological properties of *MMSCs* and mouse bone marrow *HSCs* by varying the concentration of melatonin, corticosterone and thymic serum factor (*TSF*).

Materials and methods. Mice *CBA*, *FVB*, *129/Sv* lines were used. Clonogenic potential of cultured bone marrow cells was determined by the number of colony forming units of fibroblast (*CFU-F*), and granulocyte-macrophages (*CFU-GM*). *MMSCs* ability to osteogenic differentiation was evaluated qualitatively by staining cultures with Alisarin Red S and then semiquantitatively by colorimetric method. Immunomodulatory properties of *MMSCs* were detected in the reaction of the mitogen-induced blast transformation of splenocytes. The level of melatonin, corticosterone and *TSF* was examined in the blood. In experiments in vivo melatonin was administered once at 1800, 0.1 mg/kg; surgical stress was modeled in a group of mice. In tests *in vitro*, bone marrow cells were cultured with melatonin (100 pg/mL), *TSF* (10 ng/ml), and corticosterone (25 ng/ml) (*Sigma*, USA).

Results. *1. Biological properties of MMSCs and bone marrow HSCs in mice under conditions of natural fluctuations of hormone levels.* The level of TSF at 9^{00} a.m. is 3 times higher than at 14^{00} ($p < 0.05$), whereas corticosterone is lower. Relations between activity of the epiphysis, adrenal glands and the thymus vary throughout the year and depend on the mouse strain. Melatonin and *TSF* levels decrease with the age. During cultivation of bone marrow cells obtained from adult mice in the morning, the number of *CFU-GM* is higher than while taking the material in the afternoon (48.9 ± 13.4 % and 12.4 ± 6.1 % respectively, $p < 0.05$). There is a difference between the number of *CFU-F* and *CFU-GM* at bone marrow harvesting in the morning in different seasons. Thus, in summer and winter the numbers of *CFU-F* were higher ($p < 0.05$), than in other seasons; *CFU-GM* values were the highest in spring and autumn. The ability of *MMSCs* to the formation of *CFU-F* increases with the age in mice *FVB*; the ability of cells to the osteogenic differentiation in *FVB* line mice is 1.8 times higher than in 129/v mice.

2. Properties of MMSCs and bone marrow HSC under simulation of the changes in hormone levels in vivo and in vitro. Injections of melatonin change the number of *CFU-F* in *CBA* mice bone marrow, depending on the season. Under stress conditions the orientation of changes in *CFU-F* and *CFU-GM* number in the bone marrow depends on the mouse line. Corticosterone in vitro reduces the number of *CFU-F* in the bone marrow. *TSF,* added to the culture, increases the number of *CFU-F*, enhances osteogenic differentiation of *MMSCs* and reduces their inhibitory effect on mitogen-induced proliferation of splenocytes.

Conclusion. Biological properties of *MMSCs* and bone marrow *HSCs* vary depending on the time of day, season of the year, the age and mice line. Functioning of the epiphysis, adrenal cortex and thymus is important in the implementation of these changes. The results can be useful in tissue engineering technologies and development of individualized approaches to cell therapy.

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Antitumor activity of humoral factors of mesenchymal stem cells

Mesenchymal stem cells (*MSCs*) have a broad spectrum of biological properties such as ability to differentiate into various cell histotypes and synthesis of functional activity of more than 30 molecules, including *NO*, *IDO*, chemokines, and other cytokines. The ability of *MSCs* to the synthesis of various humoral factors is not a constitutional property; it is determined by their microenvironment and developmental stage. Many problems associated with the production of *MSC* humoral factors are not studied yet, including the possibility of their practical use in the inhibition or stimulation of other cell functions.

The **purpose** of this study was to obtain *MSCs* humoral factors at the various terms of cultivation and study their antitumor activity.

Materials and methods. We studied the biological activity of the supernatants 4-7 days (first) passage and the 18-25-day period of cultivation (3-4 passage) of adherent cells of the bone marrow or adipose tissue. Cultivation of adherent cells was performed according to a standard protocol. Nutrient culture medium was collected and combined into a common pool and stored at t = -20 °C.

A study of humoral factors effect on the tumor process was performed on an experimental model of intracerebral glioma 101.8 in rats, as well as in the glial cells culture of human tumors in vitro in the *MMT* assay.

Results. Introduction of the «early» or «late» supernatants on 3rd, 5th, 7th, 10th day in rats with a brain tumor practically had no effect on survival time – the average life span of rats was 25-28 days, in both experimental and control groups. In some series of experiments, in particular, at combined administration into the brain, there was statistically insignificant slowing of tumor growth.

Supernatants of both early and late-term of cultivation in culture in vitro with brain tumors cells 1.5-1.6 times stimulated proliferation of tumor cells according to the *MTT* assay. Stimulation of tumor cells proliferation by the supernatants manifested itself in 48 hours and was increasing up to 96 hours cultivation term. Supernatants of late cultivation term had higher catalytic activity than early supernatants. Cells of malignant tumors, such as glioblastoma and anaplastic astrocytoma, proliferate under the effect of factors faster than benign tumor cells.

Conclusion. Thus, in *MSCs* culturing, since the first passage, active molecules are allocated into the culture medium. They are not able to inhibit but stimulate proliferation of human tumor cell cultures. At the same time, when administered into animals with brain tumors, there was not revealed their stimulatory action on the tumor, which can be explained by insufficient dose of these factors in tumor lesions in rat brain, and their lack of an indirect inductor mechanism of action. By their nature, these *MSCs* humoral factors can be categorized as a group of cytokine growth factors. It can be argued that these factors can be already allocated from the 1st passage by immature *MSCs*, and their progenitors.

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Morphological and functional heterogeneity of mesenchymal stem cells

It is generally known that mesenchymal stem cells of adult individuals (*MSCs*) are not only able to differentiate into various cell types, but also regulate the activity of proliferative and immune processes. The source of *MSCs* is adult tissue, which contains large amounts of adhesive to the plastic fibroblast cells. During their prolonged cultivation *MSCs* are formed. There is no single standard protocol of *MSCs* obtaining from adherent cells of the bone marrow or adipose tissue. Duration of cultivation and number of adhesive proliferating cell passages are not the same in works of different authors and amounts to tens of days, suggesting the passage of several stages of transition (transformation) of adherent cells in *MSCs* during cultivation. We have allocated (*Lisyanyy M. I.*, 2013) at least 4 stages of adherent *MSCs* development and 4 different types of cells: primary, adherent fibroblast cells («sleeping» *MSCs*), activated proliferating fibroblasts, *MSCs* precursor (*pro-MSCs*) and activated *MSC* (mature *MSCs*), which are capable to differentiate into other cell types.

Each of these stages of *MSCs* development from adherent cells has not been clearly described yet. Every type of cells involved in *MSCs* generation, probably, have their phenotypic characteristics and different functional activity, which can be realized either by the contact interaction or by synthesis of active molecules (cytokines, *NO*, and *IDO*).

There are numerous data on the various, sometimes contradictory, properties of *MSCs* and their precursors, in particular, on the effects on the immune, regenerative processes in the body. The investigations (*Lisyanyy M. I. et al.*, 2013) showed that supernatants of 24-48 hour culture of the first passage of adipose tissue adherent cells inhibit lymphocyte proliferation with phytohemagglutinin (*PHA*); and supernatants of 3-4-day culture or second passage cells stimulate both spontaneous and induced by *PHA* lymphocyte proliferation.

Functional differences between various types of *MSCs* progenitors were shown in cutaneous wound healing model in the experiment with mice and rats. Thus, two-fold administration of adherent cells of 24 or 48-hour incubation primary passage («dormant» *MSCs*) into a skin wound on the back of mice or rats resulted in a significant inhibition of healing. Whereas an administering of long cultured cells (15-25 days of culture or cells of passage 3-4) resulted in stimulation of skin regeneration and more rapid healing of a skin wound (*Lisyanyy M. I. et al.*, 2013). Consequently, cells and supernatants of short-term (24-48 hours) cultures of primary passages inhibit lymphocyte proliferation and

regeneration of skin cells, delays wound healing. Cells and supernatants of prolonged adherent cell culturing stimulate these processes, which coincides with the published data on *MSCs* ability to inhibit or stimulate the immune and proliferative processes.

Our experimental data coincide with numerous published data and indicate a functional heterogeneity of adipose tissue and bone marrow adherent cells, depending on the duration and amount of culture passages. This indicates a few stages of their development, transitioning into each other, from progenitors to *MSCs*.

The influence on regenerative processes in skin and immune system function can be performed directly by cells or by humoral factors produced by them.

Further researches in this area will clarify certain stages of development, types of progenitors and *MSCs*, and their biological activity. That has an important theoretical and practical value and expands the possibility of using *MSCs* or their precursors for regulation of immune and regenerative processes in the body, study of the nature of regulatory humoral factors, synthesized by *MSCs*, and providing biotech analogs suitable for clinical application.

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11 years of cord blood banking in Ukraine

In Ukraine the first cord blood transfusion for the correction of blood loss is dated 1933, and the first cord blood samples were frozen in 1984. At those time, little was known about cord blood value as stem cells source. It was considered as accessible alternative to the donors' blood.

The development of cord blood banking in the world after the first cord blood transplantation in 1988 performed by Eliane Gluckman, also induced the launching of this industry in Ukraine. In 2003, the first cord blood bank in Ukraine was created in the Institute of Cell Therapy. In coming years 3 more cord blood banks were founded, all of family type. Today about 15 000 cord blood samples are stored in the Ukrainian banks (about half of them in the Institute of Cell Therapy). Actually the population of Ukraine makes 48 millions and cord blood is collected in about 0.6 % births, in neighboring Russia – in 0.3 %. All cord blood banks in Ukraine are required to have appropriate license.

In 2008, the Institute of Cell Therapy Clinic got permission of the Ministry of Health for the conduction of clinical trials on the evaluation of the efficacy of cord blood stem cells in the treatment of pancreonecrosis and lower limbs ischemia. Consequently in 2012, the stem cells based methods of treatment of these diseases, developed by the scientists of the Institute of Cell Therapy were approved for clinical use. Actually Institute of Cell Therapy is conducting 3 more clinical trials on the treatment of diabetes mellitus, liver cirrhosis and cardiomyopathies using cord blood stem cells. The experience of cord blood application in Ukraine counts more than 200 allogeneic transplantations in clinical trials. There are also sporadic reports on the autologous cord blood application, mainly for the treatment of neurologic disorders.

Hence, during the last decade Ukraine made a huge advance in creation of the cord blood banking industry and bringing into clinical practice the methods of stem cells based treatment of a number of diseases. The next step in the development of this field in Ukraine should implicate the creation of the public cord blood bank, especially due to high rates of oncologic morbidity and ethnic diversity of the population.

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Activity of various types of multipotent cells in the treatment of post-radiation bone marrow syndrome in mice

Currently the study of different stem cells properties is one of the fastest growing and promising research areas in biology and medicine. The role of hematopoietic stem cells (*HSCs*) and multipotent stromal cells (*MSCs*) in physiology and pathology is being studied from the new points. It is considered that *HSCs* are the lymphocytes precursors and auxiliary cells; and *MSCs* are more involved in the formation of bone marrow and thymic niches of *HSCs* and make up the lymphocytes microenvironment at their differentiation, proliferation and apoptosis in normal and pathological conditions. Multipotent cells play a major role in the processes of regeneration and reparation. However, works on comparative characterization of activity in the direction of various types of multipotent cells are virtually absent. Meanwhile, these aspects are important for theoretical immunology and practical approaches in cell therapy.

The **purpose** of the study is to investigate the effect of different types of hematopoietic and multipotent stromal cells on the survival and the average life expectancy of lethally irradiated mice.

Materials and methods. We used immunology, radiobiology, cultural and statistics methods. Lethally irradiated mice *CBA* line on the following day were administered intravenously with $0.5 \cdot 10^6$ syngeneic cells of 13-14 dpc fetal liver (*FL*); or $0.5 \cdot 10^6$ cells of human *FL* (9-week gestation); and such cells precultivated for 24 hours in vitro with mouse thymus *MSCs* colonies; and 10^4 of untreated thymus *MSCs* collected from the cultures with a large number of fibroblast colony forming units.

Results. A group of animals receiving normal cells of syngeneic or human fetal liver had higher survival rate and average life expectancy. These figures increased with the administration of fetal liver cells of mouse and human precultivated with thymus *MSCs*. It is also important that thymus *MSCs* showed a significant activity. The effect of *MSCs* may be caused by contact stimulation of *HSCs* and their precursors, which leads to faster recovery of the immune system with post-radiation bone marrow syndrome treatment.

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Evaluation of respiratory function failure in renal transplant recipient

The constancy of bronchopulmonary temperature homeostasis is one of conditions for normal work of some important non-respiratory functions of the respiratory system, in particular, of the local immune system. A detailed study of the conditioning function of the respiratory system (*CFRS*) can improve the diagnostics, evaluation of the treatment effectiveness, prognosis, and prevention of respiratory diseases.

For identifying and differential assessing the *CFRS* failure we used a method for measuring the respiratory heat exchange. To determine the diagnostic significance of this function of the respiratory system we compared clinical-anamnestic data (cases of inflammatory lung disease during the last year), depending on the degree of *CFRS* failure.

We examined 49 renal allograft recipients aged from 17 to 56 years that made up the main group. The criteria for inclusion in the group were the end-stage renal failure, prolonged hemodialysis, and lack of acute inflammatory diseases of the respiratory system or chronic diseases in the acute stage.

All patients had different degrees of *CFRS* failure. *CFRS* failure stage I was detected in 5 (10.2 %) patients (subgroup 1). In this subgroup 1 (2 %) patient had an acute bronchitis, 4 (8.2 %) patients had no lung disease in the previous year. *CFRS* failure stage II had 30 (61.2 %) patients (subgroup 2). Among this subgroup 23 (46.9 %) patients had an acute bronchitis, 3 (6.1 %) patients had an acute lobar or segmental pneumonia of different localization, 4 (8.1 %) people had no diseases of bronchopulmonary system during the previous year. 15 (30.6 %) patients had *CFRS* failure stage III (subgroup 3). Among this examined subgroup there were 8 (16.3 %) patients with an acute bronchitis, 7 (14.3 %) with an acute lobar or segmental pneumonia of different localization. One patient with *CFRS* failure III suffered an acute lobar pneumonia and an acute bronchitis. In this subgroup there were no patients without lung disease in the previous year.

Based on these data we can conclude that with the amplification of *CFRS* failure there increases the frequency of clinical manifestations of bronchopulmonary inflammatory diseases ($r = + 0.726$, $p < 0.05$) and their severity ($r = + 0.643$, $p < 0.05$).

Thus, the differential evaluation of *CFRS* failure may be one of the noninvasive diagnoses of the respiratory system condition in order to determine the need for treatment and preventive measures aimed at reducing the frequency and severity of complications in the post-transplant period.

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The first experience of autologous chondrocytes transplantation in the intervertebral disc after lumbar microdiscectomy

The cells of nucleus pulposus and annulus fibrosus of the intervertebral disc (*ID*) have no regenerative nature, so the treatment of these structures lesions is considered to be mainly symptomatic. The priority should be technologies that can stop the development of degenerative process with the restoration of biomechanical properties of the intervertebral disc. One of the most modern and promising areas of biotechnology is the transplantation of autologous chondrocytes into intervertebral disc of the patients after microdiscectomy.

The **purpose** of the study is to develop and implement in clinical practice a high-tech method of herniated disc treatment at the lumbar level with autologous chondrocytes.

Materials and methods. During 2012-2014 the study included 6 patients, three men and three women. Primary surgery was performed for *ID* hernia at the lumbar spine. In 4 patients it was performed in the *L5-S1* location, in 2 patients – in the *L4-L5* location and *L3-L4* location, respectively. In 5 patients the paramedian hernia was lateralized on the left, in only one – on the right. *ID* hernias had signs of sequestration, and had no foraminar or extraforaminar localization. The material of removed hernia was delivered in a sterile environment to the Institute of Cellular Therapy in 1-2 hours. Selection of healthy chondrocytes from hernia mass and then cultivation and cryopreservation of autologous chondrocytes culture were carried out. In 4 patients transplantation was performed during the period from 3 to 4 months after initial surgery, in the other two – in a period of 4-5 months. The first transplant was performed 16 months ago, three – 14 months, and two – a month ago.

Transplantation was performed in the operating room under X-ray control. To do this, under the local anesthesia, we performed percutaneous *ID* puncture with needle *11G*, trying to bring the tip of the needle to the center of the disk. Culture of chondrocytes was introduced with 1.5 ml of the suspension and then the needle was removed with the mandrin. The patients were permitted to move in a vertical position and leave the hospital 3 hours after the operation.

Results. Clinical evaluation of patients was carried out before primary surgery, before transplantation and at 3, 6 and 12 months after transplantation. The evaluation was based on objective status, neurological examination, and assessments by the Visual Analogue Scale (*VAS*), *NPS* and *NASS*. *MRI* examination of the lumbar spine was performed before surgery, before transplantation, and 3 and 12 months after transplantation. Currently there are available results of 4 patients *MRI* in the period after 12 months. Patients keep *ID* height and have a marked rising of hydrophilic nucleus pulposus level in all observations. Participants in this study feel well, kept a positive effect of the microdiscectomy. There were no side effects. Research is being carried out, patient enrollment continues.

Conlusion. The development of modern biotechnology offers a hope for a new stage in surgery for *ID* degenerative lesions, since surgical treatment is aimed not only at eliminating of symptoms, but also at anticipation of further progression of *MD* degeneration. The study methodology aims not only to keep the height of the intervertebral disc and partially restore its hydrophilicity, but to return lost biomechanical properties to nucleus pulposus.

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Biological properties of murine bone marrow multipotent mesenchymal stromal cells cultured under of different oxygen content

The development of cell therapy requires optimization of cultivation conditions for a sufficient number of cells with preservation of their functional properties.

Purpose. To investigate and compare the biological properties of bone marrow multipotent mesenchymal stromal cells (*MMSC*) of *FVB* mice at cultivation in standard (21 %) and low oxygen (5 %) conditions.

Materials and methods. Experiments were performed on mature females of *FVB* mice. *MMSC* cultivation was carried out by standard protocols in multigas incubator Binder *CD210* (*Binder,* Germany) with the oxygen content of 5 % and 21 %. The phenotype of *MSCs* was assessed by expression of surface markers using monoclonal antibodies (*BD Biosciences*, USA) by flow cytometry. Osteogenic differentiation was evaluated by Alisarin Red S staining and by the degree of mineralization with semiquantitative colorimetric method. Potential to differentiate in adipogenic direction was evaluated by detection of lipid inclusions with Oil Red O dye. Proliferative activity of cells was determined by number of population doubling.

Results. There was discovered a stimulating effect on the rate of cell proliferation under conditions of 5 % oxygen. Cumulative number of population doubling at cultivation for 4 passages was 12.36 at 5 % oxygen content and 8.99 at 21 % oxygen content.

Determining the phenotype of murine bone marrow *MMSC*, an expression of surface markers, characteristic to progenitor cells, remains under conditions of low oxygen content. Thus, at the 2nd passage under conditions of 5 % O_2 *CD90* expressed from 91.3 to 97.3 % cells, under conditions of 21 % O_2 – from 94.1 to 99.4 %. This high level of expression was characteristic to the surface marker *CD44* – from 95.2 to 98.4 % (5 % O_2) and from 93.8 to 98.5 % (21 % O_2); and for *Sca-1* – 91.0 to 94.1 % (5 % O_2) and from 95.1 to 98.8 % (21 % O_2). Expression of *CD73* increased from 84.3 to 95.0 % cells at 5 % oxygen, and from 25.1 to 56.6 % cells at 21 % oxygen.

MMSCs of mice bone marrow are capable to aimed osteogenic differentiation both at 5 % and at 21 % oxygen. At the same time, osteogenic differentiation was more effective at 21 % oxygen: index of optical density at the 5 % oxygen amounted 3.19 ± 0.04 cu at the 21 % oxygen – 3.42 ± 0.06 conventional unit. The difference in the ability to stimulate adipogenic differentiation was not identified at a qualitative level.

Conclusion. Reduced oxygen content has a stimulating effect on the proliferation *MMSC* of mice bone marrow. Osteogenic differentiation was more effective at 21 % oxygen. These results can be used in tissue engineering technologies to improve the efficiency of bone marrow progenitor cells expansion and their directed differentiation.

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Plasticity of bone marrow-derived stromal cells at grafting onto neural tissue after ischemic injury *in vitro*

Bone marrow-derived stromal cells (*BMSCs*) are able to confer beneficial effects after transplantation into neural tissue with ischemic brain injuries. This effect is probably mainly caused by the release of trophic factors, though the possibility of dead neural cells being replaced by *BMSCs* cannot be excluded.

The aim of this study was to determine whether the neuroprotective effects in question are depended on direct cell-cell contacts between BMSCs and injured tissue.

Materials and methods. We therefore investigated that interplay in an in vitro model of hippocampal organotypic slice culture (*OHCs*), in order to avoid the interference due to immunological rejection processes following *in vivo*. To induce ischemia the oxygen-glucose deprivation (*OGD*) of *OHCs* during 10 minutes was carried out. The hippocampal slices of *FVB* mice (*P8*) were cultivated in the *MEM* medium. At day *7 OGD* was induced and after 2 hours of reoxygenation *BMSCs* were transplanted/grafted onto the slices ($2.5 \cdot 10^5$ cells per slice). *BMSCs* were isolated from the transgenic *FVB-Cg-Tg(GFPU)5Nagy/J* mice. On 3, 7 and 14 days after the transplantation the fluorescent immunohistochemical staining was carried out to analyze the differentiation of *BMSCs*.

The hippocampal slices were incubated with following primary antibodies: *anti-GFAP* (marker of astrocytes, 1:1000) (*DAKO*, Denmark); *anti-NeuN* (marker of neuronal nuclei, 1:1000) (*DAKO*, Denmark); *anti-Iba-1* (marker of microglia, 1:1000) (*Wako*, Japan); *anti-olig-2* (marker of oligodendrocyte nuclei, 1:1500) (*Chemicon*, Great Britain); *anti-nestin* (marker of neural stem cells, 1: 750) (*Chemicon*, the USA); *anti-GFP* (1: 500) (*Molecular Probes Inc.*, USA). Slices were incubated with secondary antibody against appropriate species: *anti-rabbit Alexa Fluor 647*, *anti-goat Alexa Fluor 488, anti-mouse Alexa Fluor 568* (1: 500) (*Molecular Probes Inc.*, THE USA) during 2 hours at 4 ^{0}C. Confocal images of grafted GFP-positive *BMSCs* were acquired with *FV1000* (*Olympus*, USA) laser scanning microscope.

Results. We have shown that *BMSCs* also are able to confer beneficial effects after transplantation into neural tissue with ischemic injury. This effect is probably caused by the release of trophic factors, although the possibilities of replacement of dead neural cells by *BMSCs* are not excluded. The potential *BMSCs* to differentiation on neural-like was evaluated for 14 days after ischemic injury. At day 7 after the oxygen-glucose deprivation and subsequent transplantation cells differentiated into microglial cells, and at day 14 *BMSCs* differentiated just as into microglial cells so too differentiated into mature oligodendrocytes. These findings suggest that the transplanted stem cells respond to signals from the microenvironment of the injured tissue of the recipient, which in turn may trigger and regulate cell differentiation as well as to determine the direction of migration.

Conclusions. Thus co-culturing of the *OGD*-treated slices with *BMSCs* significantly improves the morphological status of the ischemic neural tissue. Stem cells in our model have shown neuroprotective effects. The direct mechanism of stem cells action in ischemic brain is still unknown. But we assume that such an improvement of morphological and functional state of the ischemic tissue takes place through activation of neuroprotection, neurogenesis, synaptogenesis and due to growth factors.

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Quality control of cryopreservation and testing of cord blood samples. Experience of Cryobank of Institute of Cell Therapy

Cord blood banking is widely spread and integrated in many national health programs for treating inherent and malignancy disorders. Cord blood units (*CBUs*) are cryopreserved by different methods using various cryobags, vials, cryoprotectant compositions thus appropriate freezing programs should be developed. Cooling speeds on different steps of freezing has significant influence on viability of hematopoietic stem cells (*HSCs*). Thus quality control of the main critical steps of processing and testing of cord blood units is very important for routine banking and releasing samples for clinical application.

Cord blood was collected *in utero* in the blood collection bags with *CPDA-1*, erythrocytes were depleted by *HES*-sedimentation, and plasma was reduced by centrifugation, cord blood cells (*CBCs*) were cryopreserved with *DMSO/Dextrane 40* solution in vials using software based freezer. Colony-forming units (*CFU*) analysis was performed with culture medium *MethoCult* (*StemCell Tech.*, Canada). *CFU* number was calculated as number of colonies on 10^5 seeded *CBC, CFUs* recovery was calculated as the ratio (%) of *CFU* number of thawed *CBCs* to *CFU* number of prefreeze *CBCs*. Data was expressed as median and interquartile range. P values less than 0.05 considered statistically significant.

Microbiological analysis was performed with charcoal-based media in blood culture bottles (*Biomerieux*, France). Cord blood was tested on total antibodies against *HIV1/2*, *HCV*, *HBcor*, *Treponema pallidum*; *DNA HBV*, *DNA HIV1/2*, *DNA CMV* and *RNA HCV*.

CFU number of *CBUs* cryopreserved with 10 % *DMSO* and freezed with slow cooling (-1 °C/min) from seeding (crystal formation) to -30 oC significantly higher compared with the fast cooling (-5 °C/min) such as 103 (88-115; n = 13) to 54 (19-96; n = 30). Similar difference was observed in case cryopreservation *CBUs* with 5% *DMSO*, such *CFU* number of samples freezed with slow cooling was 103 (94-150; n = 10) significantly higher than ones freezed with fast cooling 45 (12-96; n = 13).

Furthermore the number of *CFU* of prefreezed *CBUs* was correlated with final *CPDA-1* concentration in the blood collection bag (r = -0.54, n = 18). *CFU* recovery of *CBUs* depends of shipping time of CBUs that were cryopreserved with 5 % (r = -0.57, n = 13) and 10% (r = -0.63, n = 29) *DMSO* when samples were freezed by fast cooling in contrast to slow cooling where no significant correlation were observed. *CFU* number of *CBUs* cryopreserved with 5 % and 10 % *DMSO* and slow freezing did not significantly differ.

Thus we showed that *CBUs* cryopreserved in vials has better viability when freezed by slow cooling from seeding to -30 oC. Controlled crystal initiation gather with slow cooling give opportunity to decrease concentration of *DMSO* from 10 % to 5 %.

Microbiological analysis was shown 12.6 % of CBUs (n = 419) were non sterile especially about 6 % of CBUs contained Staphylococcus spp., 4 % and 1 % of *CBUs* contaminated by Enterococcus spp. and Escherichia coli respectively. Infection screening revealed 6.4 % of *CBUs* (n = 419) were positive for antibodies against *HBcor*, 1.6 % – *HCV*, 1.9 % – Treponema pallidum . Nucleic acids of Hepatitis B, C and *HIV1/2* were not detected by real time *PCR* in the *CBUs*. *DNA* of *CMV* was detected in the 1 *CBU* (n = 2012).

Therefore in the Cryobank of Institute of Cell Therapy about 22 % of CBUs (n = 670) could be discarded by the microbiological and the infection screening as a potentially infected units.

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Formation of multicellular aggregates in the survival of isolated neural cells of rats

The **purpose** of study is to investigate the effect of multicellular aggregates formation on a survival of heterogeneous suspension of neural cells (NCs) of fetal brain and neonatal rats after their isolation and low temperature storage.

Materials and methods. NCs total suspension was obtained from brain tissue of 15 days gestation fetuses and newborn rats, and then cultured in vitro at a concentration of $4 \cdot 10^6$ cells/ml in the medium *DMEM/F12*, supplemented with 10 % rat serum. Hypothermic storage was carried out at a temperature of +6-8 °C. NCs were frozen under the protection of 10 % *DMSO* with subsequent storage at -196 °C. As a storage medium we used *DMEM/F12* in the presence or absence of 10 % serum.

Results. Freshly isolated heterogeneous *NCs* of fetuses and newborn rats formed multicellular aggregates after a few hours of culturing at the presence of serum. In this case the viability of cells in the composition of aggregates increased 1.5-2 times compared to original cells. After aggregates attachment to the substrate their cells migrated and flattened, forming neurons, glial cells and later a monolayer, which formed such neuroblastoma cells and colonies of stem/progenitor cells. During cultivation of individual cells most flattened cells had glial morphology. At the same time there was no formation of a monolayer, neuroblastoma cells and undifferentiated cells colonies.

Hypothermic storage in aggregates provided a higher effective storage time of *NCs* of fetuses and newborn rats in which the functional active neurons, glial cells and stem/progenitor cells retained, compared with a suspension, 2.7 and 2 times, respectively. During *NCs* storage in aggregates, the presence of serum in the medium did not affect the efficiency of storage.

Cryopreservation of *NCs* in aggregates was accompanied by retention of the number of functionally active neurons, glial cells and stem/progenitor cells at the level of the initial cells. This also allowed to refuse the use of serum, which was mandatory for cryopreservation of suspensions.

Thus, our study suggests that the association of *NC* in aggregates leads to a reconstruction of cell microenvironment lost in the selection process. This provides more preferable, compared with a cells suspension, survival conditions and efficient functioning of differentiated and stem/progenitor cells after separation and subjected to low temperature storage. Cultivation of *NCs* in the form of aggregates is probably accompanied by the accumulation of the E-cadherin adhesion protein, which presumably plays an important role in protecting cells from apoptosis.

Conclusion. Aggregates are multicellular three-dimensional structures, in which create the conditions for the recovery of non-lethal damage, survival, and effective functioning of both differentiated and stem/progenitor *NCs*. These aggregates could potentially be used as micromodels of neural tissue for biological, pharmacological and medical investigations.

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Transplantation of multipotent mesenchymal stromal cells of adipose tissue in perinatal pathology of CNS

Perinatal *CNS* injury is one of the most relevant medical and social problems of modern neurology and pediatrics, and results in both high mortality of young children with pre- and perinatal *CNS* disorders and a large proportion of this pathology in the structure of children's disability.

To simulate perinatal damage in the nervous tissue we used a periventricular leukomalacia injury (*PVL*). *PVL* model was realized by unilateral coagulation of common carotid artery in *FVB* mice six days after birth (*P6*), which corresponds to the human perinatal period. An hour after coagulation animals were placed in a hermetic chamber with 6.0 % O_2 for 35 minutes. To create hypoxic-ischemic injury in combination with inflammation, we administered 0.015 ml endotoxin lipopolysaccharide (*LPS*, 1 mg/kg) abdominally.

After the *PVL* modeling, operated animals lagged in development compared to the control pseudo-operated mice. Animal with *PVL* model had lower weight and height and disorders of statokinetic reflex, which provides the body to maintain the balance in active or passive displacement of it in space.

Mouse line F*VB-Cg-Tg (GFPU) 5Nagy/J*, transgenic gene by *GFP*, were used as donors of multipotent mesenchymal stromal cells (*MMSCs*) that were isolated from subcutaneous adipose tissue of animals and transferred into a culture. Fibroblast-like cells with high adhesiveness diameter 80 mm with a large number of vacuoles and granules, dominated in *MMSCs* culture at the 2nd passage. When phenotyping *MMSC* culture of adipose tissue of the 2nd passage by flow cytometry using a laser fluorimeter cyto-sorter FACSAria (*«Becton Dickinson»*, USA) we revealed a high expression level of markers *CD44* (96.6 %), *CD73* (63.4%), *CD90* (92.1 %), while the relative content of cells expressing hematopoietic markers *CD45* and *CD117* was less than 2 %. At early passages *CD34* expression ranged from 8-12 %, which is typical for adipose tissue *MMSCs* and can be a sign of a higher potential of cell differentiation in endothelial direction. There was demonstrated the ability of cells to differentiation in osteogenic and adypogenic directions. Cell viability was 92.4 % after transfer of adhesive culture into suspension for transplantation.

Syngeneic transplantation of *GFP*-positive *MMSCs* suspension into seven days (*P7*) animals with *PVL* model was performed stereotacticly (1.5 mm behind and 2.0 mm aside from bregma and 2.0 mm in depth from the skull surface) in the right hemisphere of the brain under intraperitoneal calypsol-xylazine anesthesia 24 hours after *PVL*. For transplantation there was selected an optimal amount of culture medium *RPMI-1640* (*Sigma*, USA) and a dose of cells in it. The optimal dose was $5 \cdot 10^5$ cells in 2 ml medium. The animals of the control group were administered with culture medium in appropriate volume.

Syngeneic stereotactic *MMSCs* transplantation improved neurological status of experimental animals after *PVL* modeling, contributed to the restoration of behaviors and stato-kinetic reflex. Thus, the data suggest that *MMSCs* transplantation may have a therapeutic effect in the treatment of perinatal lesions of central nervous system in the experiment.

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Arrhythmogenic risks in patients with myocardial lesions during transplantation of cord blood stem cells in short terms

Accumulated experience of cell therapy has shown that certain types of stem cells (*SCs*) may have arrhythmogenic effect. In particular, they are skeletal myoblasts. Mesenchymal *SCs* have also had a significant risk of arrhythmias. The promising ones, in terms of arrhythmological security, can be *SCs* of umbilical cord blood (*CB*), because they have a genetic program of differentiation into cardiomyocytes.

The **purpose** of the study was to investigate the presence of arrhythmogenic effects of cord blood stem cells transplantation in patients with reduced myocardium contractility. That goal led to the following objectives: 1) to conduct Holter monitoring before and after transplantation of cord blood stem cells; 2) to make a comparative analysis of the results.

Materials and methods. Daily ECG monitoring was performed for 6 patients before of cord blood *SCs* transplantation (*SCT*) and in short time after *SCT*. Registering of *Holter-ECG* was performed with *Microvit-Shiller* 101 using software *MT200*.

Results and discussion. Observation revealed that, before *SCT*, all patients had mostly their own sinus rhythm. Fluctuations in heart rate witnessed violations due to decompensation of heart failure. The average number of ventricular extrasystoles (*ES*) was 0.43 ± 0.12 % of the total *QRS* complexes. They met class V by Lown-Wolf in 3 patients and IVa class in 2 ones. In 1 patient the amount of *ES* was < 0.01 % and there were no life-threatening form of *ES*. Supraventricular *ES* and supraventricular tachycardia paroxysms were not clinically significant; conductivity

disorders and episodes of atrial fibrillation were not registered. Depression of *ST* segment (using level ST > 1 mm) was found before *SCT* in 50 % of patients mainly at night and in the morning.

Thus, 83.3 % of patients were at a risk of sudden death due to high grade arrhythmogenic *ES*. Amiodarone was administered at a dose of 400-600 mg/day per os as an antiarrhythmic drug.

To treat a severe heart failure, cell transplant «Cryopreserved human umbilical cord blood» was injected intravenously in all patients, according to the clinical trial program. Monitoring of the patient during *SCT* was carried out at an intensive care department.

At 1 and 3 months of observation after SCT the basic rhythm and heart rate remained constant. Ventricular *ES*, in comparison with the initial state, decreased by 55 % after 1 month, and by 73 % after 3 months. A month after *SCT* 2 patients met 4-B class by *Lown-Wolf*, and 2 patients met 4-A class. Sporadic *ES* were registered in 2 patients. In 3 months after *SCT* ventricular *ES* in 3 patients resulted in 4 B class by *Lown-Wolf,* while the other 3 patients – single *ES*, which corresponded to the class 2 by *Lown-Wolf*.

A month after *SCT* a relative number of supraventricular *ES* decreased and by the end of 3 months their number was insignificant.

Conclusion. Thus, the *ECG* pattern before *SCT* was characterized by excitation disorders, which is typical for patients with chronic cardiac and heart failure. Before *SCT* 83.3 % of patients were at a high risk of sudden arrhythmogenic death. Preliminary comparative analysis demonstrated that cord blood *SCT* in 1 and 3 months do not lead to negative consequences, which may indicate electrophysiological safety of this *SC* class.

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Diagnosis of acute renal allograft rejection

Polyamines are low-molecular regulators of cell metabolism, their concentration increases in differentiated tissues and cells of active growth. Significant changes in their concentrations are of considerable interest for the diagnosis of acute renal allograft rejection.

Purpose: to evaluate the possibility of the diagnosis of acute renal allograft rejection crisis by studying the concentration of polyamines (spermine, spermidine, putrescine) and the total polyamine content (*TPC*) in blood of renal transplant recipients.

Materials and methods. The study includes analysis of concentration of polyamines and *TPC* in 176 recipients of cadaveric renal allograft (*CRAG*). In the early postoperative period 45 *CRAG* recipients had an acute crisis of rejection. Determination of the polyamines content was performed according to the procedure of *N. K. Berdinskih* in the modification of *E.M. Berko*. Investigations were carried out before the donor kidney allotransplantation, and then every 72 hours during the first month after *CRAG*.

Results. We discovered that potential kidney transplant recipients have an increase in polyamines concentration (*TPC 30.7 ± 2.9 nmol/ml*) compared with healthy individuals (*TPC 19.6 ± 3.6 nM/ml*).

At the primary transplant function the concentration of polyamines decreased (*TPC 23.2 ± 0.5 nM/ml*) since the first days after the operation; reduction passed together with a decrease of creatinine or outpaced it for 3-5 days.

During the crisis of rejection we marked a reliable increase of polyamines (*TPC 42.9 ± 3.7, $p < 0.05$*). At the development of rejection with oligoanuria, polyamines increased in 90 % of cases 1-2 days before the other features of rejection crisis.

Conclusion. A method for diagnosing of acute renal allograft rejection was developed. It is based on the determination of the polyamines concentration in blood plasma in the early postoperative period. This allows to assess the condition of the renal allograft in the dynamics, determine the cause of graft dysfunction and solve the problem of immunosuppressive therapy optimization. It also enables more precise diagnose of rejection crisis and timely start of therapy and, thereby, improves results of *CRAG*.

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Predialysis living donor renal transplantation

Throughout the world there is a trend in increase of patients' number with terminal chronic renal failure (*TCRF*). The optimal treatment for this disease is recognized a renal transplantation, because it allows to achieve better survival, quality of life and social rehabilitation of patients. In addition, it is less expensive than dialysis therapy. This is very important, especially for countries with limited financial resources. In nephrological practice in the treatment of patients with *TCRF* different methods of substitution therapy are usually combined, but the most often methods are peritoneal dialysis or hemodialysis with the following renal allotransplantation (*RAT*). The question is whether such tactics is justified? Which method is better to start renal replacement therapy with? When to perform renal transplantation?

Recently, the global practice of transplantation increases the number of so-called predialysis transplants performed in patients with a decrease in glomerular filtration rate (*GFR*) 20 ml/min.

The **purpose** of this study is to develop optimal treatment in patients with terminal renal failure by a comparative analysis of the results of predialysis renal allotransplantation (*PD-RAT*) and transplantation, performed after a hemodialysis (*HD*).

Materials and methods. We analyzed the results of 70 *RAT* from relative donor performed from January 2006 to February 2012 in the department of renal transplantation and hemodialysis of A. Shalimov National Institute of Surgery and Transplantology of *NAMS* Ukraine. Experimental group (group I) was 35 patients after *PD-RAT* from living relative donor. Comparison group (group II) was 35 patients also after *RAT* from living relative donor, but it was preceded by hemodialysis for more than 2 years. Both groups were comparable for demographic parameters and character of primary renal disease. The age of recipients ranged from 17 to 52 years. All patients in the pre-and postoperative period underwent clinical and laboratory examination, which also included determining of blood lipid spectrum, *EchoCG* and duplex scan of transplant. In the period after transplantation all recipients received 4-component immunosuppressive therapy (*CD25*-receptor blocker, neuronal calcium inhibitors, mycophenolic acid preparations, methylprednisolone). Comparative evaluation criteria were acute rejection crises frequency, one and three-year graft survival, level of blood creatinine 12 months after *RAT*, indexes of calcium-phosphorus metabolism, blood lipids spectrum, *EchoCG*.

Results and discussion. Comparing results of pretransplant recipients status we determined that hemoglobin level < 100 g/l was found in 20 % of recipients of group I and in 48.5 % of patients of group II ($p < 0.025$). Hyperphosphataemia (P+ > 1.55 mmol/l) was in 62.8 % of recipients in group II and 17.1 % of patients in group I ($p < 0.001$). Increase of total cholesterol above 6.5 g/l was in 31.4 % of patients in group II and 8.5 % of patients in group I ($p < 0.01$).

In pretransplant period *RAT* recipients in group I showed less associated diseases such as ulcerous stomach disease and 12 duodenal ulcers (11.4 % versus 34.2 %). There were much more patients with hepatitis B and C in group II (48.5 %) than in group I (17.1 %). The number of sensitized patients (*PRA* > 30 %) in group I was significantly lower (8.5 %) than in group II (31.4 %), $p < 0.01$.

In pretransplant period according to the *EchoCG* data the patients in group I had much less features of left ventricular hypertrophy (11.4 %) than the patients in group II (40 %). Ejection fraction (EF) < 50 % in group II was more often (22.8 %) than in group I (5.7 %), $p < 0.01$.

Survival of *RAT* during one and three years in recipients of group I was slightly higher than in group II (97.1 and 91.4 %; 91.4 and 80 %, respectively, $p > 0.05$).

Episodes of acute *RAT* rejection within a year after surgery in recipients of group I appeared less frequently than in recipients of group II (11.4 and 22.8 %, respectively). Indicators of blood creatinine in recipients of I group were lower than in group II 12 months after *RAT*, but the difference is not significant (108 ± 45 and 139 ± 15 mM/l, respectively). Indicators of central hemodynamics, blood lipid spectrum and phosphorus-calcium metabolism 12 months after the *RAT* in group I were also positively different from such indicators in group II.

Conclusion. Thus, our study showed that the experimental group patients, who underwent renal transplantation in predialysis period, have, in particular, much better indicators: higher survival and *RAT* function, less acute rejection episodes, fewer violations of the cardiovascular system, phosphorus-calcium parameters and lipid metabolism. This may be due to better pretransplant state of recipients, less infection with hepatitis B and less sensitization. Given the lack of dialysis beds, *PD-RAT* from living relative donor can increase the number of patients with *TCRF* that can get adequate renal replacement therapy.

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