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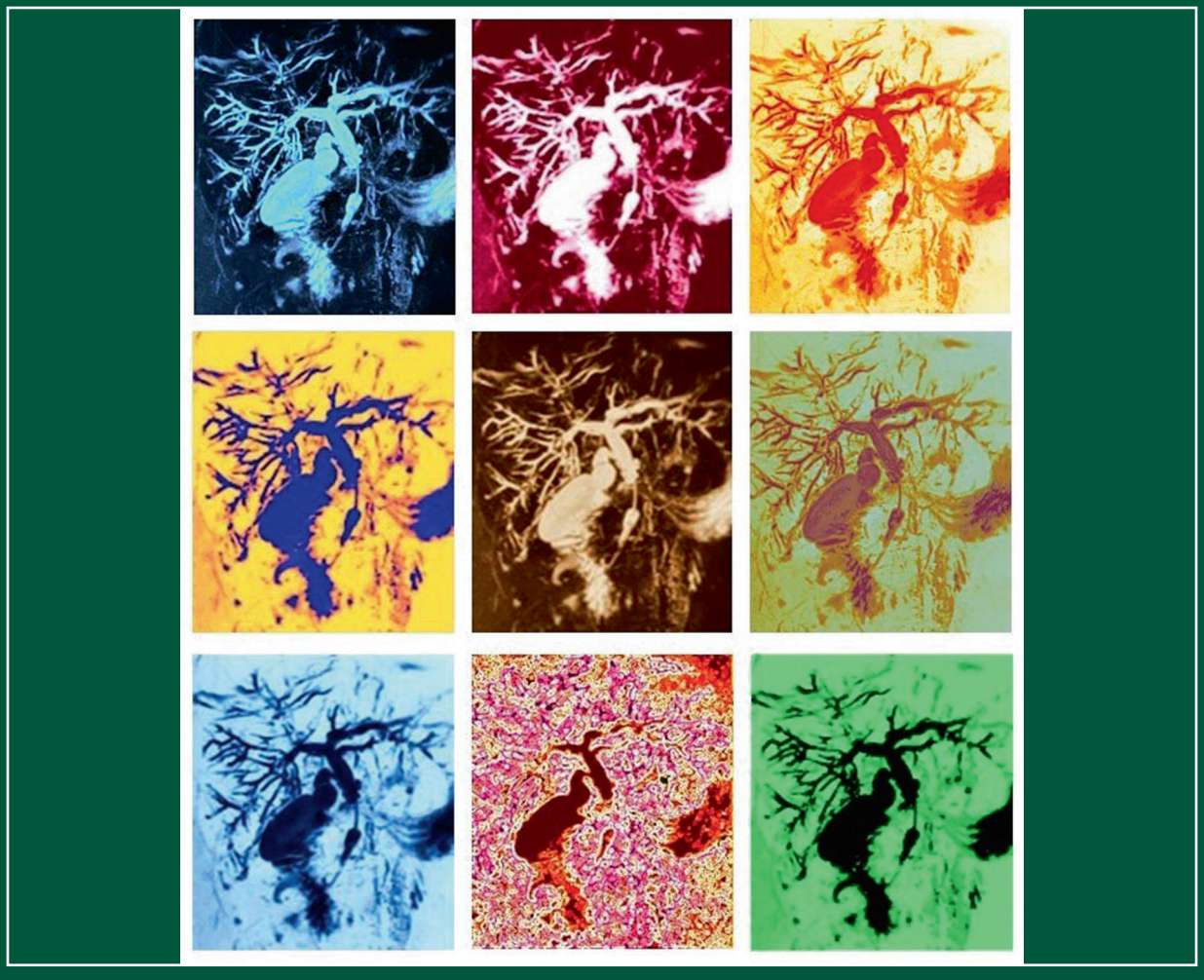
European Journal of Clinical Investigation

56TH ANNUAL SCIENTIFIC MEETING –
8–10 June 2022, Bari, Italy



Cholangiocarcinoma - 9 faces of the killer
It shows cholangiocarcinoma, an aggressive bile duct tumour with dismal prognosis,
It was captured during magnetic resonance cholangiopancreatography (MRCP)
Piotr Milkiewicz, Warsaw Poland

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ESCI EUROPEAN SOCIETY FOR CLINICAL INVESTIGATION

Official journal of the European Society for Clinical Investigation

European Journal of Clinical Investigation

THE JOURNAL OF THE EUROPEAN SOCIETY FOR CLINICAL INVESTIGATION

Aims & Scope

The European Journal of Clinical Investigation (EJCI), in publication since 1970, is a peer-reviewed general-interest biomedical journal with a broad readership. It is the official journal of the European Society for Clinical Investigation (ESCI) and it is published monthly by Wiley. It considers any original contribution from the most sophisticated basic molecular sciences to applied clinical and translational research and evidence-based medicine across a broad range of subspecialties. The EJCI publishes reports of high-quality research that pertain to the genetic, molecular, cellular, or physiological basis of human biology and disease, as well as research that addresses prevalence, diagnosis, course, treatment, and prevention of disease. We are primarily interested in studies directly pertinent to humans, but submission of robust in vitro and animal work is also encouraged. Interdisciplinary work and research using innovative methods and combinations of laboratory, clinical, and epidemiological methodologies and techniques is of great interest to the journal. Several categories of manuscripts (for detailed description see below) are considered: editorials, original articles (also including randomized clinical trials, systematic reviews and meta-analyses), reviews (narrative reviews), opinion articles (including debates, perspectives and commentaries); and letters to the Editor.

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THE JOURNAL OF THE EUROPEAN SOCIETY FOR CLINICAL INVESTIGATION

European Society for Clinical Investigation

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Officers and members of the ESCI Council 2020

Objectives

The objectives of the Society are the advancement of medical practice through science; the cultivation of clinical research by the methods of the natural sciences; the correlation of science with the art of medical practice; the fostering of high standards of ethical practice and investigation; and the diffusion of a spirit of fraternity and international co-operation among and through its members.

Membership

Any person who has been actively involved in medical research in Europe, for example by involvement in original investigations in clinical or allied sciences of medicine, can apply for membership of ESCI. Members will receive the *EJCI* monthly. Any company, corporation, organisation, or trust can apply for corporate membership in the Society.

Meetings

The Society holds one Annual Scientific Meeting each year, usually in April/May. Meetings are held in different cities in Europe. Other Society's activities include the sponsorship of Workshops and Postgraduate Courses and the encouragement of the exchange of Scientists between Laboratories.

Contact information ESCI

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$P > 0.05$). Expression of miR-149-5p and miR-32-5p were significantly higher in patients with CAD with MetS than without MetS group ($P < 0.05$). Among MetS components abdominal obesity (1.3, 1.18-1.65, CI 95%, $P < 0.05$), insulin resistance (1.26, 1.11-1.59, CI 95%, $P < 0.05$) and dyslipidemia (1.15, 1.04-1.45, CI 95%, $P < 0.05$) were positively associated with increased circulating level of miR-149-5p whereas only abdominal obesity (1.21, 1.13-1.55, CI 95%, $P < 0.05$) and dyslipidemia 1.14, 1.05-1.51, CI 95%, $P < 0.05$ were positively associated with increased level of circulating miR-32-5p in patients with CAD.

Conclusions: Circulating exosomal miR-149-5p and miR-32-5p might be prognostic biomarker in patients with CAD and MetS.

56ASM-0006 | Multicistronic plasmids encoding VEGF and FGF2 stimulate local angiogenic processes in vivo: in a matrigel plug assay and a rat model of hind limb ischemia

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Background: Peripheral arteries disease (PAD) is characterized by poor blood supply, blockage of blood vessels, which may ultimately lead to limb amputation. The presence of additional vascular disease and lack of suitable vessels for bypass surgery limits the effectiveness of surgical and physiological treatments. Gene therapy with angiogenic factors, which restores the blood supply to the limb, is a promising alternative to conservative treatment methods.

Materials and Methods: In this study, we developed plasmid constructs that provide simultaneous overexpression genes of vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF2), and reporter protein (DsRed). We applied the system of 2A-peptides of picornaviruses supplemented with a cleavage site by furin proteinase (Fu-2A) to achieve co-expression of genes.

Results: First, we showed high expression and secretion of recombinant VEGF and FGF2 proteins in imported (HEK-293T) and primary cells (HUVEC) *in vitro*. Luminex-Based Multiplex Assay showed that overexpression of VEGF and FGF2 did not affect the secretion of other cytokines and growth factors by both cell types. When transplanting transfected HEK-293 cells, as part of Matrigel plaques, into immunocompromised mice, the expression of recombinant proteins VEGF and FGF2 activated vascular formation processes and also promoted endothelial cell recruitment. Next, we injected the synthesized plasmid constructs into ischemic rat muscle with a two-step surgical model of ischemia induction. At this stage, we observed a gradual decrease in VEGF and FGF2 transgenes expression in the ischemic muscle on 14 and 21 days. Despite this, quantitative analysis of the limb perfusion ratio demonstrated a significant restoration of blood flow on day 21 in the experimental groups. Histological methods showed decreased fibrosis and increased capillary density at 14 and 21 days after injection.

Conclusions: Thus, our study demonstrates the functionality of the constructed plasmid constructs *in vitro* and *in vivo*. Expression of recombinant VEGF and FGF2 proteins as part of multicistronic constructs activated local angiogenesis and recruitment of various cell types. These results suggest the possibility of using this gene therapy construct to stimulate angiogenesis and treat PAD, however, further studies using more representative experimental models and clinical trials are needed.

This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0008 | Functional properties of langendorff-isolated rat heart recovery after hypodynamia

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Background: The study of the influence of motor activity limitation on the body is an urgent problem of physiology. Restriction of motor activity (hypodynamia) causes changes in contractile function and weakening of the heart muscle, as well as weakening of venous and arterial vessels. We studied the features of rat heart-isolated work according to Langendorff in the recovery period after 30 days of hypodynamia.

Materials and Methods: Restrictions of motor activity were achieved by placing rats in cell cages: the first two days, the time of inactivity was 1 hour, and then increased by 2 hours every 2 days. By day 25, the time spent by animals in the cage-cases reached 23 hours. The parameters of an isolated heart were recorded immediately after physical inactivity, as well as after 2 weeks of the recovery period after 30-day hypodynamia. HR, left ventricular developed pressure (LVP), and coronary flow (CF) were calculated. The recording was carried out on the PowerLab 8/35 apparatus (ADInstruments) using the LabChart Pro software.

Results: During the recovery period, there was an increase of isolated heart LVP by 32.4% ($p < 0.05$), and decrease in heart rate by 6.3% and a decrease in CP by 39.4% ($p < 0.01$) compared with the 30- day hypodynamia.
Conclusions: Recovery after limitation of motor activity is accompanied by an increase in cardiac contraction force (LVP), and a decrease in heart rate and coronary flow. The results can be associated with recovery processes in the heart, since immediately after hypokinesia there was a general tendency to heart activity desadaptation, the force contraction decrease and increase of heart rate. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0009 | Influence of Alpha1(A)–adrenoreceptors stimulation on isolated rat heart coronary flow in ontogenesis

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Background: In the mammalian heart, alpha1-adrenergic receptors (α_1-AR) perform many functions: they participate in the regulation of myocardial contractility, heart chronotropy, coronary blood flow, as well as in various pathological processes. Earlier, we showed that non-selective stimulation of α_1-AR in adult rats reduces coronary circulation, and in 1-week-old rat pups it enhances it. According to scientists, the α_{1A}-AR can mediate a positive inotropic effect in stressful and pathological situations. In this regard, the study of the role of this receptor subtype in the regulation of the blood supply to the heart is gaining relevance. The aim of this study was to investigate the effect of α_{1A}-adrenoreceptors stimulation on coronary flow in isolated hearts of rats in ontogenesis
Materials and Methods: Isolated hearts were perfused in the Langendorff system (ADInstruments). The experiments we used selective agonist α_{1A}-AR A-61603 (10^{-9}mol/L). The degree of coronary circulation was evaluated using the indicator-coronary flow (CF), which was calculated by measuring the amount of perfusate flowing through the coronary vessels of the isolated heart for 1 minute. Statistical processing of the obtained results was performed using the Student's *t*-test.
Results: Perfusion of A-61603 10^{-9}mol/L caused an increase CF isolated heart of 20- and 6-week-old rats by 12% ($p < 0.01$), and 10% ($p < 0.05$), respectively. The speed of isolated heart CF in 3 and 1 week old rats did not change in response to A-61603.
Conclusions: Stimulation of α_{1A}-AR with A-61603 changed the CF in rats of 6 week-old, as well as in adult animals. The absence of changes in CF in 1-week and 3-week-old rats on the introduction of A-61603 is probably due to the lack of sympathetic innervation of the heart of animals at this period of postnatal development. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0010 | Stimulation of α2-adrenergic receptors change heart rate and coronary flow in the newborn rats isolated heart

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Background: Controversial data on the role of alpha2-adrenergic receptors (α_2-AR) in the regulation of coronary blood supply are presented in the modern literature. It was reported that α_2-AR agonists exert a vasoconstrictor effect. On the other hand, the results of the vasodilator effect on coronary vessels of the α_2-AR agonist clonidine hydrochloride are presented in the studies. It is known that α_2-AR is present on the myocardiocytes membranes, and in vascular smooth muscles. The aim was to investigate the effect of different concentrations of the α_2-AR agonist on coronary flow (CF) and heart rate (HR) in the isolated heart of newborn rats.
Materials and Methods: Experiments were performed on 28 newborn rat, they do not have adrenergic innervation of the heart. Isolated hearts were perfused in a Krebs-Henseleit solution - Langendorff (ADInstruments) installation. The CF and HR were calculated. The signals were recorded in a PowerLab system. 10^{-9}–10^{-6}M concentrations range of clonidine hydrochloride (Sigma) were used for the stimulated of α_2-AR. The data were processed statistically using Microsoft Excel software and Student's t test.
Results: Application to a perfuse solution of clonidine hydrochloride (10^{-9}M) decreased CF and causes tachycardia in the newborn rat isolated heart. The α_2-AR agonist (10^{-8}M) caused decreased CF and bradycardia was observed. Clonidine hydrochloride (10^{-7}M) had a different effects CF and HR. The α_2-AR agonist (10^{-6}M) had no effect on the CF, and caused a different effects HR.
Conclusions: Stimulation of alpha2-AR with low concentrations of clonidine hydrochloride (10-9, 10-8 M) leads to a decrease in coronary flow and bradycardia of the newborn rats isolated heart. High concentrations of the α2-AR agonist (10-7, 10-6 M) resulted in opposite