Myocardial preconditioning by short ischemia-reperfusion cycles and levels of the peptide interleukin-8

R. Kalfin[1][*], R. Alexandrova[2]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. G. Bonchev St., Block 23, Sofia 1113, Bulgaria*
[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev St., Block 25, Sofia 1113, Bulgaria*

Coronary angiogenesis and collateral growth are chronic adaptations to myocardial ischemia. In human coronary atherectomy tissue significant expression and angiogenic activity of the pro-inflammatory chemoattractant peptide interleukin-8 (IL–8) was observed. The aim of the present study was to introduce a method for measuring the IL–8 levels in rat heart tissue and to study whether ischemic preconditioning enhances IL–8 level in the infarcted heart. We stimulated myocardial angiogenesis in Sprague Dawley rats through ischemic preconditioning in the form of *in vivo* 4 short repetitive cycles of coronary artery occlusion (5 min) each followed by reperfusion (10 min). Rats were randomly divided into 5 groups: baseline control; normoxia + sham surgery; ischemic preconditioning + sham surgery; normoxia + myocardial infarction; ischemic preconditioning + myocardial infarction. Tissue samples from left ventricle were homogenized and sonicated. Aliquots of homogenate supernatants were obtained and frozen at –70° C until thawed for assay by specific IL-8 ELISA. Samples were assesed in duplicate using cellular communication assay kit for rat IL-8 (GRO/CINC-1). Arteriolar and capillary density was evaluated by the standard deparaffinization protocol. We found that concentrations of IL–8 in the left ventricle were significantly elevated after 2, 4, 7, 14 and 28 days of left coronary artery occlusion in the ischemic preconditioning + myocardial infarction group as compared to the normoxia + myocardial infarction and/or baseline control. The nonparametric correlation showed that IL–8 concentration correlates significantly ($P < 0.05$) and negatively with arteriolar and capillary density. Therefore, IL-8 could be one of the angiogenesis "promoters" in the infracted heart.

Key words: peptide, interleukin-8, myocardium, ischemia, heart

INTRODUCTION

The peptide Interleukin-8 (IL-8) is a chemoattractant cytokine for neutrophils [1], lymphocytes [2], and fibroblasts [3]. It is a representative of α-chemokines, and was also shown to induce migration and proliferation of endothelial cells [4, 5] and smooth muscle cells [6]. IL–8 is produced by monocytes, endothelial cells, fibroblasts, lymphocytes, neutrophils, keratinocytes, epithelial cells, hepatocytes, and lung macrophages. IL–8 is a potent cytokine involved in mitogenesis [6] and angiogenesis [5]. It was shown that at physiological concentrations human IL-8 induces transient neovascularization in a rabbit corneal pouch model, accompanied by a modest lymphocytic infiltrate [7]. In addition, IL-8 was reported to take part in neovascularization in a variety of tumors [8, 9].

On the other hand, coronary angiogenesis and collateral growth are chronic adaptations to myocardial ischemia. Simonini *et al.* [10] observed significant expression and angiogenic activity of IL-8 in human coronary atherectomy tissue. Yang *et al.* [11] demonstrated that IL-8 promotes a pro-angiogenic response in endothelial cells. Evidence suggests that like other angiogenic factors, IL–8 may be down-regulated by the hypoxia cascade. Recently, an increase of plasma IL-8 level in patients with chronic heart failure was reported [12]. Having in mind all these facts, we examined a novel method of stimulating myocardial angiogenesis through short cycles of ischemic preconditioning followed by reperfusion.

EXPERIMENTAL METHODS

All animals used in this study received humane care in compliance with the principles of laboratory animal care formulated by the National Society for Medical Research and Guide for the Care and Use of Laboratory Animals published by NIH. The experimental protocol was performed after

* To whom all correspondence should be sent:
E-mail: reni_kalfin@abv.bg

receiving approval from the institutional Animal Care Committee.

Surgical procedures

Male Sprague Dawley rats weighting between 250 and 300 g were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.). As preoperative antibiotic cover we administered cefazolin (25 mg/kg i.p.). Rats were randomly divided into 5 groups: baseline control; normoxia + sham surgery; ischemic preconditioning + sham surgery; normoxia + myocardial infarction; ischemic preconditioning + myocardial infarction. After tracheotomy and initiation of ventilation, the heart was exposed through a left lateral thoracotomy. Non-traumatic occluder was applied on the left anterior descending coronary artery (LAD). The myocardium was preconditioned by carrying out a short duration of temporary regional ischemia (5 min) followed by a period of reperfusion (10 min), repeated 4 times. Myocardial infarction was produced by permanent LAD occlusion. The rats in the sham group underwent the same procedure except for the LAD ligation. After completion of all surgical procedures the chest wall was re-closed, rats received buprenorphine (0.1 mg/kg s.c.) and were placed on a heating pad while recovering from anesthesia.

IL-8 enzyme-linked immunosorbent assay (ELISA)

Tissue samples from left ventricle were homogenized and sonicated in 1 ml antiprotease buffer consisted of 1 x PBS with 2 mM phenylmethylsulfonyl fluoride, and 1 μg /ml each of leupeptin and pepstatin A. Total protein concentrations were determined using bicinchoninic acid protein assay kit (Pierce Chemical Company, Rockford, IL, USA). Aliquots of homogenate supernatants were obtained after centrifugation at 10 000 x g for 10 min and frozen at –70° C until thawed for assay by specific IL–8 ELISA. Samples were assesed in duplicate using commercially available cellular communication assay kit for rat IL–8 (GRO/CINC–1) from Amersham Pharmacia Biotech Inc., NJ, USA. The sensitivity of the assay was 0.49 pg/ml. Absorbency was measured by a plate reader at 450 nm. In the heart tissue IL-8 levels were scalar (amount per milligram protein).

Arteriolar and capillary density

The standard deparaffinization protocol was used. Endothelial cells were labeled using mouse monoclonal anti-CD31/PECAM-1 (1:100,

Pharmingen, San Diego, CA, USA) followed by a biotinylated horse anti-mouse secondary antibody (1:200 dilution). The reaction product (brown) was visualized with DAB substrate using the Vector ABC Vectastatin Elite Kit (Vectorlabs, Burminghame, CA, USA) and was counterstained with methyl green (Vectorlabs). On separate slides, vascular smooth muscle cells were labeled using mouse monoclonal anti-smooth muscle actin (1:50, Biogenex, San Ramon, CA, USA) followed by a biotinylated rat-adsorbed horse anti-mouse secondary antibody (1:200 dilution). The reaction product (violet) was visualized with VIP substrate using the Vector ABC Vectastatin Elite Kit (Vectorlabs). Images were captured and stored in digital ".tiff" format for later image analysis. Counts of artetiolar and capillary density per mm^2 were obtained after superimposing a calibrated morphometric grid on each digital image using Adobe Photoshop Software (Adobe Systems Inc., San Jose, CA, USA).

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Differences between groups were tested by one-way ANOVA followed by Newman-Keuls post hoc multiple comparison test. Correlation of arteriolar or capillary density vs. IL–8 concentration in IPMI-group was performed according to the non-parametric statistical method of Spearman. Differences were considered significant when $P < 0.05$. All statistical analyses were performed using GraphPad Prism 3.03 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

Concentrations of IL-8 in the left ventricle were significantly elevated after 2, 4, 7, 14 and 28 days of left coronary artery occlusion in the ischemic preconditioning + myocardial infarction group as compared to the normoxia + myocardial infarction and/or baseline control or sham groups, which we found to decrease gradually with time (Fig. 1).

Ischemic preconditioning increased capillary and arteriolar density in our experiments. The nonparametric correlation found by plotting of IL-8 levels vs. arteriolar or capillary density is shown in Figures 2 and 3, respectively. In both cases IL-8 concentration correlated with arteriolar and capillary density.

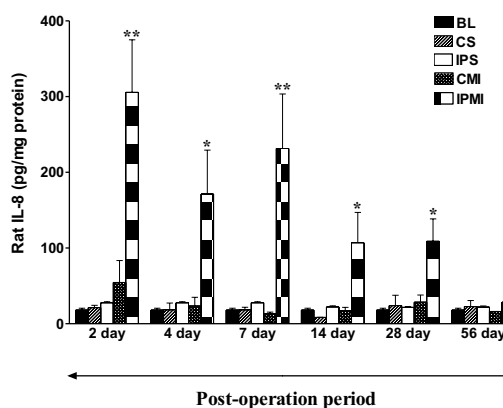Significant experimental and clinical research has focused on protection of ischemic myocardium.

Fig. 1. Concentration of the peptide interleukin-8 (IL-8) in left ventricle of rats, randomly divided into five groups: baseline control (BS); normoxia + sham surgery (CS); ischemic preconditioning + sham surgery (IPS); normoxia + myocardial infarction (CMI); ischemic preconditioning + myocardial infarction (IPMI). Results are expressed as mean ± S.E.M. of five hearts per group. Significant differences from the CMI group are indicated: *$P < 0.05$; **$P < 0.01$.
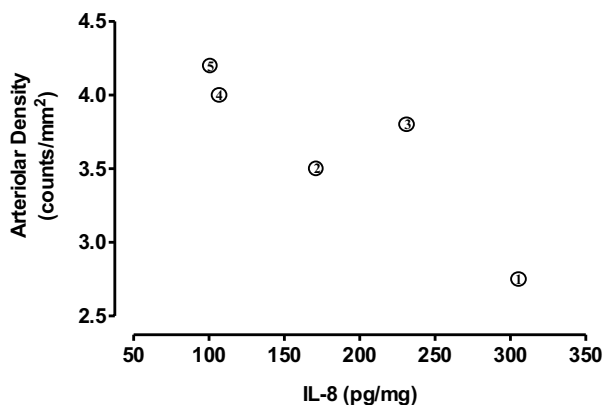

Fig. 2. Non-parametric Spearman correlation of the arteriolar density vs. interleukin-8 concentration in the left ventricle of IPMI (ischemic preconditioning followed by myocardial infarction) group: $P < 0.05$, $r = -0.91$.

Successful protection strategies are diverse and have included sublethal ischemia and certain pharmacological approaches. The biological process now recognized as "preconditioning" enhances endogenous cellular mechanisms within the myocardium, and results in protection against postischemic injury. Angiogenesis, i.e. the growth of new blood vessels from pre-existing vessels, is induced when the metabolic requirements of the tissue exceed the perfusion capability of existing vessels. It is a sequence of events and appears to be a tightly regulated process, for example by various
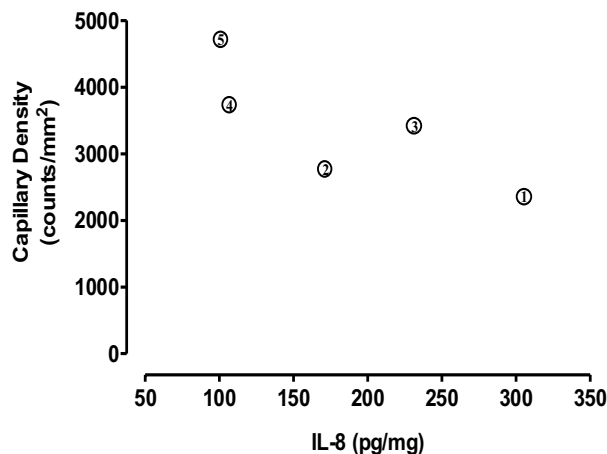

Fig. 3. Non-parametric Spearman correlation of the capillary density vs. interleukin-8 concentration in the left ventricle of IPMI (ischemic preconditioning followed by myocardial infarction) group: $P < 0.05$, $r = -0.90$.

growth factors and cytokines. However, the initiation of angiogenesis still remains unclear. Among the various triggers of angiogenesis, tissue hypoxia has been identified as being a particularly important stimulus for the induction of new vessel growth [13, 14]. For example, for a large series of angiogenesis factors it has been shown that these were strongly induced by tissue hypoxia. Based on currently available data, hypoxia inducible angiogenesis factors are VEGF, IL-8, angiogenin, FGF and PDGF [15].

The cytokine interleukin-8 is considered to be involved in angiogenesis, since a structural amino acid ELR motif (Glu-Leu-Arg), present in CXC chemokoines as IL-8, is suggested to be associated with stimulation of new blood vessels formation: chemokines that lack this motif, such as IL-10, appeared to be angiostatic [16, 17]. Our findings, described in the present study reveal that in the ischemic preconditioned infracted heart increased IL-8 levels correlate significantly and negatively with arteriolar and capillary density, hence IL-8 could play a role of angiogenesis "promoter".

REFERENCES

1 T. Yoshimura, K. Matsushima, I.J. Oppenheim, E.J. Leonard, *J. Immunol.* **139**, 788 (1987).

2 C.G. Larsen, A.O. Anderson, E. Appella, J.J. Oppenheim, K. Matsushima, *Science,* **243**, 1464 (1989).

3 J.R. Dunlevy, J.R. Couchman, *J. Cell. Sci.,* **108**, 311 (1995).

4 Z. Szekanecz, M.R. Shah, L.A. Harlow, W.H. Pearce, A.E. Koch, *Pathobiology,* **62,** 134 (1994).

5 A.E. Koch, P.J. Polverini, S.L. Kunkel, L.A. Harlow, L.A. DiPietro, V.M. Elner, S.G. Elner, R.M. Strieter, *Science,* **258,** 1798 (1992).

6 T. Yue, X. Wang, C. Sung, B. Olson, P.J. McKenna, J.L. Gu, G.Z. Feuerstein, *Circ. Res.,* **75,** 1 (1994).

7 Strieter R.M., Kunkel S.L., Elner V.M., C.L. Martonyi, A.E. Koch, P.J. Polverini, S.G, Elner, Interleukin-8, a corneal factor that induces neovascularization, *Am. J. Pathol.,* **141,** 1279 (1992).

8 E. Barcz, R.E. Skolinska, P. Kaminski, U. Demkow, K. Bobrowska, L. Marianowski, *Int. J. Gynaecol. Obstet.*, **79,** 229 (2002).

9 A. Yuan, C.J. Yu, K.T. Luh, S.H. Kuo, Y.C. Lee, P.C. Yang, *J. Clin. Oncol*., **20,** 883 (2002).

10 A. Simonini, M. Moscucci, D.W.M. Muller, E.R. Bates, F.D. Pagani, M.D. Burdick, R.M. Strieter, *Circulation*, **101,** 1519 (2000).

11 Y.H. Yang, H. Zhou, N.O. Binmadi, P. Proia, J.R. Basile. *PLoS One*, 6(10):e25826, (2011).

12 C. Cappuzzello, L. di Vito, R. Melchionna, G. Melillo, L. Silvestri, E. Cesareo, F. Crea, G. Liuzzo, A. Facchiano, M.C. Capogrossi, M. Napolitano. *J. Transl. Med*., **9**, 28 (2011).

13 O. Hudlicka, M. Brown, S. Egginton. *Physiol. Rev.*, **72**, 369 (1992).

14 A. Rivard, J. M. Isner, *Mol. Med.,* **4**, 429 (1998).

15 M. Kunz, S. M. Ibrahim, *Mol. Cancer*, **2**, 23 (2003).

16 R.M. Strieter, P.J. Polverini, S.L. Kunkel, D.A. Arenberg, M.D. Burdick, Kasper J., Dzuiba J., Van Damme J., Walz A., Marriott D, *J. Biol. Chem.,* **270,** 27348 (1995).

17 J.A. Belperio, M.P. Keane, D.A. Arenberg, C.L. Addison, J.E. Ehlert, M.D. Burdick, R.M. Strieter. *J. Leukoc. Biol.,* **68**, 1 (2000).

НИВА НА ПЕПТИДА ИНТЕРЛЕВКИН-8 ПРИ ПРЕКОНДИЦИЯ НА МИОКАРДА ПОСРЕДСТВОМ КРАТКИ ЦИКЛИ НА ИСХЕМИЯ-РЕПЕРФУЗИЯ

Р. Калфин[1]*, Р. Александрова[2]

[1]*Институт по невробиология, Българска академия на науките, ул. «Акад. Г. Бончев», блок 23, София 1113, България*
[2]*Институт по експериментална морфология, патология и антропология с музей, Българска академия на науките, ул. «Акад. Г. Бончев», блок 25, София 1113, България*

(Резюме)

Миокардът се приспособява към хроничната исхемия посредством растеж на малките кръвоносни съдове. Наблюдавано е, че коронарната атеректомия при човек значително увеличава експресията и ангиогенната активност на про-възпалителния хемоатрактантен пептид интерлевкин-8 (IL-8). Целта на настоящата работа беше да се въведе метод за измерване нивата на IL-8 в сърце на плъх и да се изследва дали исхемичната прекондиция повишава концентрацията на IL-8 при инфаркт на миокарда. Ние стимулирахме ангиогенезата в миокарда на плъхове от породата Sprague Dawley посредством исхемична прекондиция ин виво под формата на 4 къси повтарящи се цикъла на коронарна артериална оклузия (5 мин) следвана от реперфузия (10 мин). Плъховете бяха разделени на 5 групи: контролна; нормоксия + отваряне на гръдния кош; исхемична прекондиция + отваряне на гръдния кош; нормоксия + инфаркт на миокарда; исхемична прекондиция + инфаркт на миокарда. Тъканните проби взети от лявата камера бяха хомогенизирани и соникирани с ултразвук. Течността от супернатантата беше разпределена в епендорфки и съхранявана при -70˚ С до провеждането на IL-8 специфичния ензим-свързан имуносорбентен анализ. Пробите бяха изследвани в дупликат чрез специфичен кит за определяне на плъши IL-8 (GRO/CINC-1). Плътността на артериолите и капилярите беше определяна посредством стандартен депарафиниционен метод. Установихме, че концентрацията на IL-8 в лявата камера е значително повишена на 2, 4, 7, 14 и 28 ден след оклузията на лявата коронарна артерия в групата с исхемична прекондиция + инфаркт на миокарда в сравнение с групата нормоксия + инфаркт на миокарда или контролната група. Непараметричният корелационен анализ показа значителна (*P* < 0.05) негативна корелация между концентрацията на IL-8 и плътността на артериолите и капилярите. Следователно, IL-8 може да бъде един от „промоторите" на ангиогенезата при сърце в състояние на инфаркт.