Int J Clin Exp Med 2015;8(3):3318-3324
www.ijcem.com /ISSN:1940-5901/IJCEM0004773

Original Article

Balanced oxidative status by nesfatin-1 in intestinal ischemia-reperfusion

Ceylan Ayada[1], Ümran Toru[2], Osman Genç[1], Raziye Akcılar[1], Server Şahin[3]

[1]Department of Physiology, Faculty of Medicine, University of Dumlupınar, Kütahya, Turkey; [2]Department of Thoracic Medicine, Faculty of Medicine, University of Dumlupınar, Kütahya, Turkey; [3]Department of Medical Biology, Faculty of Medicine, University of Dumlupınar, Kütahya, Turkey

Received December 12, 2014; Accepted January 30, 2015; Epub March 15, 2015; Published March 30, 2015

Abstract: Objective: Ischemia causes reversible or irreversible cell or tissue damage and reperfusion can exaggerate cellular damage. Microvascular dysfunction is induced and causes enhanced fluid filtration in capillaries. At the acute phase of reperfusion more oxygen radicals are activated. Nesfatin-1 protects brain against oxidative damage and heart against ischemia/reperfusion damage. In our study, we aimed to investigate the acute effect of chronic peripheral nesfatin-1 administration in intestinal ischemia/reperfusion created rats. Method: Two-months-old, 28 *Wistar Albino* male rats, weighing an average of 200-250 g, were used and randomly divided into four experimental groups (n=7) as; Laparotomy, Ischemia/Reperfusion, Nesfatin-1+Laparotomy, Nesfatin-1+Ischemia/Reperfusion. Serum levels of total oxidant status (TOS) and total antioxidant status (TAS) were determined by colorimetric measurement method. The plasma levels of endotelin-1 and endothelial nitric oxide syntheses (eNOS) were analyzed by rat ELISA assay kits. Results: Plasma levels of endothelin-1 significantly increased, plasma level of eNOS, serum levels of TOS and TAS significantly decreased in nesfatin-1 applied groups. Additionally, The oxidative stress index (OSI) parameters decreased significantly in three groups compared to laparotomy. Conclusion: Chronic peripheral nesfatin-1 administration can decrease eNOS level and OSI at the acute phase of ischemia/reperfusion. We suppose that it can be protective for ischemia/reperfusion injury by balancing oxidant capacity. On the other hand, this effect of nesfatin-1 is not related with micro-circular compensation and increases anti-oxidant capacity.

Keywords: Nesfatin-1, ischemia/reperfusion, endothelin-1, eNOS, total oxidant capacity, total antioxidant capacity

Introduction

Ischemia, which is developed by deficiency in blood flow of perfused organs or tissue, causes reversible or irreversible cell or tissue damage [1]. Restoring blood flow after ischemia is described as reperfusion. Restoration of blood flow must be provided to prevent irreversible cell damage and to regain organ function. On the other hand, reperfusion can exaggerate cellular damage [2, 3]. Ischemia/reperfusion (I/R) induces micro-vascular dysfunction which can cause unbalanced oxidant-antioxidant capacity. Altogether; micro-vascular dysfunction and unbalanced oxidant-antioxidant capacity induced by I/R cause serious problems in several serious medical and surgical procedures [4].

Nesfatin-1 is an anorexic hypothalamic peptide and derived from the nucleobindin-2. Nesfatin-1 inhibits feeding independently from leptin pathway [5]. It is involved in the regulation of homeostasis [6]. Intracerebroventricular administration of nesfatin-1 causes hypertension [7]. Intravenous administration of nesfatin-1 can also increase blood pressure through inhibition of nitric oxide (NO) production [8] and eNOS inhibition [9]. It has been shown that nesfatin-1 protects heart against I/R injury [10].

In the present study, we aimed to identify the effect of chronic systemic nesfatin-1 application at the acute phase of intestinal I/R created rats. Plasma levels of endothelin-1, endothelial nitric oxide syntheses (eNOS), serum levels of total oxidant status (TOS) and total antioxidant status (TAS) were measured. We aimed to clarify the effect of chronic nesfatin-1 treatment on I/R in relation with microcirculation effectors and oxidant-antioxidant statuses.

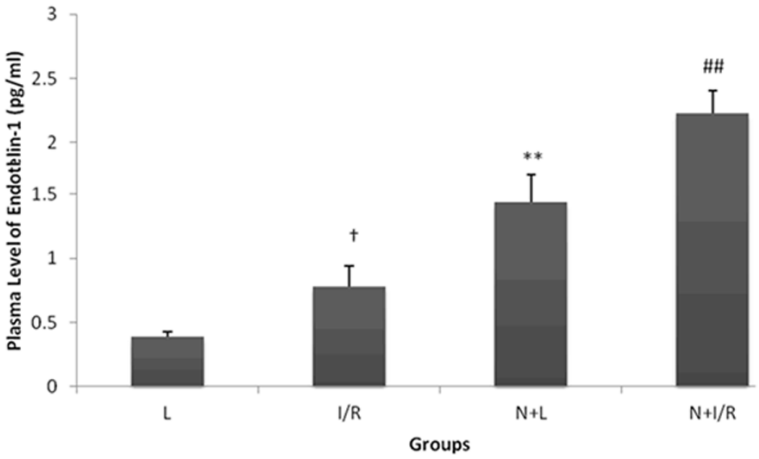

Figure 1. Plasma levels of endothelin-1 in L, I/R, N+L and N+I/R groups. †: The significance between L and the other groups, P<0.05 (Mann Whitney U test). **: The significance between L and the other groups, P<0.01 (Mann Whitney U test). ##: The significance between I/R and the other groups, P<0.01 (Mann Whitney U test).

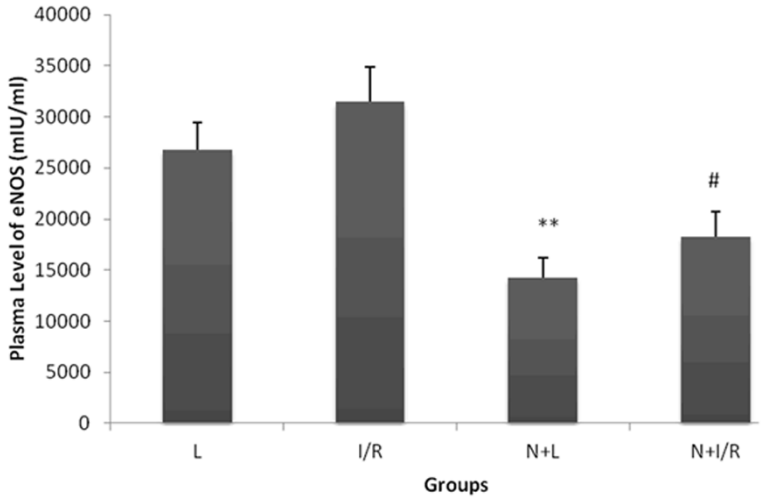

Figure 2. Plasma levels of eNOS in L, I/R, N+L and N+I/R groups. **: The significance between L and the other groups, P<0.01 (Mann Whitney U test). #: The significance between I/R and the other groups, P<0.05 (Mann Whitney U test).

Materials and methods

Animals and experimental conditions

All experimental protocols conducted on animals were consistent with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23) and approved by the Dumlupınar University Ethics Committee of Animal Care and Usage. In this study, two-months-old 28 male *Wistar Albino* rats weighing 200-250 g were used. They were reared under the supervision of a veterinarian, kept in a well-ventilated, noiseless environment, and allowed free access to food and water. The rats were housed in a room with controlled temperature (23±1°C) and relative humidity (50±5%), and they were kept in transparent plastic cages (42×26×15 cm), each containing three or four rats, exposed to a 12:12 light/dark cycle.

Experimental design

The rats were randomly divided into four experimental groups (n=7). The groups were described as; sham (L) rats were underwent laparotomy without any injection, ischemia/reperfusion (I/R) rats underwent occlusion of superior mesenteric artery for 30 min followed by 2 h reperfusion (I/R) [11] without any injection, nesfatin-1+laparotomy (N+L) rats were treated with rat nesfatin-1 segment (Phoenix Pharmaceuticals Cat No 003-22A) intraperitoneally (i.p.) (0,25 nmol/gr), which is peripherally effective dose [12], for 10 consecutive days and underwent laparotomy (N+L), nesfatin-1+ischemia/reperfusion (N+I/R) rats were treated with nesfatin-1 as in N+L group and underwent occlusion of superior mesenteric artery for 30 min followed by 2 h reperfusion. All rats were anesthetized with ketamine (75 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) during experimental processes [11].

ELISA, TAS-TOS analysis and OSI calculation

At the end of the experimental period, all animals were anesthetized with ketamin/xylazine HCl (75 mg/kg/10 mg/kg i.p.). Blood samples were collected in tubes with and without EDTA.

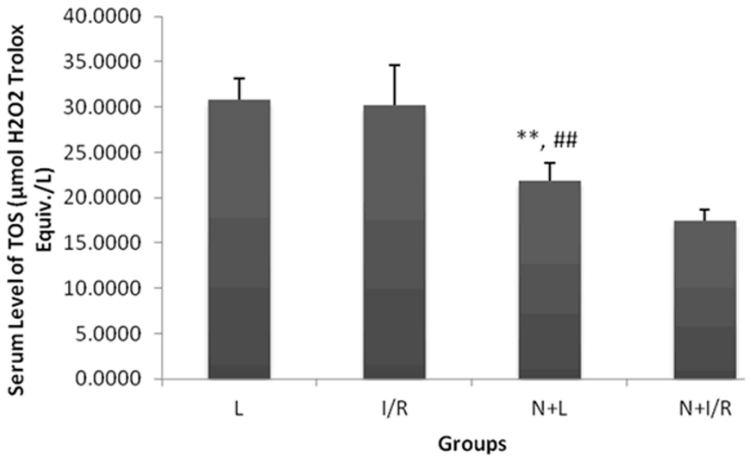

Figure 3. Serum levels of TOS in in L, I/R, N+L and N+I/R groups. **: The significance between L and the other groups, P<0.01 (Mann Whitney U test). ##: The significance between I/R and the other groups, P<0.01 (Mann Whitney U test).

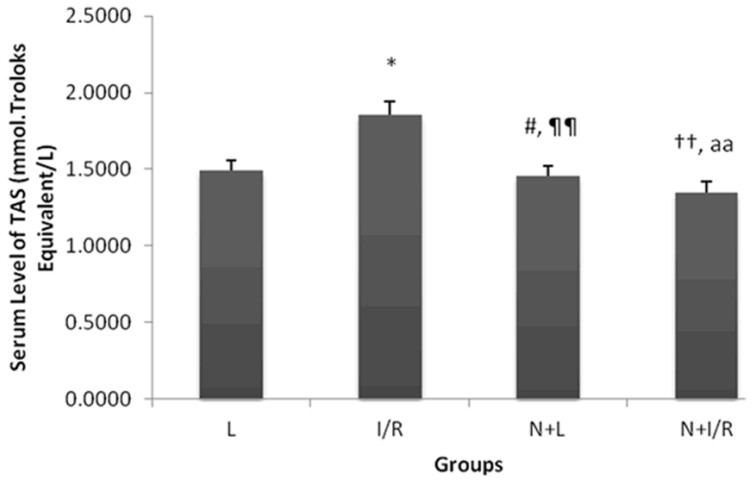

Figure 4. Serum levels of TAS in L, I/R, N+L and N+I/R groups. *,#: The significance between L and the other groups, P<0.05 (Mann Whitney U test). ††: The significance between L and the other groups, P<0.01 (Mann Whitney U test). ¶¶, aa: The significance between I/R and the other groups, P<0.01 (Mann Whitney U test).

After centrifugation, plasma of each rat was stored at -80°C until ELISA analysis. Plasma concentrations of endothelin-1 (Cusabio Biotech, Cat No CSB-E06979r) and eNOS (Cusabio Biotech, Cat No CSB-E08323r) were analyzed by rat ELISA assay kits. Concentration of each sample was calculated according to their chemiluminescence data. Chemiluminescence data were analyzed by an ELISA microplate reader (das, Digital and Analog Systems, Vimercate, MI, Italy).

Serum levels of TOS-TAS were determined by colorimetric measurement method (*Ral Assay* Diagnostics). The oxidative stress index (OSI) is calculated according to the following formula [13]: OSI (arbitrary unit)=TOS (µmol H_2O_2 Equiv./L) /TAS (mmol.Trolox Equiv./L).

Statistical analysis

Statistical analyses were done by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 16.0 package program. All data were given as mean±standard error of the mean (SEM). Statistical significances among all groups and between two groups were analyzed by Kruskal-Wallis and Mann-Whitney U tests, respectively. Differences were considered significant at $P<0.05$.

Results

Plasma levels of endothelin-1 and eNOS

Statistically significant difference was found for the plasma level of endotelin-1 among the L (0.38±0.04 pg/ml), I/R (0.77±0.16 pg/ml), N+L (1.44±0.21 pg/ml) and N+I/R (2.22±0.17 pg/ml) groups (P=0.000).

The plasma levels of endotelin-1 in N+L and N+I/R groups were significantly higher compared to L and I/R groups, respectively; P=0.002, P=0.003 (**Figure 1**). The plasma level of endotelin-1 was significantly increased in I/R group compared to L group (P=0.048) (**Figure 1**).

We observed statistically significant difference for the plasma level of eNOS among the L (26.79±2.59 mIU/ml), I/R (31.48±3.37 mIU/ml), N+L (14.23±2.00 mIU/ml) and N+I/R (18.30±2.45 mIU/ml) groups (P=0.002) (**Figure 2**). The plasma levels of eNOS in N+L and N+I/R

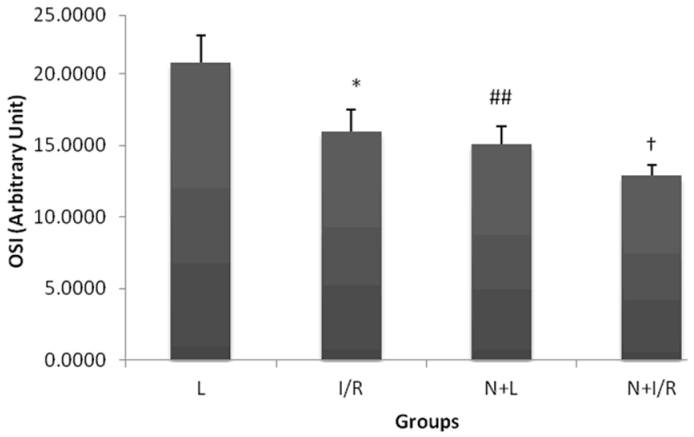

Figure 5. The oxidative stress index (OSI) of L, I/R, N+L and N+I/R groups. *, †: The significance between L and the other groups, P<0.05 (Mann Whitney U test). ##: The significance between L and the other groups, P<0.01 (Mann Whitney U test).

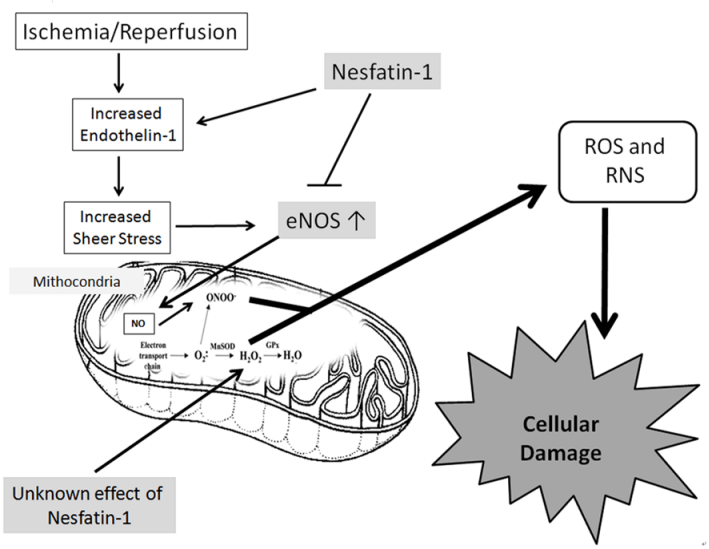

Figure 6. Suggested mechanism for the action of nesfatin-1 on the balanced oxidative status induced by ischemia/reperfusion. eNOS; endothelial nitric oxide syntheses, MnSOD; Manganese superoxide dismutase, GPs; Gypenosides, ONOO⁻; Peroxynitrite, ROS; Reactive oxygen species, RNS; Reactive nitrogen species.

groups were significantly decreased compared to L and I/R groups, respectively; *P*=0.006, *P*=0.013 (**Figure 2**).

Serum levels of TOS and TAS

We have observed statistically significant difference for the serum level of TOS among the L (30.78±2.42 μmol H_2O_2 Trolox Equiv./L), I/R (30.23±4.44 μmol H_2O_2 Trolox Equiv./L), N+L (21.88±1.88 μmol H_2O_2 Trolox Equiv./L) and N+I/R (17.40±1.26 μmol H_2O_2 Trolox Equiv./L) groups (*P*=0.002). The decrease for the serum level of TOS in N+L was statistically significant compared to L and I/R groups (*P*=0.002, *P*=0.002) (**Figure 3**).

We have observed statistically significant difference for the serum level of total antioxidant status (TAS) among the L (1.49±0.05 mmol. Trolox Equiv./L), I/R (1.85± 0.09 mmol. Trolox Equiv./L), N+L (1.45±0.07 mmol. Trolox Equiv./L) and N+I/R (1.34±0.07 mmol. Trolox Equiv./L) groups (*P*=0.000). The serum level of TAS in I/R group was significantly increased compared to L group (*P*=0.021). The decreased serum level of TAS in N+L was statistically significant compared to L ans I/R groups, respectively; *P*=0.048, *P*=0.002. The decrease for the serum level of TAS in N+I/R group was statistically significant compared to L and I/R groups res pectively; *P*=0.002, *P*=0.003 (**Figure 4**).

We have observed statistically significant difference for the OSI among the L (20.77±1.87 TOS/TAS), I/R (15.95±1.51 TOS/TAS), N+L (15.08±1.22 TOS/TAS) and N+I/R (12.92±0.70 TOS/TAS) groups (*P*=0.014). The decrease for OSI parameters in I/R, N+L and N+I/R groups were statistically significant compared to L group, respectively; *P*=0.048, *P*=0.003, *P*=0.025 (**Figure 5**).

Discussion

Intestinal I/R injury can cause important problems for surgical and transplantation processes because of its association with high morbidity and mortality. Restoring blood flow after ischemia is required to prevent tissue necrosis and regain organ function. On the other hand, restoration of blood flow can increase cellular damage [2]. Thus, reperfusion can limit the recovery of organ function. I/R is paired with microvascular dysfunction that cause unbal-

anced oxidant-antioxidant capacity [4]. Nesfatin-1 has received rising attention for therapeutic processes, because experimental studies indicate its biological action to suppress food intake [12] and modulate insulin secretion [14]. Recently nesfatin-1 has been described as a protective agent for I/R injury in heart [10]. If nesfatin-1 is considered as a therapeutic agent we believe that it is neccessary to know more about effects of nesfatin-1 in different conditions. For this purpose, we aimed to identify the acute effect of chronic peripheral nesfatin-1 application on intestinal I/R. We tried to explain this effect of nesfatin-1 by its effect on serum level of TOS-TAS parameters and on microcirculation regulators such as endothelin-1 and eNOS.

I/R can increase synthesis of some bioactive compounds such as endothelin and thromboxane A_2 [4]. Endothelin-1 (ET-1) belongs to endothelin peptide family and described as a strong vasoconstrictor agent. It is produced by endothelial cells. Vasoconstrictor effect occurs via ET-A receptor. On the other hand, ET-B receptor is responsible for vasodilator action of ET-1 [15]. It is known that endothelin level is increased during I/R [16]. In correlation with the literature, we also found increased plasma level of ET-1 in I/R group (see **Figure 1**). In the literature there is no information about the effect of nesfatin-1 administration on plasma level of endothelin-1. Present results indicate that chronic peripheral nesfatin-1 administration increases plasma level of endothelin-1 under laparotomy and I/R conditions (see **Figure 1**). This can also mean that nesfatin-1 has a possible vasoconstrictor effect through increased endothelin-1 under these conditions. However, up to now it is has been shown that nesfatin-1 plays role as a vasoconstrictor agent via inhibition of NO production [8]. We cannot assess how ET receptors, especially ET-B, are affected by increased plasma level of ET-1 due to the limitation of our experimental design. However, if we consider vasoconstrictor and aggregator effects of ET-1, chronic peripheral nesfatin-1 administration can cause deterioration of injury especially at the initial phase of I/R.

NO is known as a vasodilator. Its vasodilator action occurs via inhibiting the effects of vasoconstrictors such as AngII and ET-1 [17, 18]. It is synthesized by eNOS which is sourced from endothelial and red blood cells [19]. NO reacts with O_2 and produces peroxynitrite. This formation of NO contributes to I/R injury [20] (see **Figure 6**). NO and ET-1 control each other via autocrine feedback mechanisms [15, 21]. It has been shown that inhibition of NO synthesis reduces I/R damage related with this feedback mechanism [22]. In the literature it has been shown that nesfatin-1 inhibits NO production [8]. Furthermore, we found that nesfatin-1 reduces plasma level of eNOS under chronic stress conditions [9]. In the present study, although not significantly, I/R created rats have increased plasma level of eNOS compared to laparotomy group as expected according to the literature. Additionally, nesfatin-1 administration reduced plasma levels of eNOS in both nesfatin-1 applied groups compared to their controls. We can conclude that nesfatin-1 can be protective for I/R injury by inhibiting the production of reactive nitrogen species (RNS) provided by eNOS in spite of increased plasma level of ET-1 (see **Figure 6**).

Ischemia impairs oxidative phosphorylation and increases precursors of reactive oxygen species (ROS) in cells. Reperfusion provides molecular O_2 and induces ROS production more rapidly than ischemia. Especially at the initial stage of reperfusion production of ROS changes dramatically [4]. Nesfatin-1 can prevent brain damage induced by oxidative mechanisms [23] and heart for I/R injury [10]. We observed that nesfatin-1 reduced TOS and also TAS levels under laparatomy and I/R conditions. We can say that chronic peripheral nesfatin-1 administration can reduce oxidant level in both experimental conditions in parallel with inhibition of NO production. Additionally, OSI parameters significantly decreased in nesfatin-1 applied groups. We think that the decrease of OSI parameter in nesfatin-1 applied groups is related with inhibition of eNOS which also means inhibition of NO production (see **Figure 6**).

Conclusion

Damaged microcirculation caused by I/R can not be compensated by chronic peripheral nesfatin-1 administration especially at the initial stage of I/R. On the other hand, nesfatin-1 application can balance oxidative status which is unbalanced by I/R. This effect of nesfain-1 is not related with induced anti-oxidant capacity. Nesfatin-1 can balance oxidative status via

inhibiting NO production by decreasing eNOS level. We can conclude that chronic peripheral nesfatin-1 administration can provide protection against I/R injury. If nesfatin-1 has therapeutic potential for clinical treatments, we believe its chronic or acute effects should be identified in different clinical situations by further experimental studies.

Acknowledgements

This study was supported by Dumlupınar University Research Fund (Project no: 2013/8).

Disclosure of conflict of interest

None.

Address correspondence to: Ceylan Ayada, Department of Physiology, Dumlupınar University, Faculty of Medicine, Kütahya, Turkey. Tel: +905056331263; +902742652031-2731; Fax: +902742652285; E-mail: ceylanayada@yahoo.com

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