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Use of C-type natriuretic peptide as an indicator in detection of inducible peripheral ischemia

İndüklenebilir periferik iskeminin saptanmasında C-tip natriüretik peptidin gösterge olarak kullanımı

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Background: In this experimental study, alterations in plasma C-type natriuretic peptide (CNP) levels were evaluated during the critical initial hours of peripheral ischemia.

Methods: Forty male Sprague-Dawley type rats (aged 8 to 12 weeks, weighing 230±30 g) were included in the study. Four groups were created with 10 rats namely control group, group 1, group 2, and group 3. Baseline plasma CNP levels were detected in the control group without any intervention, while the plasma CNP levels were determined in the second hour of peripheral ischemia in group 1. Plasma CNP levels were determined in the fifth hour of peripheral ischemia for group 2, whereas plasma CNP levels were determined in group 3 at the eighth hour of peripheral ischemia.

Results: The baseline plasma CNP levels were 0.285±0.011 pmol/L in the control group. In rats with peripheral ischemia, a significant time-dependent increase was detected in plasma levels of CNP ($p<0.05$). The plasma CNP levels were detected as 0.350±0.015, 0.486±0.084, and 0.534±0.048 pmol/L in group 1, 2, and 3 respectively.

Conclusion: Plasma CNP, an endothelium-derived vasodilator, is associated with the cellular response in ischemic tissues in a time-dependent manner.

Keywords: C-type natriuretic peptide; ischemic response; peripheral ischemia.

Amaç: Bu deneysel çalışmada, periferik iskeminin başında kritik saatlerdeki plazma C-tip natriüretik peptid (CNP) düzeylerindeki değişiklikler değerlendirildi.

Çalışma planı: Çalışmaya 40 adet Sprague-Dawley cinsi erkek sıçan (8-12 haftalık ve ortalama ağırlıkları 230±30 g) alındı. Her grupta 10 sıçan olacak şekilde dört grup oluşturuldu: kontrol grubu, grup 1, grup 2 ve grup 3. Kontrol grubunda herhangi bir işlem yapılmadan başlangıç plazma CNP değeri tespit edilirken, grup 1'de periferik iskeminin ikinci saatinde plazma CNP düzeyine bakıldı. Grup 2'de periferik iskeminin beşinci saatindeki plazma CNP düzeyi tespit edilirken, plazma CNP düzeyleri grup 3'te periferik iskeminin sekizinci saatinde belirlendi.

Bulgular: Başlangıç plazma CNP düzeyi kontrol grubunda 0.285±0.011 pmol/L olarak tespit edildi. Periferik iskemi oluşturulan sıçanlarda, plazma CNP düzeyinin zamana bağlı olarak anlamlı olarak arttığı tespit edildi ($p<0.05$). Plazma CNP düzeyleri grup 1, 2 ve 3'de sırasıyla 0.350±0.015, 0.486±0.084 ve 0.534±0.048 pmol/L olarak tespit edildi.

Sonuç: Endotel kaynaklı vazodilatatör olan plazma CNP zamana bağlı olarak iskemik dokularda hücresel yanıt ile ilişkilidir.

Anahtar sözcükler: C-tip natriüretik peptid; iskemik yanıt; periferik iskemi.

The C-type natriuretic peptide (CNP) is a novel member of the natriuretic peptide family which also includes the atrial natriuretic peptide (ANP) and the brain natriuretic peptide (BNP).[1] This family of peptides has similar structural and functional specialties, but CNPs feature a vascular endothelial release, shorter circulatory half-life, and low circulatory concentration.[1,2] Natriuretic peptides show their potential effects by binding their

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respective receptors through a coupling reaction involving guanylate cyclase (GC). Natriuretic peptide receptors (NPRs) have two forms that have been designated as A and B, and studies that have focused on the vascular smooth muscles suggest that CNP is a potent agonist of NPR-B.[1,3]

Previous reports have examined the effects of natriuretic peptides in different types of ischemia.[4-6] Swärd et al.[4] studied the recombinant human atrial natriuretic peptide (h-ANP) with regard to ischemic acute renal failure and reported that infusion of h-ANP improves the renal excretory function. Another study by Staub et al.[5] investigated the role of BNP in inducible myocardial ischemia and determined that it was associated with inducible ischemia. Furthermore, screening for BNP proved beneficial in the detection of ischemia.[5] In addition, Nadir et al.[6] demonstrated that natriuretic peptides can be helpful for determining the efficacy of anti-ischemic therapy and risk factor classification. In addition, Kumakura et al.[7] found high levels of BNP in the limbs of critical ischemia patients.

In the current study, we investigated CNP levels during the critical hours of peripheral ischemia using inducible experimental rat models.

PATIENTS AND METHODS

In this interventional animal study, 40 male Sprague-Dawley rats (aged 8-12 weeks and weighing 230±30 g) were included in the experiment, which received ethical approval from the Laboratory Animal Production Unit of Dicle University. The animals were obtained from the same unit, and standard temperature- (22±2 °C) and humidity (50±5%)-controlled rooms were used for preserving the rats until the start of the experiment. In addition, a standard diet and tap water were provided ad libitum for the rats.

The rats were divided into four groups containing 10 rats each, and the following procedures were applied to the groups consecutively:

- *Control group:* The baseline characteristics of the CNP levels were determined without any application in this group. Intracardiac blood samples were taken from each rat, and they were then euthanized.
- *Group 1:* The right femoral artery was located via a simple femoral incision, and the femoral artery was clamped. Three milliliters of intracardiac blood samples were taken at the second hour of ischemia without declamping, and the rats were then sacrificed.
- *Group 2:* The same procedure was followed except that the intracardiac blood samples were taken at the fifth hour of ischemia.
- *Group 3:* The same procedure was followed except that the intracardiac blood samples were taken at the eighth hour of ischemia.

During the interventions, the rats were anesthetized with 130 mg/kg of ketamine hydrochloride (HCL) (Ketalar®, Pfizer, Inc., İstanbul, Turkey) and 20 mg/kg of Xylasine (Rompun®, Bayer Healthcare AG, Leverkusen, Germany) through an intraperitoneal line. The ketamine HCL (50 mg/kg) was also used to maintain the anesthesia, and the vital parameters were continuously monitored.

All blood samples were placed in citrated tubes and centrifuged at 4,000 rpm at 4 °C for 10 minutes. The plasma was subsequently transferred into small microcentrifuge Tubes (Eppendorf AG, Hamburg, Germany) and stored at -80 °C until further processing.

The CNP was assayed in plasma using a commercially available CNP-22 radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). For the solid phase extraction step, 1 ml of plasma was eluted with 1 ml of a solution containing 60% acetonitrile in 1% trifluoracetic acid (TFA) as previously described by Del Ry et al.[2] The samples were then dried in a rotary evaporator and reconstituted with 300-500 µl of the assay buffer. For the immunometric assay, 100 µl of the buffer was used.[2] The mean CNP recovery of the extraction procedure was 74.8%.

Statistical analysis

Values were presented as mean ± standard deviation (SD), and the CNP parameters of the animal subjects were compared using a paired t-test. In addition, the Lilliefors test was used to check the normal distribution of the parameters. Statistical analysis was performed with the SPSS version 15.0 for Windows software program (SPSS Inc., Chicago, IL, USA), and a p value of <0.05 was established as the threshold for significance.

RESULTS

The basal values of the CNP were 0.285±0.011 pmol/L in the control group, and the obtained CNP values were compared in the each of the ischemia groups according to these base values. The CNP values were 0.350±0.015 pmol/L at the second hour of ischemia in group 1, 0.486±0.084 pmol/L at the fifth hour

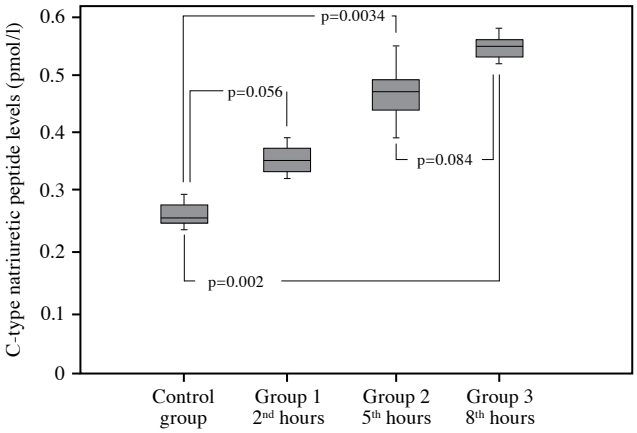

Figure 1. Variations in serum C-type natriuretic peptide levels in prolonged ischemia.

of ischemia in group 2, and 0.534±0.048 pmol/L at the eighth hour of ischemia in group 3. As shown in Figure 1, a time-dependent increase in these values was obtained in the inducible ischemia groups, and this was found to be statistically significant ($p<0.05$).

DISCUSSION

The main finding of this experimental study was that the plasma CNP concentrations with prolonged ischemia were elevated when compared with the basal values. Furthermore, the elevated levels seemed to be strongly related to ischemia duration. To our knowledge, this is the first study that has documented CNP levels during inducible peripheral ischemia, and we believe that the higher CNP concentrations may be an indicator of ischemic exposure.

Blood supply, vascular resistance, and hemodynamic variations are regulated by sensitive interactions between the heart and kidneys. Ischemic muscles can trigger the release of substances via humoral or neurogenic pathways.[8] For example, catecholamines and other mediators might be released by the intramyocardial sympathetic nerve to activate the sympathetic system. During chronic or prolonged ischemia, these pathways are activated by integrating ischemic preconditioning or adaptive mechanisms during low perfusion.[9] The main problem occurs after the reperfusion of ischemic tissues because the tissue and systemic responses are determined by the duration of ischemia. Prolonged peripheral ischemia can lead to more skeletal muscle necrosis which can then result in myonephropathic metabolic syndrome (MNMS).[10,11] Thus, determining the ischemia duration is important for estimating clinical outcomes. In particular, biomarkers have gained importance in determining critical ischemia duration, and recent studies have focused on identifying the values of biomarkers associated with different types of ischemia.[11]

Since first isolated from a porcine brain in 1990, CNP has attracted the attention of researchers because of its important physiological features,[2,12] but its efficacy has been neglected in cardiovascular system regulation due to the unsuccessful determination of CNP levels in initial studies.[2,13] Additionally, NPR-B, a receptor of CNP, has demonstrated various effects on the blood vessel wall upon its expression and cell surface localization in the vascular smooth muscle.[3,14] Moreover, CNP also shows anti-atherogenic properties on vessel walls with the inhibition of leukocyte migration and suppression of P-selectin expression.[14,15] Furthermore, Costa et al.[16] demonstrated that infusion of CNP increases inducible nitric oxide synthase (iNOS) expression. Interestingly, some researchers have also claimed that CNP has a vaso-relaxant impact through the increased production of endothelial NO via elevated iNOS activity.[16,17]

Several studies have investigated NPR-B as it relates to inducible ischemia types.[5,18] Staub et al.[5] suggested that it might have diagnostic value in the determination of inducible myocardial ischemia, and Kumakura et al.[7] reported that high NPR-B levels correlated with a number of affected distal arteries in critical limb ischemia. Furthermore, Montagnana et al.[19] found that NT-prohormone-brain natriuretic peptide (NT-proBNP) was substantially increased in peripheral arterial disease (PAD) patients. Tokudome et al.[20] studied the possible interactions of ANP and BNP in hind-limb ischemia in an experimental model and claimed that natriuretic peptides demonstrate diuresis, natriuresis, and blood vessel dilation effects via the GC pathway which can play an important role in the angiogenic effect of these peptides. They also suggested that ANP and BNP may have a predominate role in the vascular remodeling in cardiovascular events. In another study, Park et al.[21] suggested that recombinant human ANP injections provide more blood flow recovery along with an increase in capillary density in limb ischemia. Guanylyl cyclase activates signal production via the conversion of GTP to the second messenger, cyclic GMP (cGMP), which is responsible for vasorelaxation via interactions of cellular mechanisms such as inositol 1,4,5-trisphosphate generation inhibition, the inhibition of carbonic anhydrase II (Ca2)+ influx, and membrane Ca2+ ATPase stimulation.[22] Furthermore, CNP has

a regulatory effect on the GC enzyme system via the NPR-B, one of the seven particulate GCs cloned from mammalian tissue, and it has an autocrine/paracrine role in vasorelaxation and vascular remodeling because it affects this system.[3,23] Unfortunately, we could not find sufficient data regarding CNP and its relationship with peripheral ischemia, and relatively little data is available in reference to CNP and cardiac events, including heart failure. For example, Passino et al.[24] studied CNP levels in patients with chronic heart failure during physical exercise and claimed that alterations in the levels of CNP from plasma after physical exercise might reflect an enhancement in endothelial function.

In conclusion, our findings suggest that CNP might play an important role on the vascular endothelium as it affects hypoperfusion or ischemia. Hopefully, our results will provide the impetus for more research on this topic since CNP may be useful in the determination of ischemia duration. With this in mind, further studies should be designed to define the role of CNP in peripheral vascular ischemia.

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