Effect of Isatin on Ischemia and Reperfusion Injury: An Experimental Study in the Isolated Heart

İsatinin İskemi ve Reperfüzyon Hasarı Üzerine Etkisi: İzole Kalpte Deneysel Bir Çalışma

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ABSTRACT

Introduction: Isatin is an endogen indole that is found in body fluids and reported as a neuroprotective, anticonvulsant and sedative. The aim of this study was to investigate the effects of isatin on left ventricle functions before and after low-flow ischemia in the isolated rat heart.

Materials and Method: Male Wistar rats were separated in four groups. Isatin (50 mg/kg i.p.) was administered in the I and I-ANP groups, twenty minutes before the hearts were placed on the Langendorff apparatus. Serum physiologic was administered to the control (C) and ANP groups. Low flow ischemia was applied in all groups. ANP (0.1 µM/L) was added to perfusion solution in ANP and I+ANP groups fifteen minutes before ischemia. Left ventricle development pressure (LVDP), and maximum and minimum pressure changes were recorded before and after ischemia. cGMP levels were measured in the perfusate in all groups.

Results: LVDP and minimum pressure change values of I group was found similar to C group at the 60th minute of the reperfusion. The level of cGMP in I group was similar to C group but lower than the ANP and I+ANP groups before and after ischemia.

Conclusion: Administration of isatin prior to cardiac ischemia does not significantly alter cardiac function during reperfusion period in rat heart. The results of this study showed that isatin may not seem to have a disturbing effect on cardiac functions after low flow ischemia.

Keywords: İsatin; iskemi; atriyal natriuretik peptid; cGMP

ÖZET

Giriş: İsatin, vücut sıvılarında endojen olarak bulunan, sinir sisteminde koruyucu, antikonvülzan ve sedatif rolü olduğu bildirilen bir indoldür. Bu çalışmanın amacı, izole sıçan kalbinde oluşturulan iskemi reperfüzyon modelinde isatinin sol ventrikül işlevi üzerine etkisini incelemektir.

Hastalar ve Metod: Erkek Wistar türü sıçanlar dört gruba ayrıldı. I ve I-ANP gruplarına kalpler Langendoff düzeneğine yerleştirilmeden once isatin (50 mg/kg i.p.) verildi. C ve ANP gruplarına serum fizyolojik verildi. Tüm gruplara düşük akımlı iskemi uygulandı. İskeminin 15 dk öncesinde, ANP ve I+ANP gruplarında perfüzyon solüsyonuna ANP (0.1 μM/L) eklendi. İskemi öncesi ve sonrasında sol ventrikül gelişim basıncı (LVDP) ve maksimum ve minimum basınç değişimleri kayıt edildi. Tüm gruplarda perfüzyon sıvısından cGMP düzeyleri ölçüldü.

Bulgular: Reperfüzyonun 60. dakikasında I grubunun LVDP ve mimimum basınç değişim değerleri C grubuna benzer bulundu. İskemi öncesinde ve sonrasında, I grubunun cGMP değerleri C grubuna benzer ancak ANP ve I+ANP gruplarından daha düşük bulunda.

Sonuç: İskemi öncesinde isatin verilmesi sıçan kalbinde kardiyak işlevi anlamlı şekilde değiştirmemektedir. Bu çalışmanın bulguları, isatinin düşük akımlı iskemi sonrasında kardiyak işlevi bozucu etki oluşturmadığı yönündedir.

Anahtar Kelimeler: Isatin; ischemia; atrial natriuretic peptide; cGMP

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1. Introduction

Isatin (Indoledione 2,3) is an endogenous indole found in mammalian tissues and body fluids (1). It is produced in the intestine from indole, which is formed by tryptophan catabolism (2). Isatin is found in mammalian brain, heart, kidney, liver and body fluids. Plasma concentration of this indole may exceed 1µM in human (3). Tissue isatin concentrations change according to organs at the range of, 0.1–1m M. Maximum concentration of isatin in cardiac tissue is found nearly 3 µM (4). This is approximately 3 times of brain level. This finding suggests that heart is an important target organ for isatin.

Isatin has reported to have inhibitory effects on both of natriuretic peptide receptors and also monoamine oxidase enzyme (MAO) activity in the body. It has been reported that increased isatin concentration inhibits monoamine oxidase (MAO) enzyme and produces neuroprotective effect like improving role for the bradikinesia symptom of Parkinson's disease due to suppression of dopamine consumption in animals (5, 6). Sedative and anticonvulsant effects were also reported for this indole (7).

In addition to behavioral positive effects, isatin has also been reported to have inhibitory effects on cardiac function (7). A study by Medvedev et al (8) revealed that isatin inhibits guanyl cyclase-linked natriuretic peptide receptors thus it decreases cellular cGMP level. In contrary, atrial natriuretic peptide (ANP) mediates cGMP level and protects heart tissue against calcium overload and reoxygenation-induced hyper-contracture in isolated ventricular cardiomyocytes (9). When taken into account the ANP mediated increase in cGMP level in reperfusion period in isolated rat heart (10) the possible deleterious role of isatin may alter the biological and pharmacological actions of natriuretic peptides in myocardial injury. In present, it is not known clearly whether isatin affects contraction and relaxation functions of heart after ischemic condition. The role of isatin, which is a benifical non-peptid biofactor, on cardiac hemodynamic function during ischemia and reperfusion condition is needed to be understood. The aim of this study was to investigate the possible hemodynamic effects of isatin on the isolated rat heart before and after ischemia.

2. Materials and Methods

The study was conducted with male Wistar rats weighing 250-350 g kept under standard conditions (23±1◦C room temperature, 60% humidity, 12 hours light-dark cycle) in Laboratory Animals Unit of …….University. The experiments were approved by Local Ethic Committee for Animal Use (TÜHDYEK-2012/68). The rats were divided into 4 groups as the control (C; n=10), the isatin (I; n=10), the ANP (n=7), and the isatin+ANP (I+ANP; n=7) groups. Thirty minutes before ischemia, isatin was administered intraperitoneally (50 mg/kg; within 2.5 ml/kg serum physiologic) to the I and I+ANP groups (11). The C and the ANP groups administered only serum physiologic (2.5 ml/kg; intraperitoneal).

2.1. Hemodynamic study on isolated heart

Before the abdominal cavity was opened with a transverse incision, intraperitoneal heparin (Nevparin Flakon, Mustafa Nevzat İlaç Sanayii A.Ş. İstanbul/Turkey; 500 U/kg) and thiopental (I.E. Ulagay A.Ş., İstanbul/ Turkey; 100 mg/kg) were administered in all groups. Then, the hearts were rapidly excised, put in a Petri dish containing the ice-cold Krebs-Henseleit solution and located into the Langerdorff system and perfused with Krebs-Henseleit solution for 15 minutes in C and I groups. ANP was added to Krebs solution after the hearts were located into the Krebs

solution in the ANP and the I+ANP groups (Figure 1). Afterwards, 30 minutes low-flow ischemia (0.3 ml/min baseline flow) and 60 minutes of reperfusion were applied to all hearts. Krebs-Henseleit solution consisting of (mmol L-1) NaCl 118.3, NaHCO3 25.0, KCl 4.7, KH2 PO4 1.2, MgSO4 1.2, CaCl2 2.5, and glucose 11.1 saturated with 95% O2 and 5% CO2, maintained at 37 °C and pH 7.4-7.45.

A latex balloon that was filled with water was placed in the left ventricle through the mitral valve. Left ventricular developed pressure (LVDP), maximum left ventricle pressure change ratio (dp/dt_{max}), minimum left ventricle pressure change ratio (dp/dt_{min}), and heart rate were continuously monitored by a data acquisition system (Biopac MP36 System, Inc., USA) during the experiments. LVDP was calculated by subtracting the end-diastolic pressure from the systolic pressure. Dp/dt was demonstrated by using the following components; P shows LV pressure, t shows time and d shows rate of change. It is accepted that dp/dt_{max} gives valuable knowledge about the inotropic features; dP/dt_{min} shows lusitropic features that related with the relaxation function of the left ventricle.

Basal (B) hemodynamic measurements were performed at 15th minute of the experiment and the other hemodynamic measurements were performed at first minutes after reperfusion (R1) and 60th minutes after reperfusion (R60) in all groups. In addition to hemodynamic measurements, samples were obtained to detect cGMP levels from perfusion fluid dripping from isolated hearts. cGMP levels from the samples were measured with ELISA method using “Rat Cyclic guanosine monophosphate ELISA kit” (CK-E30335, China).

2.2. Determination of Ischemic Area

At the end of the hemodynamic measurements sections were obtained from left ventricle tissues to determine ischemic areas in rats. Ischemic and nonischemic areas were marked using triphenyl tetrazolium chloride (TTC) for these sections similar to the method described previously (12). One of the most commonly used method to detect the amount of necrosis in ischemic hearts is staining with tetrazolium. This method was carried out by wrapping the frozen hearts by stretch film at -80 ° C after ischemia and reperfusion. The hearts were removed from the freezer after 24 hours and separated into 2 mm slices. Ventricle slices were incubated for 15-20 minutes at a buffer containing 1% tetrazolium at a pH of 7.4 and kept at 20% formal for 20 minutes after incubation. After this procedure, the color separation in the slices became clearer. The necrotic tissues were observed in pale yellowish-brown color, and live tissues were dark red. The plates were clamped from the sides. Necrotic areas were drawn on transparent acetates. The drawings on the acetate were enlarged twice and scanned. Then, the areas of necrotic tissues were measured. Ischemic areas were determined with using computer-assisted planimetric method (13).

2.3. Statistics

Normality distribution of numeric variables was tested by one sample Kolmogorov Smirnov test. Hemodynamic parameters among groups were compared by the Kruskal-Wallis test, and then when the significant difference was obtained the Mann-Whitney U test was used for multiple comparisons. Baseline, 1^{th} min and 60^{th} min measurements of hemodynamic parameters in each group were compared by the Freidman Test, and then the Wilcoxon test was used for multiple comparisons when the significant difference was obtained. SPSS 20.0 package program was

used for statistical analysis. Statistical significance was determined as $p<0.05$. The data were shown as median (min-max).

3. Results

In this study, LVDP, dp/dt_{max}, dp/dt_{min} and heart rate values were found similar in B and RI measurements in all groups. However, LVDP values of R60 measurements in the ANP and the I+ANP groups were higher than the I group and rLVDP values of R60 measurements in the I+ANP group were higher than the C group.

However, LVDP values of R60 measurements in the I+ANP group were higher than the C group, R60 values of ANP and the I+ANP groups were higher than the I group (Table 1). There was also a significant difference between the groups, for the $R60_{dp/dtmin}$ values. $R60_{dp/dtmin}$ values were higher in the ANP group than the C group, and higher in the ANP and the I+ANP groups than I group.

Intragroup comparisons revealed that $R60_{dP/dtmaks}$ values in the C and I groups decreased significantly compared with $B_{dP/dtmaks}$ and $R1_{dP/dtmaks}$ values. In terms of intragroup comparisons, $R60_{dp/dtmin}$ values were found to be lower than $B_{dp/dtmin}$ and $R1_{dp/dtmin}$ values both in C and I groups. Intragroup comparisons of heart rates demonstrated that in the C group, $R1_{HR}$ value decreased significantly compared to B_{HR} and $R60_{HR}$ values. No significant difference was detected for heart rate in other groups (Table 1).

Comparison of ischemic areas showed a significant difference between the groups. Ischemic area in the ANP group was significantly lower than the C group. In addition, ischemic areas in ANP group and the I+ANP group was lower than the I group (Figure 2).

Comparison of cGMP values between the groups showed that there were significant differences between B_{cGMP} values before perfusion and $R_{1\text{-}cGMP}$, $R_{60\text{-}cGMP}$ values during reperfusion period. cGMP values in the I group was lower than the ANP and the I+ANP groups at B_{cGMP}, $R_{1\text{-}cGMP}$, $R_{60\text{-}cGMP}$ measurements (Table 2). cGMP levels in the C group were similar to the I group before and after ischemia. But, B_{cGMP} levels in the C group was lower than the ANP group.

4. Discussion

Isatin is known as a natriuretic peptide antagonist that inhibits natriuretic peptide receptors. Thus, it was suggested as a blocker on the natriuretic peptide-induced cardioprotective effects during ischemia and reperfusion (6). Besides this function, neuroprotective and anticonvulsant effects of isatin have been demonstrated (11). These behavioral functions of isatin have been mainly attributed to monoamine oxidase inhibition (6). Taking into account the positive functional effects and therapeutic roles of natriuretic peptides on the heart, it could be clinically important to understand whether the isatin as a natriuretic peptide blocker has a potential detrimental effect on the heart. Cardiac hemodynamic effects of isatin were investigated before and after low flow ischemia in the present study. The findings of this study demonstrated that isatin did not change left vetricle function following low flow ischemia in isolated rat heart. Thus, this endogeneus indol may not seem to have disturbing effect on cardiac functions after low flow ischemia. This finding may have a clinical importance in order to assess potential pharmacological actions of isatin during cardiac ischemic conditions.

The cardiac intracellular effects of both isatin and ANP were observed with using cGMP level in the present study. Protective effect of ANP on the infarction of isolated rabbit heart has been demonstrated with the activation of cGMP and protein kinase G (14). Isatin has been reported to

inhibit ANP induced guanylate cyclase activity (15). It has been suggested that intracellular mechanism of isatin was related with the formation of cGMP (2). In contrary to this finding, our study demonstrated that ANP increases left ventricle contractility by increasing cGMP however isatin did not affect ventricular hemodynamic responses and did not cause a prominent alteration on myocardial tissue with using cGMP related cellular mechanism before and after ischemia.

In previous studies, improved left ventricle function and protection of heart against infarction has been reported when ANP was administered shortly before reperfusion period (10, 14). Similar to these studies, ANP were added before ischemia in perfusion solution in our study. Isatin was also given intraperitoneally in the present study. Effects of isatin administration on behavioral and metabolic responses were investigated with previous experimental studies on animals. Low doses (5–20 mg/kg) or high doses (>50 mg/kg) have been preferred to investigate the central nervous system effects of isatin. The therapeutical role of isatin on natriuretic peptide induced hyperthermia has also been investigated in a previous study (11). Isatin (50 mg/kg) was adiministered intraperitonally, 30 min before the ANP administration in the present study, similar to the study of Pataki et al. (11). Further studies about different doses of exogenously administered isatin may be useful.

In terms of the cardiac hemodynamic effects of isatin, decreased LVDP, dp/dt_{max} and dp/dt_{min} values in R60 measurements have been observed in the I and C groups. However, no decrease was shown for R60 measurements of LVDP, dp/dt_{max} and dp/dt_{min} values in ANP and I+ANP groups in this study. According to this finding, improved left ventricle function in R60 measurements might result from the ANP administration. Although isatin is reported as a natriuretic reseptor bloker, we did not observe any alteration on improved left ventricle function of ANP. These findings were compatible to our previous results that different doses of isatin did not effect cardiac contractility and relaxation functions of ANP when administered before ischemia in isolated rat heart (16). However, some experimental studies have reported that isatin could limit protective effect of ANP and BNP following ischemia and reperfusion condition in the heart (14, 17). More studies are necessary to clarify cardiac hemodynamic effects of isatin.

Left ventricle contraction function was evaluated with dp/dt_{max} and relaxation function has been shown with using dp/dt_{min} measurements in the present study. Lower degree of relaxation in left ventricle in the C and I groups has been observed than the ANP groups. Moreover, evaluation of dp/dt_{min} values of the ANP and the I+ANP groups during reperfusion period showed no significant decrease; dp/dt_{min} values in these groups were similar to pre-ischemic values. When considering the non-changed values in I group, higher values in I+ANP group may result from the improving effect of ANP on relaxation function of the left ventricle. It has been shown that serum isatin level was increased both in heart and in brain of rats with stress condition (8). According to our findings, higher levels of isatin in stress condition may not seem to have deleterious effect on cardiac function.

Infarct areas due to ischemia were determined by using TTZ staining in all groups in our study. Yang et al (14) investigated the effects of ANP and isatin on rabbit heart by forming ischemia and reperfusion in a recent study. Decreased infarction area has been shown when ANP administered just before reperfusion period; they also reported that protective effect was disappeared in groups that were given isatin. In the present study, the level of ischemic area at left ventricle was not significantly different between C and I groups. The percent level of ischemic area was 15% (7-21) in C group and 17% (10-33) in I group. However, the ischemic area was 6% (4-10) in ANP group

and 8% (4-14) in I+ANP group. These findings suggest a significant decrease in ischemic area in the ANP and I+ANP group compared with I group. According to this finding, isatin did not cause any change in ischemic area when it was administered before ischemia.

In conclusion, it has been observed that isatin, which is known as an endogenous indol with antagonistic effects on natriuretic peptide receptors, did not significantly change left ventricle contractility and relaxation function after ischemia. In this study, hemodynamic measurements and cGMP levels were also examined to understand the role of cGMP in the cardiac hemodynamic effects of isatin and ANP. According to our findings, isatin may not seem to be effective by using cGMP as a second messenger unlike ANP in the heart and therefore, it does not significantly affect cardiac hemodynamic function and myocardial infarct area in reperfusion period. These findings indicate that isatin, which is known effective on natriuretic peptide receptors and monoamine oxidase B does not have a disturbing effect on myocardial ischemia and reperfusion. From the clinical point of view, isatin may not appear to be an effective factor for changing the beneficial effects of natriuretic peptides on the cardiac contraction and relaxation function.

References

1.Buneeva OA, Kopylov AT, Tikhonova OV, Zgoda VG, Medvedev AE, Archakov AI. Effect of affinity sorbent on proteomic profiling of isatin-binding proteins of mouse brain. Biochemistry 2012;77:1326-1338.

2. Gillam EM, Notley LM, Cai H, De Voss JJ, Guengerich FP. Oxidation of indole by cytochrome P450 enzymes. Biochemistry 2000;39:13817-13824.

3.Mawatari K, Segawa M, Masatsuka R, Hanawa Y, Iinuma F, Watanabe M. Fluorimetric determination of isatin in human urine and serum by liquid chromatography postcolumn photoirradiation. Analyst 2001;126:33-36.

4. Igosheva N, Matta S, Glover V. Effect of acute stress and gender on isatin in rat tissues and serum. Physiol Behav 2004;80:665-668.

5. Igosheva N, Lorz C, O'Conner E, Glover V, Mehmet H. Isatin, an endogenous monoamine oxidase inhibitor, triggers a dose- and time-dependent switch from apoptosis to necrosis in human neuroblastoma cells. Neurochem Int 2005;47:216-224.

6. Medvedev A, Buneeya O, Gnedenko O, Ershov P, Ivanov A. Isatin, an endogenous nonpeptide biofactor: A review of its molecular targets, mechanisms of actions, and their biomedical implications. Biofactors 2018;44: 95-108.

7. Pandeya SN, Smitha S, Jyoti M, Sridhar SK. Biological activities of isatin and its derivatives. Acta Pharm 2005;55:27-46.

8. Medvedev AE, Sandler M, Glover V. Interaction of isatin with type-A natriuretic peptide receptor: possible mechanism. Life Sci 1998;62:2391-2398.

9. Hempel A1 FM, Schlüter KD, Forssmann WG, Kuhn M, Piper HM. ANP protects against reoxygenation-induced hypercontracture in adult cardiomyocytes. Am J Physiol 1997;273:H244-H249.

10. Sangawa K, Nakanishi K, Ishino K, Inoue M, Kawada M, Sano S. Atrial natriuretic peptide protects against ischemia-reperfusion injury in the isolated rat heart. Ann Thorac Surg 2004;77:233–237.

11. Pataki I, Adamik A, Telegdy G. Isatin (Indole-2, 3-dione) inhibits natriuretic peptide-induced hyperthermia in rats. Peptides 2000;21:373-7.

12. Downey JM. Measuring infarct size by the tetrazolium method. 2003. Available from: https://www.southalabama.edu/ishr/help/help.htm

13. Brown DA, Lynch JM, Armstrong CJ, Caruso NM; Ehlers LB, Johnson MS et al. Susceptibility of the heart to ischaemia-reperfusion injury and exercise-induced cardioprotection are sex-dependent in the rat. J Physiol 2005;564:619-630.

14. Yang XM, Philipp S, Downey JM, Cohen MV. Atrial natriuretic peptide administered just prior to reperfusion limits infarction in rabbit hearts. Basic Res Cardiol 2006;101; 311–318.

15. Medvedev A, Sandler M, Glover V. The influence of isatin on guanylyl cyclase of rat heart membranes. Eur J Pharmacol 1999;384:239-241.

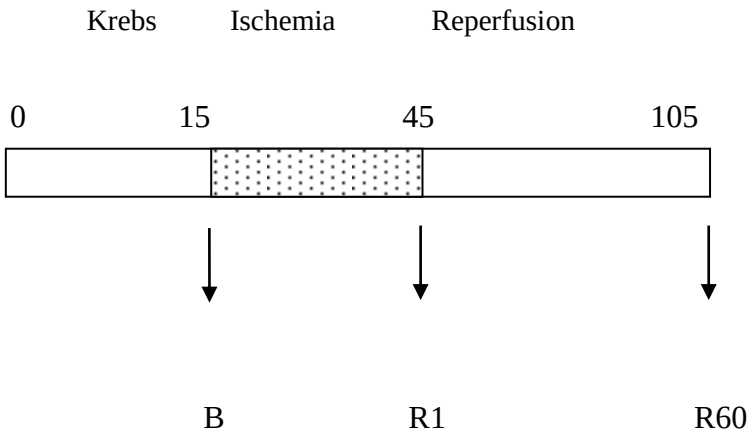
16. Vardar SA, Palabıyık O, Topuz TD, Gürel EE, Çalışkan S, Topçu Özen S, et al.

Hemodynamic effects of atrial natriuretic peptide in ischemia-repertusion injury that occurs after exercise. Turk J Med Sci 2015;45:298-305.

17. Tourki B, Matéo P, Morand J, Elayeb M, Godin-Ribuot D, Marrakchi N et al. Lebetin, a Snake Venom-Derived Natriuretic Peptide, Attenuates Acute Myocardial Ischemic Injury through the Modulation of Mitochondrial Permeability Transition Pore at the Time of Reperfusion. Plos One 2016;11:1-22.

Figure 1. Experimental procedure of the groups taken to Langendorff system.

C and I groups



ANP and I+ANP groups

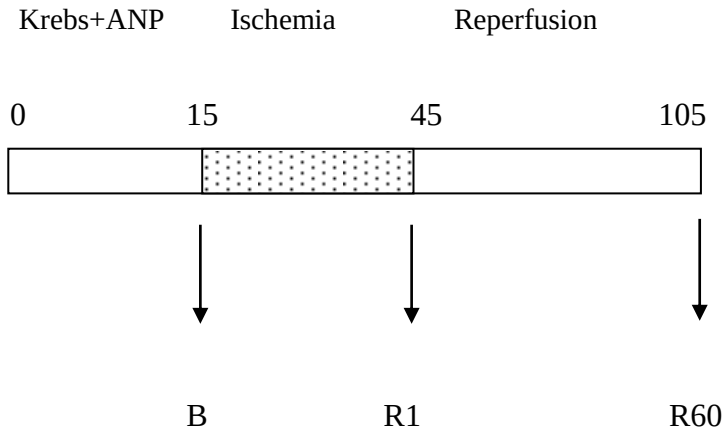
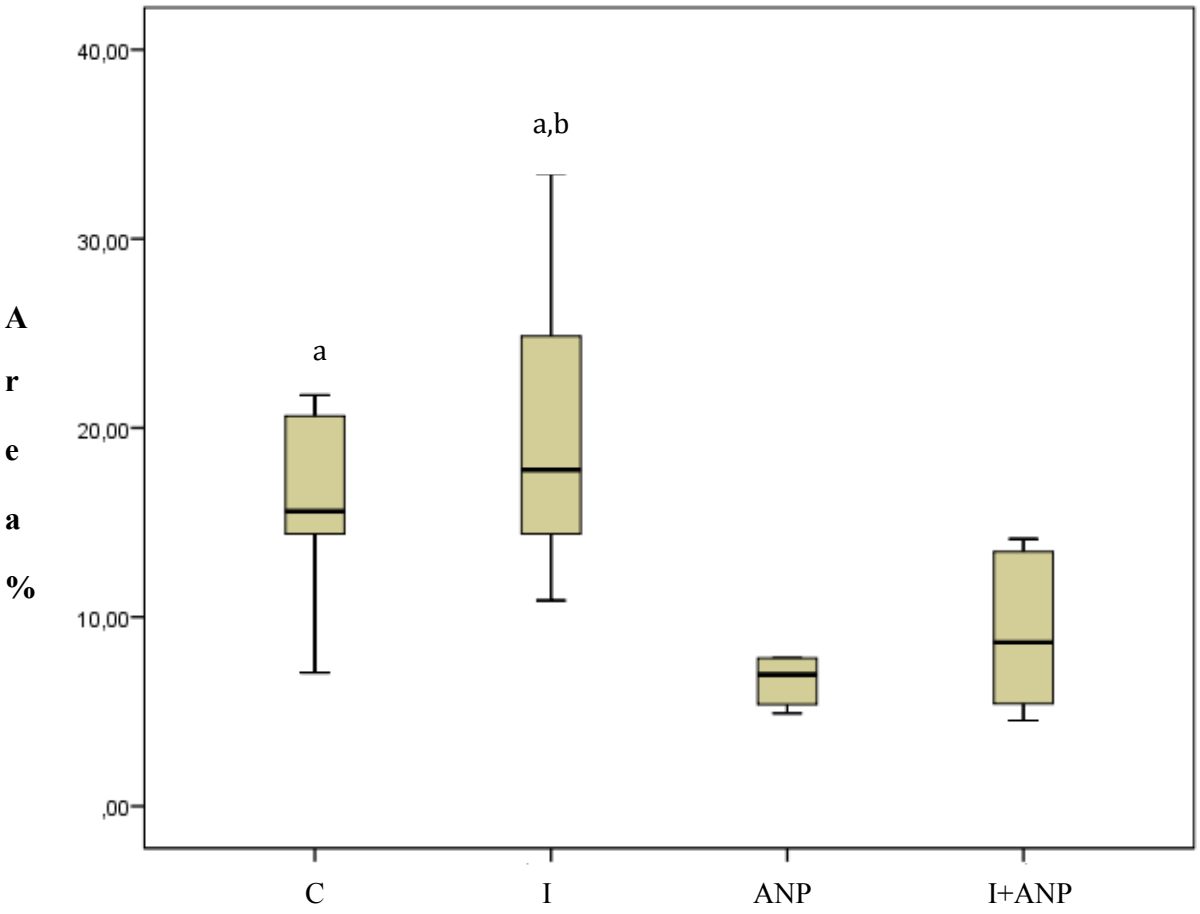


Figure 2. The ratio of ischemic areas in the groups.



The Control Group (C), the Isatin Group (I), the Atrial Natriuretic Peptide Group (ANP), the Isatin + Atrial Natriuretic Peptide Group (I+ANP) a; difference from ANP; b: difference from I+ANP

Table 1. Left ventricle hemodynamic measurements of the groups

	C	I	ANP	I+ANP	P
B_{LVDP} (mmHg)	91(48-143)	66 (5-141)	85 (61-115)	120 (89-168)	0.083
$R1_{LVDP}$ (mmHg)	82 (4-124)	39 (8-127)	72 (7-154)	143 (61-151)	0.084
$R60_{LVDP}$ (mmHg)	33 (1-125) *,#,b	13(1-49) ***,#**,a,b	61 (2-149)	71(3-135)	**0.028**
P	**0.002**	**0.006**	0.565	0.368	
$B_{dp/dtmax}$(mmHg/s)	1964(1340/4140)	1617(-36/4085)	2283(637/4025)	3061(1053/400)	0.762
$R1_{dp/dtmax}$ (mmHg/s)	1890 (-604/3491)	956 (-602/3026)	2694 (-205/4162)	2707 (2253/3806)	0.056
$R60_{dp/dtmax}$(mmHg/s)	750 (-527/4346) *,#	264 (-210/1434) *,#	1745 (-584/4900)	1932 (-359/4114)	0.071
P	**0.020**	**0.045**	0.368	0.651	

$B_{dP/dtmin}$(mmHg/s)	-1377 (-2593/-808)	-911 (-2576/-158)	-1480(-2622/-506)	-1982 (-2821/307)	0.624
$R1_{dP/dtmin}$ (mmHg/s)	-911 (-2576/-158)	-804 (-1421/-245)	-869 (-2157/-607)	-1032 (-2508/-326)	0.444
$R60_{dP/dtmin}$ (mmHg/s)	-578(-2119/-371)*,#,a	-444 (-968/-157)*,#,a,b	-1097 (-2587/-635)	-1126 (-2164/-535)	**0.002**
P	**0.001**	**0.027**	0.867	0.565	
B_{HR} (beat/min)	229 (191/277)	224 (156/263)	223 (105/272)	209 (161/241)	0.736
$R1_{HR}$ (beat/min)	171(78/234)*,^	164(107/272)	205(136/252)	189(151/223)	0.204
$R60_{HR}$ (beat/min)	239(142/300)	236(95/365)	227(144/277)	267(165/285)	0.865
P	**0.001**	0.497	0.867	0.368	

The Control Group (C), The Isatin Group (I), The Atrial Natriuretic Peptide Group (ANP), Isatin + Atrial Natriuretic Peptide Group (I+ANP)

B_{LVDP} ; Pre-ischemia baseline left ventricle developed pressure, $B_{dp/dtmax}$; Pre-ischemia baseline maximum left ventricle pressure change rate**,** $B_{dP/dtmin;}$ Pre-ischemia baseline left ventricle pressure change ratio**,** B_{KH} Pre-ischemia baseline heart rate, $R1_{LVDP}$; Left ventricle developed pressure measured at 1^{st} minute of reperfusion, $R1_{dp/dtmax}$ left ventricle maximum pressure change ratio measured at 1^{st} minute of reperfusion**,** $R1_{dP/dtmin}$ left ventricle minimum pressure change ratio measured at 1^{st} minute of reperfusion**,** $R1_{HR}$ Heart rate measured at 1^{st} minute after reperfusion, $R60_{LVDP}$ left ventricle developed pressure measured at 60^{th} minute of reperfusion, $R60_{dp/dtmax}$ left ventricle maximum pressure change ratio measured at 60^{th} minute of reperfusion, $R60_{dP/dtmin}$ left ventricle minimum pressure change ratio measured at 60^{th} minute of reperfusion, $R60_{HR}$ Heart rate measured at 60^{th} minute of reperfusion; *Difference from B; **#** Difference from R1; ^ Difference from R60, a; Difference from ANP, b; Difference from I+ANP.

Table 2. cGMP Levels of the Groups

	C	**I**	**ANP**	**I+ANP**	**P**
B_{cGMP} (pmol/ml)	1,02(0,01/1,59) a	0,91(0,16-/1,26) a ,b	1,29(1,15/1,35)	1,15(1,01/1,38)	**0.005**
R_{1cGMP} (pmol/ml)	1,03(0,01/1,64)	0,91(0,16/1,05) a ,b	1,20(1,03/1,53)	1,15(0,98/1,47)	**0.008**
R_{60cGMP} (pmol/ml)	0,99(0,18/1,61)	0,87(0,15-/1,08) a ,b	1,24(1,11/1,31)	1,24(1,06/1,44)	**0.002**
P	0.882	0.236	0.607	0.368	

The Control Group (C), The Isatin Group (I), The Atrial Natriuretic Peptide Group (ANP), Isatin + Atrial Natriuretic Peptide Group (I+ANP). cGMP value measured before ischemia (B_{-cGMP}), (R_{1cGMP}) cGMP value measured at the 1st minute after reperfusion; (R_{60cGMP}) cGMP value measured at the 60th minute after reperfusion, a; difference from ANP; b: difference from I+ANP