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

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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 agent. The aim of the present study was to investigate the effects of isatin on left ventricle functions before and after low-flow ischemia in the isolated rat heart.

Patients and Methods: Male Wistar rats were divided into four groups. Isatin (I, 50 mg/kg intraperitoneally) was administered in the I and I-atrial natriuretic peptide (ANP) groups 20 min 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 the ANP and I + ANP groups 15 min before ischemia. Left ventricular developed pressure (LVDP) and maximum and minimum pressure changes were recorded before and after ischemia. Cyclic guanosine monophosphate (cGMP) levels were measured in the perfusate in all groups.

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

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

Key Words: Isatin; atrial natriuretic peptide; ischemia; cGMP

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

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 Yöntem: Erkek Wistar türü sıçanlar dört gruba ayrıldı. I ve I-atriyal natriüretik peptid (ANP) gruplarına kalpler Langendoff düzeneğine yerleştirilmeden önce isatin [50 mg/kg intraperitoneal (ip)] verildi. C ve ANP gruplarına serum fizyolojik verildi. Tüm gruplara düşük akımlı iskemi uygulandı. İskeminin 15 dakika 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 kaydedildi. Tüm gruplarda perfüzyon sıvısından siklik guanozin monofosfate (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 bulundu.

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: İsatin; atriyal natriüretik peptid; iskemi; cGMP

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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]. It is found in mammalian brain, heart, kidney, liver, and body fluids. The plasma concentration of this indole may exceed 1 μM in humans[3]. Tissue isatin concentrations change according to organs at the range of 0.1-1 mM. The maximum concentration of isatin in cardiac tissue is found to be nearly 3 μM[4]. This is approximately three times the brain level. This finding suggests that the heart is an important target organ for isatin.

Isatin has been reported to have inhibitory effects on both natriuretic peptide receptors and also monoamine oxidase (MAO) enzyme activity in the body. It has been reported that an increased isatin concentration inhibits MAO enzyme and produces neuroprotective effect, such as improving the role for bradykinesia symptom of Parkinson's disease due to the 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]. Medvedev et al. revealed that isatin inhibits guanylate cyclase-linked natriuretic peptide receptors, thus decreasing cellular cyclic guanosine monophosphate (cGMP) level[8]. On the contrary, atrial natriuretic peptide (ANP) mediates cGMP level and protects heart tissue against calcium overload and reoxygenation-induced hypercontracture in isolated ventricular cardiomyocytes[9]. When taking into account the ANP-mediated increase in cGMP level in the reperfusion period in isolated rat heart, the possible deleterious role of isatin may alter the biological and pharmacological actions of natriuretic peptides in myocardial injury[10]. Currently, it is not known clearly whether isatin affects contraction and relaxation functions of the heart after an ischemic condition. The role of isatin, which is a beneficial nonpeptide biofactor, on cardiac hemodynamic function during ischemia and reperfusion condition is needed to be understood. The aim of the present study was to investigate the possible hemodynamic effects of isatin on the isolated rat heart before and after ischemia.

PATIENTS and METHODS

The study was conducted on male Wistar rats weighing 250-350 g kept under standard conditions (23 ± 1°C temperature, 60% humidity, and 12-hour light-dark cycle) in the Laboratory Animals Unit of Trakya University. The experiments were approved by the local ethics committee for animal use (TÜHDYEK-2012/68). The rats were divided into four groups as control (C; n= 10), isatin (I; n= 10), ANP (n= 7), and isatin + ANP (I + ANP; n= 7). Isatin was administered intraperitoneally (50 mg/kg; within 2.5 mL/kg serum physiologic) to the I and I + ANP groups 30 min before ischemia[11]. The C and the ANP groups were administered only serum physiologic (2.5 mL/kg intraperitoneally).

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., Istanbul/Turkey; 500 U/kg) and thiopental (I.E. Ulagay A.Ş., Istanbul, Turkey; 100 mg/kg) were administered in all groups. Then, the hearts were rapidly excised, placed in a Petri dish containing ice-cold Krebs-Henseleit solution, located into the Langendorff system, and perfused with Krebs-Henseleit solution for 15 min in the C and I groups. ANP was added to the Krebs solution after the hearts were located into the Krebs solution in the ANP and the I + ANP groups (Figure 1). Thereafter, 30 min low-flow ischemia (0.3 mL/min baseline flow) and 60 min reperfusion were applied to all hearts. Krebs-Henseleit solution consisted of (mmol l-1) NaCl 118.3, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.1 saturated with 95% O_2 and 5% CO_2, 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

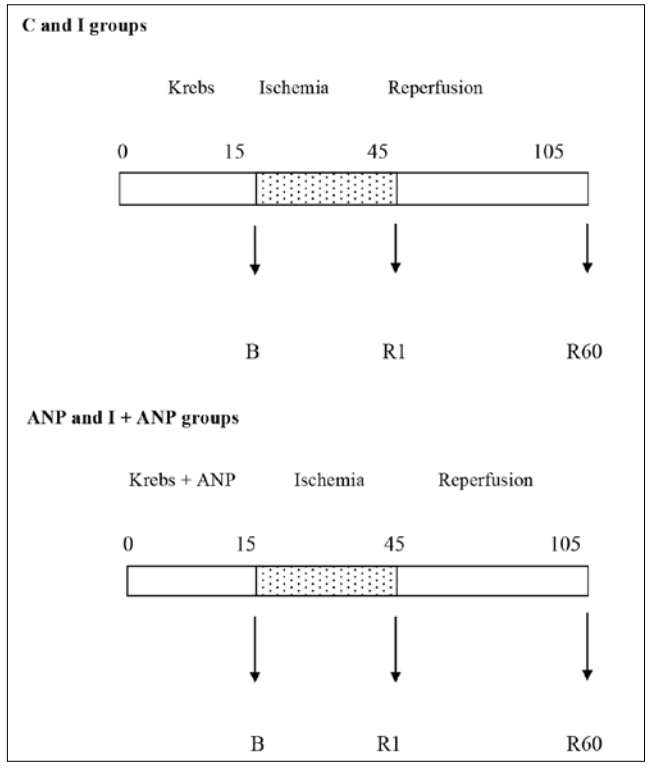

Figure 1. Experimental procedure of the groups obtained by the Langendorff system.

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} provides valuable knowledge about the inotropic features; dp/dt_{min} shows lusitropic features that are related with the relaxation function of the left ventricle.

Basal (B) hemodynamic measurements were performed at 15 min of the experiment, and the other hemodynamic measurements were performed at the first minutes after reperfusion (R1) and 60 min after reperfusion (R60) in all groups. In addition to hemodynamic measurements, the samples were obtained to detect cGMP levels from perfusion fluid dripping from isolated hearts. cGMP levels from the samples were measured by the ELISA method using Rat Cyclic Guanosine Monophosphate ELISA Kit (CK-E30335, China).

Determination of the Ischemic Area

At the end of the hemodynamic measurements, sections were obtained from left ventricle tissues to determine the ischemic areas in rats. Ischemic and non-ischemic areas were marked using triphenyl tetrazolium chloride for these sections similar to the method described previously[12]. One of the most commonly used methods to detect the amount of necrosis in ischemic hearts is staining with tetrazolium. This method was performed by wrapping the frozen hearts by a stretch film at -80°C after ischemia and reperfusion. The hearts were removed from the freezer after 24 h and separated into 2 mm slices. Ventricle slices were incubated for 15-20 min at a buffer containing 1% tetrazolium with a pH of 7.4 and kept at 20% formol for 20 min after incubation. After this procedure, the color separation in the slices became clearer. The necrotic tissues were observed in pale yellowish-brown color, and the 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 the necrotic tissues were measured. Ischemic areas were determined by using computer-assisted planimetric method[13].

Statistics

SPSS 20.0 package program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. One sample Kolmogorov-Smirnov test was used to test the normal distribution of numerical variables. Kruskal-Wallis test was used to compare hemodynamic parameters among the groups, and when the significant difference was obtained, Mann-Whitney U test was used for multiple comparisons. Baseline, 1 min, and 60 min measurements of hemodynamic parameters in each group were compared by the Freidman test, and the Wilcoxon test was used for multiple comparisons when the significant difference was obtained. Data were expressed as median (min-max). A p value < 0.05 was considered statistically significant.

RESULTS

In the present study, LVDP, dp/dt_{max}, dp/dt_{min}, and heart rate values were found to be 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 those in the I group, and rLVDP values of R60 measurements in the I + ANP group were higher than those in the C group.

However, LVDP values of R60 measurements in the I + ANP group were higher than those in the C group, and R60 values of the ANP and the I + ANP groups were higher than those of the I group (Table 1). There was also a significant difference between the groups for the $R60dp/dt_{min}$ values. $R60dp/dt_{min}$ values were higher in the ANP group than in the C group and higher in the ANP and the I + ANP groups than in the I group.

Intragroup comparisons revealed that $R60dp/dt_{maks}$ values in the C and I groups were significantly lower than Bdp/dt_{maks} and $R1dp/dt_{maks}$ values. With regard to intragroup comparisons, $R60dp/dt_{min}$ values were found to be lower than Bdp/dt_{min} and $R1dp/dt_{min}$ values in both the C and I groups. Intragroup comparisons of heart rates demonstrated that in the C group, R1HR value was significantly lower than BHR (pre-ischemia baseline heart rate) and R60HR 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. The ischemic area in the ANP group was significantly lower than that in the C group. In addition, the ischemic areas in the ANP group and the I + ANP group were lower than those in the I group (Figure 2).

Comparison of cGMP values between the groups showed that there were significant differences between BcGMP (cGMP value measured before ischemia) values before the perfusion period and R1cGMP and R60cGMP values during the reperfusion period. cGMP values in the I group were lower than those in the ANP and the I + ANP groups at BcGMP, R1cGMP, and R60cGMP measurements (Table 2). cGMP levels in the C group were similar to the I group before and after ischemia. However, BcGMP levels in the C group were lower than those in the ANP group.

DISCUSSION

Isatin is known as a natriuretic peptide antagonist that inhibits natriuretic peptide receptors. Thus, it was suggested as a blocker on natriuretic peptide-induced cardioprotective effects during ischemia and reperfusion[6]. In addition to this function, neuroprotective and anticonvulsant effects of isatin have been demonstrated[11]. These behavioral functions of

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)$^{*,\#,a,b}$	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} (beats/min)	229 (191/277)	224 (156/263)	223 (105/272)	209 (161/241)	0.736
$R1_{HR}$ (beats/min)	171 (78/234)$^{*,\wedge}$	164 (107/272)	205 (136/252)	189 (151/223)	0.204
$R60_{HR}$ (beats/min)	239 (142/300)	236 (95/365)	227 (144/277)	267 (165/285)	0.865
p	0.001	0.497	0.867	0.368	

* Difference from B, $^{\#}$ Difference from R1, ^ Difference from R60, ^{a} Difference from ANP, ^{b} Difference from I + ANP.
C: Control group, I: Isatin group, ANP: Atrial natriuretic peptide group, I + ANP, isatin + atrial natriuretic peptide group, 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_{HR}: Pre-ischemia baseline heart rate, $R1_{LVDP}$: Left ventricle developed pressure measured at 1 min of reperfusion, $R1_{dp/dtmax}$: Left ventricle maximum pressure change ratio measured at 1 min of reperfusion, $R1_{dp/dtmin}$: Left ventricle minimum pressure change ratio measured at 1 min of reperfusion, $R1_{HR}$: Heart rate measured at 1 min after reperfusion, $R60_{LVDP}$: Left ventricle developed pressure measured at 60 min of reperfusion, $R60_{dp/dtmax}$: Left ventricle maximum pressure change ratio measured at 60 min of reperfusion, $R60_{dp/dtmax}$: Left ventricle minimum pressure change ratio measured at 60 min of reperfusion, $R60_{HR}$: Heart rate measured at 60 min of reperfusion.

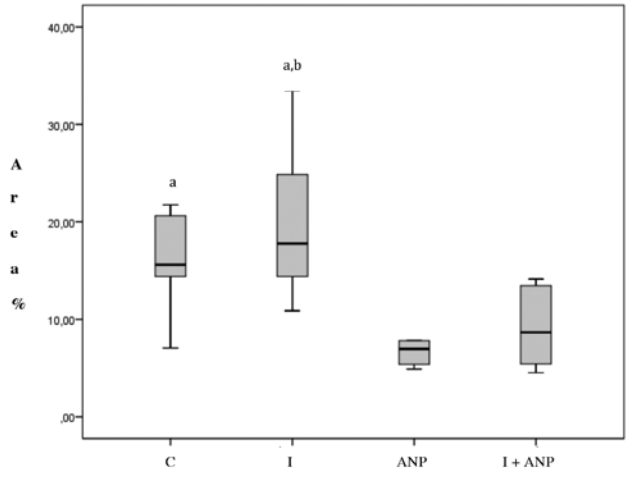

Figure 2. The ratio of ischemic areas in the groups.
C: Control group, I: Isatin group, ANP: Atrial natriuretic peptide group, I + ANP, isatin + atrial natriuretic peptide group.
[a] Difference from ANP.
[b] Difference from I + ANP.

isatin have been mainly attributed to MAO 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 isatin as a natriuretic peptide blocker has a potential detrimental effect on the heart. In the present study, the cardiac hemodynamic effects of isatin were investigated before and after low-flow ischemia. The findings of the present study demonstrated that isatin did not change left ventricle function following low-flow ischemia in isolated rat heart. Thus, this endogenous indole may not appear to have a disturbing effect on cardiac functions after low-flow ischemia. This finding may have a clinical importance to assess the potential pharmacological actions of isatin during cardiac ischemic conditions.

In the present study, the cardiac intracellular effects of both isatin and ANP were observed using cGMP level. The 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 the intracellular mechanism of isatin was related with the formation of cGMP[2]. 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 using cGMP-related cellular mechanism before and after ischemia.

Table 2. cGMP levels of the groups

	C	I	ANP	I + ANP	p
$Bc_{GMP\ (pmol/mL)}$	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
$R1_{cGMP\ (pmol/mL)}$	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
$R60_{cGMP\ (pmol/mL)}$	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	

C: Control group, I: Isatin group, ANP: Atrial natriuretic peptide group, I + ANP: Isatin + atrial natriuretic peptide group, B_{cGMP}: cGMP value measured before ischemia, $R1_{cGMP}$, cGMP value measured at 1 min after reperfusion; $R60_{cGMP}$, cGMP value measured at 60 min after reperfusion.
[a] Difference from ANP, [b] Difference from I + ANP.

In previous studies, improved left ventricle function and protection of the heart against infarction have been reported when ANP was administered shortly before the reperfusion period[10,14]. Similar to these studies, ANP was added before ischemia in perfusion solution in our study. Isatin was also administered intraperitoneally in the present study. The 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]. Similar to the study by Pataki et al., isatin (50 mg/kg) was adiministered intraperitonally 30 min before ANP administration in the present study[11]. Further studies about different doses of exogenously administered isatin may be useful.

With regard to 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 the ANP and I + ANP groups in the present 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 receptor blocker, we did not observe any alteration on the improved left ventricle function of ANP. These findings were compatible to our previous results that different doses of isatin did not affect 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 the protective effect of ANP and brain natriuretic peptide following ischemia and reperfusion condition in the heart[14,17]. More studies are necessary to clarify the cardiac hemodynamic effects of isatin.

Left ventricle contraction function was evaluated by dp/dt_{max}, and relaxation function has been shown using dp/dt_{min} measurements in the present study. A lower degree of relaxation in the left ventricle in the C and I groups has been observed than that in the ANP groups. Moreover, the evaluation of dp/dt_{min} values of the ANP and the I + ANP groups during the 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 the I group, higher values in the 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 the heart and in the brain of rats with stress condition[8]. According to our findings, higher levels of isatin in stress condition may not appear to have a 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. investigated the effects of ANP and isatin on rabbit heart by forming ischemia and reperfusion in a recent study[14]. A decreased infarction area has been shown when ANP is administered just before the reperfusion period; they also reported that the protective effect disappeared in groups that were administered isatin. In the present study, the level of ischemic area at the left ventricle was not significantly different between the C and I groups. The percent levels of ischemic area were 15% (7-21) in the C group and 17% (10-33) in the I group. However, the ischemic areas were 6% (4-10) in the ANP group and 8% (4-14) in the I + ANP group. These findings suggest a significantly lower ischemic area in the ANP and I + ANP groups than that in the 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 indole with antagonistic effects on natriuretic peptide receptors, did not significantly change left ventricle contractility and relaxation function after ischemia. In the present 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 appear to be effective by using cGMP as a second messenger in contrast to ANP in the heart; therefore, it does not significantly affect cardiac hemodynamic function and myocardial infarct area in the reperfusion period. These findings indicate that isatin, which is known to be effective on natriuretic peptide receptors and MAO 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 cardiac contraction and relaxation function.

CONFLICT of INTEREST

The authors reported no conflict of interest related to this article.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: ZG, SV

Analysis/Interpretation: ZG, SV, NS

Data Acquisition: OP, AK

Writting: ZG, SV

Critical Revision: SV, NS

Final Approval: All of authors.

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