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Co-Enzyme Q_{10} Protects Rat Heart against Oxidative Stress Induced by Ischemic Reperfusion Injury

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Abstract: Oxidative stress plays a major role in the etiopathology of myocardial Ischemic Reperfusion (IR) injury which is a common sequel of ischemic heart disease. Antioxidants have potent therapeutic effects on both ischemic heart disease and ischemic-reperfusion injury. In the present study, the effect of co-enzyme Q_{10} (CoQ_{10}) on oxidative stress associated with IR injury was investigated in a rat heart model. Eighteen rats of 200-250 g b.wt. were divided into sham-operated control group (I) (n = 6), ischemia and reperfusion group (II) (n = 6) and CoQ_{10} (1 mg kg^{-1} b.wt. daily by oral route for 7 days before induced ischemia reperfusion) treated group (III) (n = 6) used for induction of ischemia-reperfusion injury. Hearts from all the groups were then processed for biochemical and histopathological studies. All values were expressed as mean±SD. Differences in mean values were compared using SPSS 11.0 by one-way ANOVA and Student-Newman-Keul (SNK) test. A value of $p<0.05$ was considered statistically significant. There was a significant increase in myocardial catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities of these enzymes in mitochondria were maintained to near normal ($p<0.05$) level in CoQ_{10} treated groups with significant change in group (III) group when compared to group (II). Ischemia and reperfusion induced toxicity reduced remarkable amount of RNA content as compared to sham operated control rat. CoQ_{10} attenuated decrease DNA and RNA content induced by ischemic and reperfusion. Histopathology studies showed degree of myocardial damage in CoQ_{10} administered group showing normal structure of myocytes with mild edema during the cardiac IR, which reached a level comparable to sham operated rats. The study strongly suggests that CoQ_{10} administration prevents oxidative stress and associated ultrastructural changes, induced by myocardial ischemic-reperfusion injury.

Key words: Ischemic-reperfusion injury, superoxide dismutase, glutathione, ischemic heart disease, reactive oxygen species

INTRODUCTION

Cardiomyocyte apoptosis has been involved in the pathophysiology of various cardiovascular diseases such as ischemic cardiomyopathy, hibernating myocardium, heart

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failure, reperfusion injury and transplant rejection reactions (Moncada and Higgs, 1993). Apoptotic distinct form of cell death and becoming more obscure. Apoptosis indicate cell death based on DNA and cellular fragmentation requires caspase activation, as these enzymes can activate the endonucleases responsible for DNA degradation. Not only apoptosis is main cause of cell death which is responsible for different cardiovascular and other types of diseases, but also other types of damaging processes are set in motion causing the death of the cells, for that several mechanisms have been proposed, viz., new damage can be initiated by generation oxygen free radicals. Super oxide anion can be reproduced from damaged mitochondria; Cellular antioxidant defense mechanism may also be compromised by ischemia, additional injury is associated with the production of cytokines and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells in ischemic injury (Nishimura *et al*., 1998) and the highly reactive radicals causes DNA damage and alter the cellular antioxidant defense system that comprises SOD and catalase hydrogen peroxide to water, which work in a sequential manner in the disposal of super oxide radicals and conversion of changes in Glutathione (GSH) homeostasis have also been implicated in the etiology progression of number of pathological diseases. Mitochondria are the principal targets in the development of ischemia-reperfusion (I/R) induced injury (Elimadi *et al*., 2001; Detmers *et al*., 1999). The effect of free radicals are expressed by the accumulating of oxidative damage to biomolecules: nucleic acids, lipids and proteins (Polidori *et al*., 2007). Overall, it is thought that the combined effects of ROS and elevated Ca^{2+} play a critical role in the transition from reversible to irreversible reperfusion injury. In particular, they lead to the opening of the mitochondrial permeability transition pore that is now widely accepted to play a critical role in reperfusion injury (Halestrap *et al*., 2004; DiLisa and Bernardi, 2006; Solaini and Harris, 2005; Dorado *et al*., 2006). Generation of reactive oxygen species immediately upon reperfusion has been documented in experimental conditions, as well as in patients with acute myocardial infarction undergoing thrombolysis, coronary angioplasty or open heart surgery (Bolli, 1998). Upon reperfusion, molecular oxygen undergoes sequential reduction to form reactive oxygen species, including superoxide anion and hydroxyl radical, in addition to hydrogen peroxide. The interaction of oxygen-derived free radicals with cell membrane lipids and essential proteins contribute to myocardial cell damage, leading to depressed cardiac function and irreversible tissue injury with concomitant depletion of certain key endogenous antioxidant compounds, e.g., superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and glutathione peroxidase (GPx) (Ferrari *et al*., 1991). It is well established that preconditioning reduces ROS production both at the end of ischemia and during reperfusion (Kevin *et al*., 2003; Hoek *et al*., 2000; Narayan *et al*., 2001; Ozcan *et al*., 2002). It has been reported that in patients with cardiac disease such as chronic heart failure, the myocardium becomes deficient in CoQ_{10} and CoQ_{10} reductase (Mortensen, 1993). CoQ_{10} level is also reduced in other cardiovascular diseases such as cardiomyopathy (Konishi *et al*., 1984). The CoQ_{10} can protect human Low-Density Lipoprotein (LDL) from lipid peroxidation, suggesting its role in atherosclerosis (Joshi *et al*., 2006). Therefore, the study was designed to evaluate the effects of CoQ_{10} on myocardial endogenous antioxidants and on oxidative stress associated with ischemic-reperfusion injury in isolated rat heart model.

MATERIALS AND METHODS

Chemicals

The CoQ_{10} was obtained as a gift sample from Tishcon Corporation, New York, USA. Chemicals were obtained from Sigma (Sigma, St. Louis, Mo, USA). All other chemicals used were of analytical grade.

Animal Care

The study was approved by the Institute Animal Care Ethics Committee. Mature Wistar rats of both sex weighing between 200 and 250 g were procured from Laboratory Animal Resource, Animal House (Reg. No. 118/ac) IFTM, Moradabad. All rats were treated in accordance with the guideline for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised 1985).

Experimental Protocol

Male Wister rats weighing 200-250 g were used for induction of ischemia-reperfusion injury as described previously (Kato *et al*., 2004). Briefly, under ether anesthesia, a left thoracotomy was performed to expose the heart. The left coronary artery was ligated 2-3 mm from its origin with a 5-0 Prolene suture (Ethicon Inc, Somerville, NJ, USA) for 15 min and then the ligation was released. Sham operation was performed using the same procedure except for the coronary artery ligation. Eighteen Wister rats were divided into sham-operated control group (I) (n = 6), ischemia and reperfusion group (II) (n = 6) and Q_{10} treated group (1 mg kg^{-1} b.wt. daily by oral route for 7 days before induced ischemia reperfusion) group (III) (n = 6).

Infracts Size Determination

At the end of 15 min ischemia followed by 45 min reperfusion, the heart were removed and cut into thin cross sectional slices and then incubated in a 0.08% solution of 2,3,5-triphenyltetrazolium chloride dissolved in Kerbs-Henseleit buffer at 37°C for 30 min. The slices were then fixed in formalin. The cardiac ischemic zone was determined by using computer assisted plainimetry as described by Joshi *et al*. (2004).

Estimation on DNA and RNA as Method Described by Burton (1956)

- Extraction of myocardial nucleic acid
- Estimation of myocardial DNA
- Estimation of myocardial RNA
- Biochemical parameters

Isolation of Mitochondria

Two hundred milligram of cardiac tissue was weighed and homogenized with 0.35 M sucrose buffer at 4°C and centrifuged at 10,000 g for 5 min. The resultant mitochondrial pallet was then resuspended in 0.25 M sucrose solution containing 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA and made up to a final volume of 2 mL with the same.

Estimation of Myocardial Reduced Glutathione (GSH)

The GSH was determined as method described Ellman (1959).

Estimation of Catalase Activity

Catalase activity was determined as method described Aebi (1984).

Estimation Super Oxide Dismutase (SOD)

The SOD activity was determined as method described McCord and Fridovich (1969).

Histopathological Examination

Myocardial tissue was fixed in 10% formalin, routinely processed and embedded in paraffin. Paraffin sections (3 μm) were cut and stained with Hematoxylin and Eosin (H and E), examined under a microscope as per the technique of Shimamatsu.

Statistical Analysis

All values were expressed as Mean±SD. Differences in mean values were compared using SPSS 11.0 by one-way ANOVA and Student-Newman-Keul (SNK) test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The infract size shown in Fig. 1 and Table 1. Sham operated group infract size was 5% of total surface of the heart (Fig. 1a). After 15 min ischemia and 45 min reperfusion infracts size was 65% (Fig. 1b). The CoQ_{10} treated group reduced infracts size significantly ($p<0.01$) as compared to IR group which was 26% of the total surface of heart (Fig. 1c).

Table 2 represents the activity of myocardial antioxidant enzymes (GSH, SOD and CAT) after ischemic-reperfusion injury. There was a significant decrease in myocardial GSH, SOD and CAT activity in group (II) (4.53±0.60, 6.87±0.97 and 0.81±0.20 units mg^{-1} protein; $p<0.05$) as compared to sham operated group (7.15±0.73, 12.38±1.32 and 1.54±0.49 units mg^{-1} protein). Activities of these enzymes in mitochondria were maintained to near normal ($p<0.05$) level in CoQ_{10} treated groups with significant change in group (III) (7.10±0.81, 11.97±1.32 and 1.53±0.57 units mg^{-1} protein) group when compared to group (II).

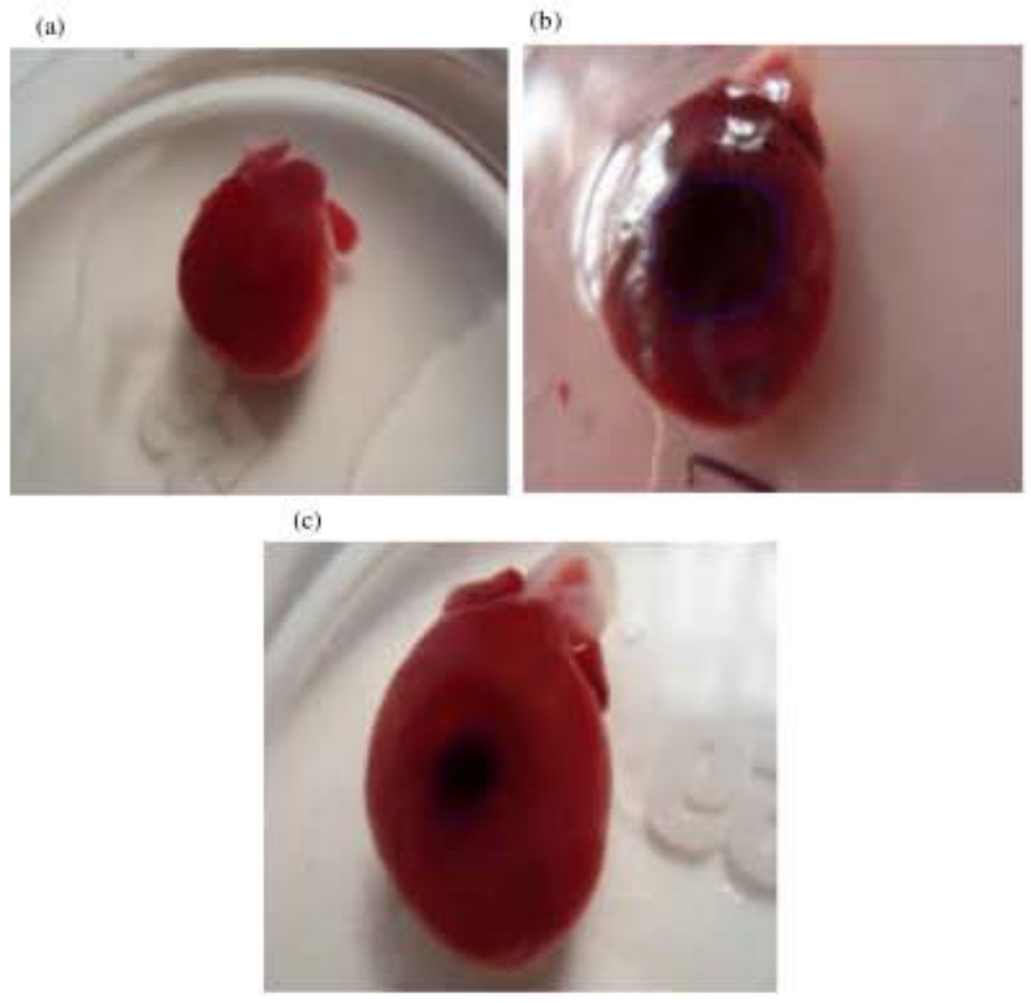

Fig. 1: Showing ischemic zone in (1A): Sham operated rat heart and (2B): Ischemia and reperfused rat heart (3C): Q_{10} treated rat heart after 2, 3, 5 triphenylterazolium chloride staining. Ischemic zone marked with blue lining

Table 1: Effect of Q_{10} after ischemia reperfusion of rat heart

Groups	Percentage infract size
Sham-operated (group I)	5.71±0.81
IR Injury (group II)	65.60±7.34**
CoQ_{10} treated (group III)	26.13±4.23$^{\#\#}$

Results are expressed as Mean±SD (n = 6). Significantly different (*p<0.05,**p<0.01) from sham operated rats and significantly different ($^{\#}$p<0.05,$^{\#\#}$p<0.01) from vehicle-treated ischemia and reperfusion were recorded

Table 2: Effect of Q_{10} on mitochondrial antioxidant level of GSH, SOD and CAT of rat suffered from acute heart injury induced by ischemic and reperfusion

Groups	GSH †	SOD †	CAT †
Sham operated (group1)	7.15±0.73	12.38±1.32	1.54±0.49
IR (group II)	4.53±0.60*	6.87±0.97*	0.81±0.20*
CoQ_{10} treated (group III)	7.10±0.81$^{\#}$	11.97±1.32$^{\#}$	1.53±0.57$^{\#}$

Results are expressed as Mean±SD (n = 6). Significant different (*p<0.05), from sham operated rats. Significant different ($^{\#}$p<0.05), from vehicle treated ischemia and reperfusion. †Activity is expressed as: nmol per 100 mg protein for GSH; unit per mg per 100 mg protein for SOD; nmol of H_2O_2 decomposed per min per mg protein for CAT

Table 3: Effect of Q_{10} on DNA and RNA of rat suffered from acute heart injury induced by ischemic and reperfusion

Groups	DNA‡	RNA‡
Sham operated (group1)	2.96±0.79	14.54±2.17
IR (group II)	2.32±0.45*	8.29±0.73*
CoQ_{10} treated (group III)	2.86±0.23$^{\#}$	13.57±1.03$^{\#}$

Results are expressed as Mean±SD (n = 6). Significant different (*p<0.05), from sham operated rats. Significant different ($^{\#}$p<0.05), from vehicle treated ischemia and reperfusion, ‡Expressed as mg g^{-1} of heart tissues

Table 3 shows the effect of CoQ_{10} on DNA and RNA of rat suffered from acute heart injury induced by ischemic and reperfusion in control and experimental groups. DNA and RNA were 2.96±1.49 and 14.54±2.17, respectively in the sham operated rats decreased to 2.32±1.01, 8.29±0.73 after ischemic followed by reperfusion, where as significantly increased in CoQ_{10} treated rats. Ischemia and reperfusion induced toxicity reduced remarkable amount of RNA content as compared to sham operated control rat. The CoQ_{10} attenuated decrease DNA and RNA content induced by ischemic and reperfusion.

In IR group, showing severe necrosis, marked edema and focal destruction of myocardial in fibers (Fig. 2b). The degree of myocardial damage in CoQ_{10} administered group showing normal structure of myocytes with mild edema (Fig. 2c). The myonecrosis was also not remarkable in this group.

Prolonged severe ischemia leads to cardiac cell death. Necrosis was regarded as the only mode of cell death, whereas, now there is accumulating evidence that in addition to overt necrosis, a subset of cells also die by apoptosis (programmed cell death). The relative contributions of necrosis and apoptosis to cell death in ischemia and reperfusion are still open to debate, although necrosis appears to dominate during ischemia and apoptosis may dominate during reperfusion. There is now evidence that apoptosis occurs during sustained ischemia and when reperfusion follows shorter periods of ischemia (Fliss and Gattinger, 1996; Moudgil *et al*., 2001).

Cardiac myocytes are the likely targets of Reactive Oxygen Species (ROS) attack in the failing heart. It is conceivable that free radicals cause damage at or near the site of there formation. Therefore, as a major source of ROS production, mitochondria could also be the major targets susceptible to ROS attack (Stadtman, 1992). The defects in mitochondrial architecture would lead to the alteration of the mitochondrial metabolism, resulting in decreased activities of mitochondrial enzymes, in the heart, injury induced by IR, thus become a key contributor to intrinsic cell dysfunction (Mimnaugh *et al*., 1984). In the present study, the profile of oxidative/antioxidative status in heart after acute injury induced

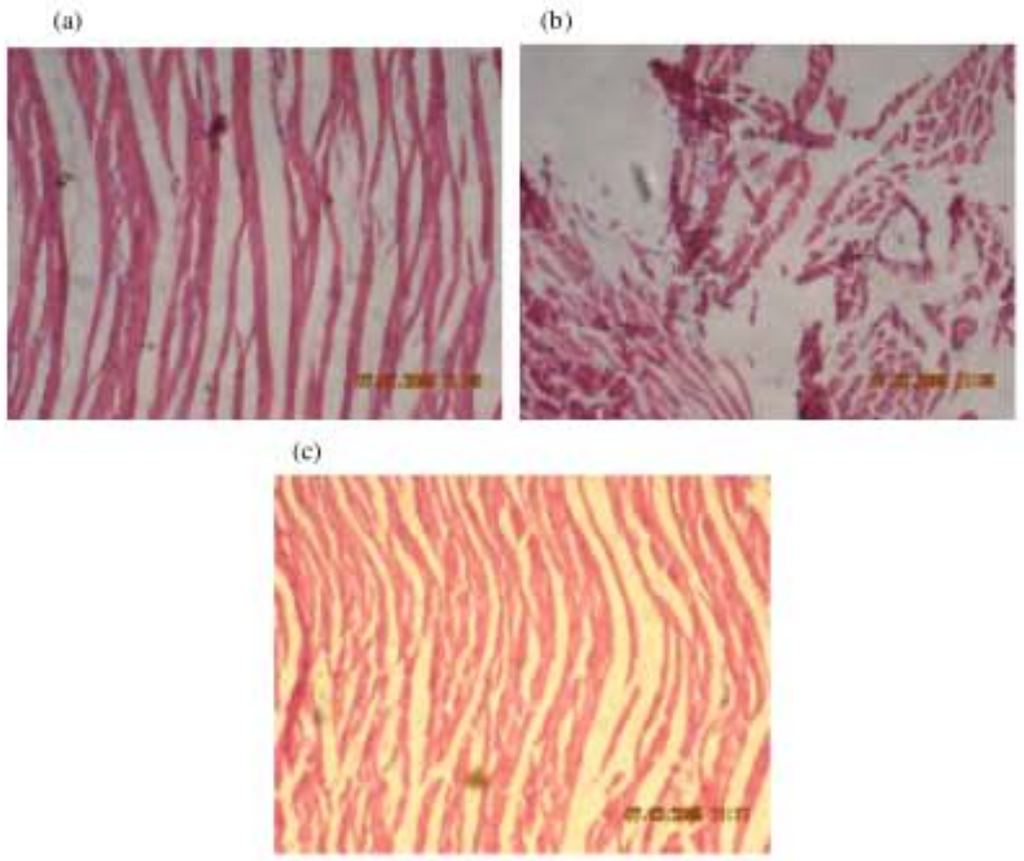

Fig. 2: Histopathology of rat heart. (a): Showing intact microfibrils and myocytes are embedded in tissue in sham operated group (H and E; x 200.), (b): showing severe necrosis, marked edema and focal destruction of myocardial in fibers in IR group (H and E; x 200) and (c): Showing normal structure of myocytes with mild edema in Q_{10} administered group (H and E; x 200)

by ischemic and reperfusion revealed marked alterations in antioxidant enzyme activities. Low activities of antioxidant enzymes such as SOD, CAT and GSH might be due to the overwhelming effects of free radicals, cellular antioxidant enzymes such as SOD, CAT and free radical scavengers like GSH protect cells and tissues against noxious radicals. An imbalance between cellular pro-oxidant and antioxidant levels results in the oxidative stress that leads to tissue damage. The antioxidant enzymes react directly with ROS to yield non-radical products. SOD, a mitochondrial as well as cytosolic enzyme, O_2^- is converted to H_2O_2 by dismutation, which is decomposed by CAT to H_2O (Hassan and Fridovicg, 1978). A number of studies have reported that the activity of SOD, CAT and GSH were decreased significantly in lung, kidney and liver after exposing for long term to chemical stimuli like smokeless tobacco (Mates *et al*., 1999). Present results concord with the earlier study in ischemic heart, Reduced glutathione is only one among many potential antioxidant defenses involved in the protection of various organs against oxidant-induced injury in inflammation (Meister and Anderson, 1983). It is a strong nucleophile and often inactivates electrophilic reactive compounds by either direct non-enzymatic conjugation or enzymatic catalysis. Glutathione has been implicated in various cellular events, such as inflammatory response, modulation of redox-regulated signal transduction, regulation of cell proliferation, remodeling of extracellular matrix, apoptosis, immune modulation and mitochondrial respiration (Rahman and MacNee, 2000). Glutathione synthesis is regulated by oxidants, antioxidants, growth factors and inflammatory and anti-inflammatory agents (Rahman and MacNee, 2000). Major antioxidants like SOD, CAT and GSH are important for cellular protection due to their ability to detoxify free radicals such as reactive oxygen species. A number of studies have reported diverse results for the changes of these antioxidant enzyme activities in animal heart, injury induced by ischemic and reperfusion. The rise in the activities of mitochondrial

antioxidant enzyme in group III rats, pre-treated with CoQ_{10} enzyme highlights the protection rendered by the CoQ_{10} enzyme in combating the oxidative insults and the best protection in histopathological evidences (Fig. 2a-c). A lipid soluble benzoquinone, CoQ_{10}, is an essential component for electron transport in oxidative phosphorylation of mitochondria. Also called ubiquinone, its principal function is to act as an electron carrier between the nicotinamide adenosine dehydrogenases (NADH) and succinate dehydrogenases and the cytochrome system (Sunamori *et al*., 1991). During mitochondrial electron transport, ubiquinone also occurs as semiquinone and ubiquinol, the fully reduced form of ubiquinone. Semiquinone has a role in the generation of superoxide anions during mitochondrial respiration. Where as ubiquinol functions as an intracellular antioxidant, presumably by preventing both the initiation and propagation of lipid peroxidation (Kagan *et al*., 1998) and protect the myocardium from ischemia and reperfused injury.

The finding showed that activities of mitochondrial enzymes (GSH, SOD and CAT) in heart were restored to near normal conditions in Q10 treated group rats, due to protective effect of CoQ_{10} on myocardium (Oliveira *et al*., 2004). Which reduced the extent of myocardium damage induced by ischemic and reperfusion and there by restricted the leakage of these enzymes from myocardium. This indicate that administration of CoQ_{10} enzyme scavenge the free radicals thereby prevent the cellular damage in the membrane. Earlier reports have shown that supplementation of antioxidants in IR induced myocardial injury has a beneficial effect (Balanehru and Nagarajan, 1992).

CONCLUSION

The CoQ_{10} treated rats showed maximum protection from ischemia followed by reperfusion injury. Thus, present study is in agreement with earlier study that ischemia and reperfusion causes severe degeneration in rat heart. Experimental as well as clinical studies with exogenous antioxidants supplementation have been shown to have protective effect in ischemic heart disease. Thus, we have evaluated the antioxidant potential of CoQ_{10} in prevention on ischemic reperfusion injury in our present studies it could be concluded that the CoQ_{10} shows cardioprotective. Further study is needed to establish molecular basis of cardioprotective effects of CoQ_{10} in ischemic-reperfusion injury.

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