Coenzyme Q_{10} treatment reduces lipid peroxidation, inducible and endothelial nitric oxide synthases, and germ cell–specific apoptosis in a rat model of testicular ischemia/reperfusion injury

In this experimental study, we assessed the preventive effects of coenzyme Q_{10} (CoQ_{10}) in a rat model of ischemia/reperfusion injury. The results of this study show that CoQ_{10} administration before the reperfusion period of testicular torsion provides a significant decrease in testicular lipid peroxidation products and expressions of inducible nitric oxide synthase, endothelial nitric oxide synthase, and germ cell-specific apoptosis. (Fertil Steril® 2010;93:280–2. ©2010 by American Society for Reproductive Medicine.)

Testicular torsion is a urologic emergency that causes testicular injury and subfertility (1). It appears that the main pathophysiology of testicular torsion is ischemia/reperfusion (I/R) injury of the testis caused by the twisted spermatic cord and its release (2, 3). Although reperfusion is essential for the survival of ischemic tissue, there is good evidence that reperfusion itself causes additional cell injury.

Coenzyme Q_{10} (CoQ_{10}) is an essential component for electron transport in oxidative phosphorylation of mitochondria (4, 5). It is a potent antioxidant, a membrane stabilizer, and cofactor in the production of adenosine triphosphate by oxidative phosphorylation. Coenzyme Q_{10} has been widely applied in food supplements and cosmetics in Japan, the U.S., and many other countries. In recent years, the frequency of studies involving CoQ_{10} has increased in both basic and clinical research areas (4–7). Several studies have demonstrated the protective effect of CoQ_{10} in various forms of tissue injury (4). However, no study has investigated the effect of CoQ_{10} in testicular I/R injury.

The present study aimed to assess the effects of CoQ_{10} administration on lipid peroxidation, inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), and germ cell–specific apoptosis in a rat model of testicular I/R injury.

The study included 21 adult male Wistar rats weighing 240–260 g. The rats were maintained on a 12-hour light/dark cycle after ethical committee on animal research approval. Institutional Review Board approval was obtained. The rats were divided into three groups of seven rats each. One group underwent 1 hour of testicular torsion, the second received pretreatment with CoQ_{10} before detorsion, and the third underwent a sham operation. Surgery was done with the subject under ketamine anesthesia (single intraperitoneal 50 mg/kg dose). All surgical procedures were performed through standard ilioinguinal incisions (8). At the end of the experiments, bilateral orchiectomies were performed and the rats were killed by pentobarbital overdose (200 mg/kg) and bilateral thoracotomy.

In the torsion-detorsion (T-D) group, the gubernaculum was divided, and the testis was freed from its longitudinal and distal pole attachement to the epididymis. Torsion was created by rotating the left testis 720° clockwise and maintained by fixing the testis to the scrotum with a 4-0 silk suture placed through the tunica albuginea. After 1 hour of torsion, the testis was counter-rotated back to the natural position and reinserted into the scrotum. The wound was closed using 3-0 catgut suture. After detorsion for 4 hours, bilateral orchiectomies were performed.

In the group receiving CoQ_{10} before detorsion, the same surgical procedure was done as in the T-D group, but CoQ_{10} (10 mg/kg; Sigma Chemical Co., St. Louis, MO) was injected intraperitoneally for 30 minutes before detorsion. The administration mode and dose of CoQ_{10} corresponded to those used in earlier experimental studies (7). After detorsion for 4 hours, bilateral orchiectomies were performed.

In the control group (sham group), a sham procedure was performed. This consisted of same procedure as in the T-D group,

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except that after rotating the testis 720° clockwise, it was immediately relieved, and a 4-0 silk suture was placed through the tunica albuginea. Bilateral orchiectomies were performed after the procedure.

Testicular lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) (9). High-performance liquid chromatographic (HPLC) analysis was performed using a Shimadzu HPLC system (Kyoto, Japan) with MDA kit (Immundiagnostik, Bensheim, Germany). Immunohistochemical evaluation of iNOS, eNOS, and germ cell–specific apoptosis were performed with iNOS Ab-1, eNOS Ab-1, and apoptosis protease–activating factor 1 (APAF-1) antibody (Lab Vision Corp., Neomarkers, CA), respectively (10). The number of stained germ cells were counted in 100 seminiferous tubules on circular cross-sections for each group.

All data are expressed as mean ± SD. Analysis of variance was used for statistical analysis of the data among the groups. Multiple comparisons were made using Tukey procedure, with $P<.05$ considered to be statistically significant.

The values of testicular MDA levels and iNOS, eNOS, and APAF-1 expressions of each group are shown in Table 1. Compared with the sham group, testicular MDA levels and expressions of iNOS, eNOS, and APAF-1 obtained from the T-D group were significanlty higher in the ipsilateral testes ($P<.001$). Ipsilateral testicular MDA levels and iNOS, eNOS, and APAF-1 expressions obtained from the CoQ_{10} treatment group were significantly lower than those obtained from the T-D group ($P<.01$). All of the parameters of contralateral testes did not reveal any statistically significant differences among the groups ($P>.05$).

In agreement with earlier studies (8, 11, 12), we found that testicular T-D caused a significant increase in testicular lipid peroxidation products and expressions of iNOS and eNOS and germ cell–specific apoptosis. The results from the present study indicated that CoQ_{10} administration before the reperfusion period of testicular torsion provided a significant decrease in testicular lipid peroxidation products and expressions of iNOS, eNOS, and germ cell–specific apoptosis.

Coenzyme Q_{10} was first introduced as an ethical drug for heart failure patients in Japan and other nations. Coenzyme Q_{10}, which functions endogenously in the mitochondrial electron transport chain, can be ingested to scavenge free radicals and contribute to antioxidant defenses in vivo (5). In recent years, the role of CoQ_{10} in disease prevention and treatment has been intensely investigated (4). The successful results of CoQ_{10} administration in different organ systems led us to attempt such treatment with a model of testicular I/R injury. To our knowledge, the effect of CoQ_{10} on testicular I/R injury has not been reported.

We demonstrated that administration of CoQ_{10} treatment before reperfusion caused significant reduction in the level of testicular MDA. It has been demonstrated that CoQ_{10} functions as an antioxidant, scavenging free radicals and inhibiting lipid peroxidation (5). The present data indicate that treatment with CoQ_{10} prevents further increase in lipid peroxidation caused by I/R; therefore, it may protect the testis against oxidative damage.

The results of earlier studies suggest that NO has an important role in damage of the testis via I/R (8, 12). It has been investigated in several organs that after ischemia, superoxide is produced during the reperfusion phase which rapidly reacts with NO and forms

TABLE 1

The values of MDA levels and iNOS, eNOS and APAF-1 expressions in ipsilateral and contralateral testes (mean ± SD).

Group	MDA (μmol/g protein)		iNOS (positive staining/tubule)		eNOS (positive staining/tubule)		APAF-1 (positive staining/tubule)	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Torsion-detorsion[a]	1.99 ± 0.45	0.38 ± 0.12	5.88 ± 2.01	1.15 ± 0.85	6.55 ± 3.08	1.12 ± 0.54	25.62 ± 6.39	10.15 ± 1.22
CoQ_{10}[b]	0.71 ± 0.21	0.4 ± 0.18	2.57 ± 0.78	1.13 ± 0.75	3.28 ± 0.95	1.16 ± 0.62	11.00 ± 1.15	9.95 ± 1.18
Sham	0.42 ± 0.15	0.39 ± 0.1	1.12 ± 0.92	1.15 ± 0.95	1.18 ± 0.42	1.14 ± 0.7	9.28 ± 0.95	9.75 ± 1.15

Note: There were no statistically significant differences in the contralateral testes among the groups ($P>.05$).
APAF-1 = apoptosis protease–activating factor 1; CoQ_{10} =; coenzyme Q_{10}; eNOS = endothelial nitric oxide synthase; iNOS = inducible nitric oxide synthase; MDA = malondialdehyde.
[a] Versus sham in the ipsilateral testes: $P<.001$.
[b] Versus torsion-detorsion in the ipsilateral testes: $P<.01$.

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reactive nitrogen species peroxynitrite (2). Peroxynitrite initiates toxic oxidative reactions, directly inhibits mitocondrial respiratory enzymes, decreases cellular oxygen consumption, and inhibits membrane sodium transport. Coenzyme Q_{10} can reverse endothelial dysfunction by preventing oxidative and nitrative stress and inflammation (5). It may act to scavenge oxidant species, thereby reducing oxidative stress and resulting in recoupling of NOS (13). We demonstrated that treatment with CoQ_{10} significantly decreases the expressions of iNOS and eNOS. In the light of these data, one may anticipate that administrating CoQ_{10} before reperfusion would protect the testis against NO-related injury in I/R.

Turner et al. (11) reported that testicular T-D in the rat resulted in germ cell–specific apoptosis. In the present study, we showed that T-D caused a significant increase in APAF-1 expression in the testis. APAF-1 plays a central role in mitochondrial apoptosis (14). The release of cytochrome c from the mitochondria leads to recruitment of procaspase 9 and APAF-1 into a protein complex that subsequently activates caspase 9. Lysiak et al. (15) found that IR of the testis stimulated a mitochondrial- and caspase 9–dependent pathway to germ cell–specific apoptosis. To our knowledge, the role of CoQ_{10} on germ cell–specific apoptosis of testis induced by I/R is undefined. Under our experimental conditions, we found that administering CoQ_{10} before reperfusion caused a significant decrease in the testicular APAF-1 expression compared with that in the T-D group.

Recent investigations have emphasized the antiapoptotic effect of CoQ_{10} and the protective effect of CoQ_{10} against apoptotic changes in several tissues and cells (16, 17). Emerging evidence indicates that a central event of apoptosis is opening of the mitocondrial permeability transition pore. Opening of the permeability transition pore is responsible for disruption of the mitochondrial transmembrane electrochemical gradient and is accompanied by extrusion to cytoplasm of several molecules, including cytochrome c and APAF-1, responsible for caspase cascade activation. The antiapoptotic activity of CoQ_{10} is mediated by hindering mitochondrial depolarization, cytochrome c release to cytoplasm, and activation of caspase 9 (16, 17). Coenzyme Q_{10} may be endowed with antiapoptotic activity as a modulator of permeability transition pore opening. On the other hand, we demonstrated that CoQ_{10} may confer protective effect against lipid peroxidation in testicular I/R injury, and germ cell–specific apoptosis may be suppressed by this antioxidant action of CoQ_{10}. However, this study is the first report in the literature to show that the apoptotic changes seen in germ cells were reduced by CoQ_{10} in the rat model of testicular torsion.

To date, several enzymes and drugs have been studied to alleviate testicular damage in animal models of I/R injury (1, 10, 18). However, none have been tested in clinical trials, apart from cooling the scrotum. Coenzyme Q_{10} was first introduced as a drug for heart failure patients; its present status in most countries is that of a compound aimed at improving cellular bioenergetics, counteracting oxidative stress, and slowing down some age-related pathologies; it is also used as a preventive support measure.

In conclusion, the use of CoQ_{10} in humans in both earlier studies and clinically without significant side effects can make its potential use in testicular torsion more attractive. The results of the present experimental study show that administration of CoQ_{10} may be a novel approach for the therapy of I/R injury of the testis.

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