407

Dae Yong K[1], Kang Sup K[1], Ju Hyun S[1], Jae Sung L[1], Hongsik K[1], Yong Gil N[1], Chong Koo S[1]
1. *Chungnam national university hospital, Urology department*

KOREAN RED GINSENG REDUCES ISCHEMIA/REPERFUSION-INDUCED OXIDANT STRESS AND DYSFUNCTION IN THE BLADDER VIA INCREASED SOD EXPRESSION IN RATS.

Hypothesis / aims of study
Ischemia and reperfusion (I/R) of the bladder are observed in age-related disorders such as urinary retention, atherosclerosis, vasospasm, embolization and thrombosis. Bladder dysfunction due to I/R injury was proved by organ bath techniques and histological studies(1). I/R mediated bladder injury induces high reactive oxygen species which is likely to play an important role in bladder damage(1, 2). Therefore, antioxidants may have a potential therapeutic effect in bladder dysfunction.
Korea red ginseng (KRG) has been used in alternative medicine due to its antistress and antioxidant activity. Although KRG has been investigated for multiple purposes(3), its effect on I/R injury of the bladder has not yet been elucidated. The present study was performed to determine whether KRG protected a rat's bladder against dysfunction induced by I/R injury.

Study design, materials and methods
Forty 12-week-old male Sprague-Dawley rats were divided into four groups: sham operated group (C), sham-operated pretreated KRG group (KRG), 3 hours ischemia then 3days reperfusion group (I/R), ischemia-reperfusion and pretreated KRG group (I/R+KRG). Blood flow in the bladder was monitored with a laser doppler flowmeter to confirm the I/R operation was successful. The bladder physiologic functions were estimated by cystometric studies. Contractile responses to carbachol and KCl through isolated organ bath studies were recorded. Blood levels of reactive oxygen species (ROS) were determined using the free oxygen radical tests (FORt). Superoxide generation was measured based on lucigenin-enhanced chemiluminescence in the bladder tissue. We also used oxidative fluorescent microtophotography to evaluate the in situ concentration of superoxide. We performed western blot analysis to evaluate Mn SOD, Cu/Zn SOD, Ec SOD activity.

Results
In the I/R group, the contractile responses were significantly lower than the other groups and were reversed by the pretreatment with KRG, whereas the responses to KCl were not affected by KRG. The mean level of FORT, which is an oxidative stress marker, showed a marked increase following I/R compared with the C group. KRG administration prevented an increase in bladder FORT level. Basal bladder superoxide and NADPH-stimulated superoxide production were significantly increased in the I/R group and were significantly decreased by pretreatment with KRG. The C and KRG group demonstrated minimal fluorescence in the detrusor muscle. In the I/R group, there is a marked increased in ethidium bromide fluorescence reflecting increased superoxide levels in the detrusor muscle. Dihydroethidien (DHE) staining from the I/R group was markedly decreased by pretreating it with KRG. Western blot showed Mn SOD and Cu/Zn SOD expressions improved after KRG pretreatment.

Table 1. Comparison of voiding frequncy and cystometrogram parameters

Parameter	Group			
	C	KRG	I/R	I/R+KRG
Voiding frequency/6hour	2.36±0.42	2.10±0.20	4.76±0.72*	3.42±0.41+
MP (mmHg)	49.36±4.57	47.84±3.41	38.93±2.83‡	44.25±3.10
BC (ml)	0.72±0.13	0.75±0.04	0.63±0.04	0.69±0.07
MV (ml)	0.68±0.07	56±0.08	0.38±0.05‡	0.59±0.10
RV (ml)	0.04±0.01	0.09±0.02	0.25±0.07‡	0.10±0.02

MP, Micturition pressure; BC, Bladder capacity; MV, Micturition volume; RV, Residual volume.
Data shown as mean ± S.E.M.

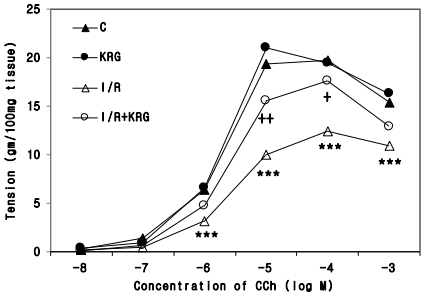

Fig. 1. Concentration-contraction curves for carbachol (CCh) in detrusor strips from four groups.

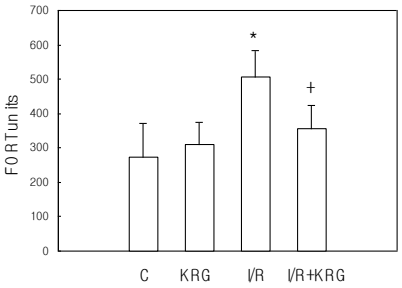

Fig. 2. Assessment of oxidative stress markers :Free oxygen radical tests(FORT)

* : vs C and KRG group, p<0.05. *** : <0.001
+ : vs untreated I/R group, p<0.05. ++ : p<0.01
‡: significantly different from other groups. p<0.05

Interpretation of results

We have demonstrated that I/R leads to bladder dysfunction as well as to the induction of superoxide. We found that I/R of the bladder was associated with a significant generation of superoxide radicals compared with control and I/R+KRG group. Increased superoxide formation in the I/R group was nearly normalized by KRG pretretment.

The superoxide dismutase (SOD) plays an important role in protecting against free oxygen radicals. SOD represents a major celluar defence against superoxide and peroxynitrate formation which contribute to tissue injury and organ dysfunction. In this experiment we confirmed that pretreated KRG recovered SOD activity and thus reversed bladder dysfunction and oxidative damage.

Concluding message

Treatment with KRG reversed the contractile responses of rat bladder and prevented oxidative stress following I/R injury.

References

1. Effect of age on hydrogen peroxide mediated contraction damage in the male rat bladder. J Urol, 2003
2. The role of free radicals and nitric oxide in the ischemia-reperfusion injury mediated by acute bladder outlet obstruction. Int Urol Nephrology, 2007
3. Ginseng pharmacology : multiple constituents and multiple actions. Biochem Pharmacology, 1999

Specify source of funding or grant	**none**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Chungnam national university animal ethics committee**