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LONG-TERM CALORIE RESTRICTION PROTECTS AGAINST AGE-RELATED OXIDATIVE AND FIBROTIC CHANGES IN THE RAT BLADDER

Hypothesis / aims of study

A recent *in vitro* functional study showed that contractile property in response to muscarinic-and nerve mediated-stimulations are impaired with aging in rats, and that these impairments can be prevented by long-term calorie restriction (CR) (1). However, the pathophysiology of these age-related impairements and mechanisms involved in the preventative effect of CR have not yet been determined. In previous studies, calorie restriction (CR) shows a potentially antioxidant effect and a preventative effect on the fibrotic changes associated with aging in the rat aorta and heart (2). We hypothesized that such age-related changes in the oxidative stress and fibrosis occur also in the rat bladder, which contributes to its age-related contractile impairments. Therefore, the aims of the present study were to investigate possible changes in oxidative stress, antioxidant enzymes and fibrotic changes in aged male rat bladder, and to determine whether CR can protect against the age-related changes.

Study design, materials and methods

Fischer 344 male rats were divided into three groups: young (6 months-old, Y: n=8), old (26 months-old) fed fully ad libitum with standard food (O+AL: n=7) or CR (O+CR: n=8). The O+CR group has been fed the same standard food only three days a week since 6 weeks-old.

Malondialdehyde (MDA) level, an index of lipid peroxidation, and glutathione (GSH) level, an antioxidative substance, were determined with fluorescence measurement at 490 nm and 425 nm, respectively. For the analysis of cDNA microarray, 4 samples of the whole bladder in each group were examined. The genes which have been generally defined as a player related to the peroxidation and/or antioxidant were evaluated.

In separate bladder tissues, amount of fibrosis in both detrusor layer and whole bladder layer was evaluated by using Masson-trichrome staining. The images were analyzed by using Adobe and Image J software, and calculated the collagen-deposition rate.

Results

The MDA level was significantly higher in the bladder of O+AL (1.94 ± 0.24 nmol/mg protein) group than those of Y (0.49 ± 0.14 nmol/mg protein) and O+CR (0.27 ± 0.09 nmol/mg protein) groups (Figure 1A). On the other hand, there were no significant differences in GSH level between Y (6.98 ± 0.50 nmol/mg protein) and O+AL (8.09 ± 0.98 nmol/mg protein) groups, although the value of O+CR (3.99 ± 0.23 nmol/mg protein) group significantly lower than those of Y and O+AL groups (Figure 1B).

The cDNA microarray analysis revealed a gene expression of glutathione S-transferase alpha 2 (Gsta2), a modulator of glutathione activity, was up-regulated in O+AL group compared with Y group. In contrast, the expression of Gsta2 was rather down-regulated in O+CR group compared with Y group (Table 1). Except for Gsta2, the expressions of other genes related to oxidative stress showed no large differences among the three groups.

The Masson-trichrome staining showed that the collagen-deposition rates significantly increased in both detrusor and whole bladder layers of O+AL group compared with those of Y group. However, no significant differences on the rate in either layer were observed between Y and O+CR groups (Figures 2, 3A and 3B).

Interpretation of results

The present study suggests that lipid peroxidation increases with aging in the male rat bladder and long-term CR can prevent the age-related increase in peroxidation. These findings are in line with the previous study showing that MDA level significantly increased with aging in the bladder of male Wistar rats (3). On the other hand, regarding the antioxidative activity, GSH level showed no significant differences with aging although the expression of Gsta2 was up-regulated with aging in the cDNA microarray analysis. These results suggest that GSH production has been activated in response to increased oxidative stress at gene level, but not at protein level. In contrast, the results of the decreased GSH level and gene expression of Gsta2 in O+CR group suggest that under long-term CR condition antioxidative substances did not need to fully exert against peroxidation caused by aging because there have been less oxidative stress. On the basis of the results of Masson-trichrome staining, fibrosis progresses with aging. Furthermore, long-term CR might have a role in prophylactic effect on their age-related fibrosis.

Concluding message

Aging increases oxidative stress and fibrotic progression in the bladder of the male rats, and CR can protect against these age-related oxidative stress and fibrosis. These age-related peroxidation and fibrotic changes might contribute to the contractile impairments of the bladder associated with aging.

Gene Symbol	Gene name	Fold change		
		O+AL vs Y	O+CR vs Y	O+AL vs O+CR
Gsta2	glutathione S-transferase alpha 2	1.90471131	0.664292776	2.867276868
Hmox1	heme oxygenase (decycling) 1	1.670469	1.243925773	1.342900868
Gpx2	glutathione peroxidase 2	1.495660671	1.296595469	1.153529151
Nqo1	NAD(P)H dehydrogenase, quinone 1	1.263365991	1.474344685	0.856900021
Nfe2l2	nuclear factor, erythroid derived 2, like 2	1.207566659	1.168095355	1.033791167
Gclc	glutamate-cysteine ligase, catalytic subunit	1.087916243	1.111127975	0.979109758
Prdx1	peroxiredoxin 1	1.056681253	1.066481215	0.990810938
Sod2	superoxide dismutase 2, mitochondrial	1.045131492	1.010134296	1.034646083
Keap1	Kelch-like ECH-associated protein 1	1.0163894	1.083453726	0.938101348
Gpx1	glutathione peroxidase 1	0.987362354	0.943906498	1.046038306

Table 1. Representative genes which play a key role in responding to oxidative stress and antioxidation, and their expression in the bladder of the three groups by using cDNA microarray analysis.

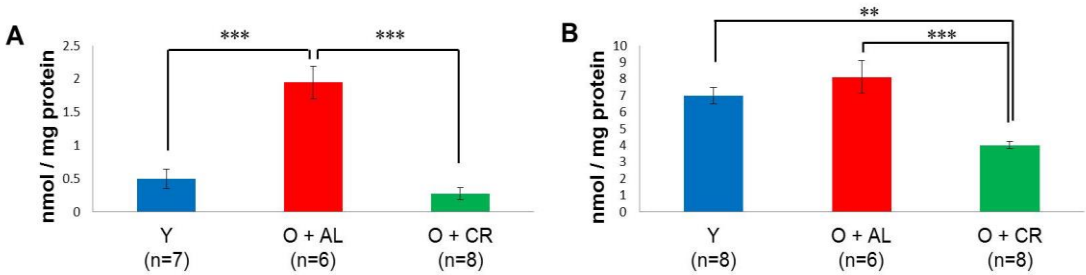

Figure 1. Malondialdehyde (MDA) level (A) and glutathione (GSH) level (B) in the bladder of the three groups
**P<0.01, ***P<0.001: significant differences between groups (Tukey test)

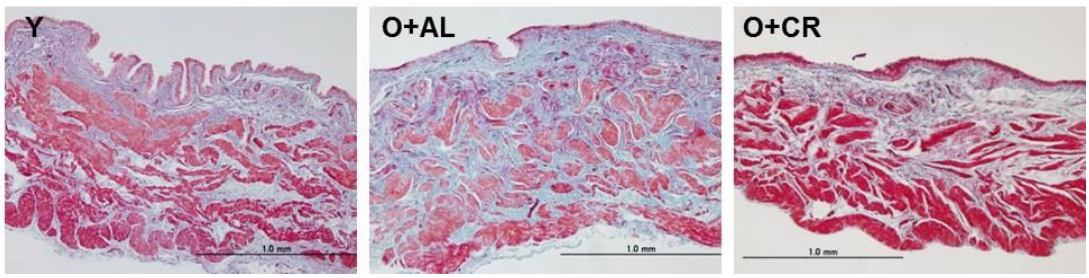

Figure 2. Representative images of Masson-trichrome staining of the bladder in the three groups

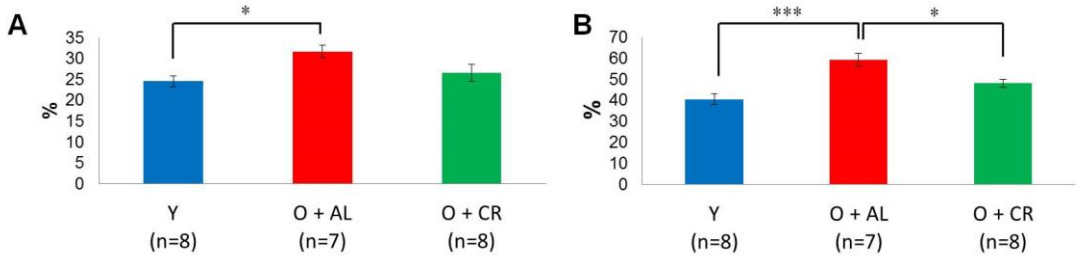

Figure 3. The collagen-deposition rate in detrusor layer (A) and whole bladder layer (B) in the three groups
*P<0.05, ***P<0.001: significant differences between groups (Tukey test)

References

1. Ito H, Aizawa N, Fujita Y, et al.: Age-related changes in detrusor properties and gene expression in the bladder and dorsal root ganglion and preventative effect of low caloric diet against these changes. Moderated poster #28, 43rd Annual meeting of International Continence Society, August 28 2013, Barcelona
2. Chiarpotto E, Bergamini E and Poli G: Molecular mechanisms of calorie restriction's protection against age-related sclerosis. IUBMB Life. 2006;58(12):695-702
3. Aybek H, Aybek Z, Abban G, et al.: Preventative effects of vitamin E against oxidative damage in aged diabetic rat bladders. Urology. 2011;77(2):508.e10-14

Disclosures

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