

## PROTECTIVE EFFECTS OF GRAPE SUSPENSION ON IN VIVO ISCHEMIA, ISCHEMIA FOLLOWED BY REPERFUSION, AND PARTIAL OUTLET OBSTRUCTION OF THE RABBIT BLADDER

### Hypothesis / aim of study

Ischemia / reperfusion (I/R) is proposed to be a major etiological factor in bladder dysfunctions of men with obstructive bladder, women with postmenopausal incontinence and of both sex in association with aging. The current studies investigated the effects of a standardized grape suspension on both a model of *in vivo* bilateral ischemia / reperfusion, and on partial bladder outlet obstruction.

### Study design, materials and methods

(A) 24 NZW rabbits were separated into 6 groups of 4. Groups 1-3 were treated by gavage with aqueous grape suspensions (dose); groups 4-6 received sugar-water vehicle. Groups 3 and 6 were controls. Groups 1 and 4 were subjected to bilateral ischemia for 2 hours (I). Groups 2 and 5 received bilateral ischemia for 2 hours and reperfused (recovery) for 1 week (I/R). The effect of ischemia +/- reperfusion on cystometry, *in vitro* contractile responses, morphology, and activities of Cu-Zn SOD and catalase were evaluated.

(B) Twenty-four male New Zealand White rabbits were separated into 4 groups of 6 each. Rabbits in-group 1 and 3 were treated by gavage with 10 ml aqueous grape suspension daily (20 mg/ml standardized grape powder obtained from the California Table Grape Commission). Those in groups 3 and 4 were given sugar-water vehicle by gavage (10 mg sucrose + 10 mg fructose / ml). After 3 weeks of daily oral administration each rabbit was anesthetized with pentobarbital and moderate outlet obstruction was created in groups 1 and 3, while sham operations were performed in groups 2 and 4. Treatment was continued for an additional 3 weeks. At the end of the 3 week obstructed period each rabbit was anesthetized with pentobarbital and the bladder excised, weighed, and 4 full thickness longitudinal strips of detrusor were prepared for contractility studies. Two additional full thickness strips were taken for immunohistochemistry and the remaining bladder body was separated between muscle and mucosa, frozen in liquid nitrogen and stored at -70°C for biochemical analyses.

### Results

(A) Ischemia resulted in significant decreases in the responses to all forms of stimulation in the vehicle-fed rabbits whereas there were no decreases in the responses of the grape-fed rabbits. Ischemia followed by reperfusion resulted in significant decreases in the responses to FS in both groups. However, there was a greater decrease in the response to 2 Hz in the vehicle-fed rabbits than that in the grape-fed rabbit. There were no reductions in the responses to ATP, carbachol or KCl in the grape-fed rabbits, however there were significant decreases in the responses to ATP, carbachol and KCl in the vehicle-fed rabbits. In the ischemia alone there was severe hypoxia showing in the mucosa and detrusor of the vehicle-fed rabbits whereas there was only mild hypoxia showing in the grape-fed rabbits. In the I/R rabbits, there was moderate hypoxia showing in the mucosa and detrusor of the vehicle-fed rabbit whereas there was virtually no hypoxia showing in the detrusor and only mild hypoxia showing in the mucosa in the grape-fed rabbits. In studies of SOD and CAT activities, grape up-regulated both SOD and CAT activity of bladder muscle and mucosa in control condition, which implies a greater antioxidant capacity against ischemia and I/R damage.

(B) Bladder weights of the sham vehicle and sham grape groups were similar. Although the bladder weights of both obstructed groups were greater than weights of sham groups, the bladder weight of the obstructed vehicle group was nearly double that of the obstructed grape group. The contractile responses to all frequencies of field stimulation and carbachol were significantly reduced in both obstructed groups. However, the responses of the obstructed-vehicle group were significantly lower than the responses of the obstructed-grape group (please note that only the response to 32 Hz is given in the table). The contractile response to

KCl were reduced equally in both the obstructed sham and obstructed grape groups (data not shown)

ChAT is the enzyme that synthesizes acetylcholine and is a marker enzyme for cholinergic transmission. In the vehicle-treated rabbits, obstruction mediated a significant decrease in ChAT activity, whereas in the grape treated rabbits there was no decrease in ChAT activity. There was a significant reduction in the density of nerve tracks within the detrusor smooth muscle of both obstructed groups. However, the density of nerve tracks in the grape-treated obstructed rabbits was significantly greater than in the vehicle-treated obstructed rabbits. This data matches the contractile responses to field stimulation, which is also a measure of the integrity of the neuronal innervation of the smooth muscle. Lastly, there were no changes in the activity of citrate synthase (marker for mitochondrial function) in the muscle; however, the activity was reduced to a significantly greater degree in the obstructed vehicle group than in the obstructed grape group.

#### Interpretation of results

The bladder strips contractile function tests demonstrated that the grape suspension provided protective effects against obstructive, ischemia, and I/R damage, which are consistent with histological studies showing that the grape suspensions protected against the severity of tissue hypoxia induced by obstruction, ischemia, and I/R; and is also consistent with the biochemical studies performed.

#### Conclusions

Feeding rabbits the grape suspension provided significant protection against the detrimental effects of bilateral ischemia, ischemia followed by reperfusion, and partial outlet obstruction. Up-regulation of SOD and CAT activities by grape suspension participate in the protective effects. Daily grape consumption might benefit patients with urinary bladder obstructive dysfunction.

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