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Comparative biochemical responses and antioxidant activities of the rabbit urinary bladder to whole grapes versus resveratrol

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Abstract The objective of this study is to compare the antioxidant activity of a whole-grape suspension with the antioxidant activity or pure resveratrol on the effect of hydrogen peroxide (H_2O_2) on malondialdehyde (MDA) generation, choline acetyltransferase (ChAT) activity, calcium ATPase activity, and sarcoendoplasmic reticular ATPase (SERCA) of the male rabbit urinary bladder. MDA was used as a model for the effect of H_2O_2 on lipid peroxidation. ChAT, SERCA, and calcium ATPase were evaluated based on their importance in urinary bladder physiology and pathology. Four male rabbit bladders were used. Each bladder was separated into muscle and mucosa, frozen under liquid nitrogen and stored at −80 °C for biochemical evaluation. The effect of H_2O_2 on the enzymes listed above was determined in the presence and absence of either resveratrol or a whole-grape suspension. (1) Resveratrol was significantly more effective than the grape suspension at protecting the bladder muscle and mucosa against peroxidation as quantitated by MDA formation. (2) The grape suspension was significantly more effective at protecting ChAT activity against oxidative stress of the muscle than resveratrol. (3) Neither the grape suspension nor resveratrol were particularly effective at protecting the bladder muscle or mucosa calcium ATPase or SERCA against oxidative stress. (4) ChAT was significantly more sensitive to oxidative stress than either calcium ATPase or SERCA. These data support the idea that the grape suspension protects the mitochondria and nerve terminals to a significantly greater degree than resveratrol which suggests that the activities of the grape suspension are due to the combination of active components found in the grape suspension and not just resveratrol alone.

Keywords Bladder · Smooth muscle · Mucosa · Antioxidants · Resveratrol · Grapes

Introduction

Several journal articles have been published on the antioxidant activity of whole grapes highlighting their ability as a natural product to significantly inhibit oxidative stress in several models of ischemia/reperfusion and hypoxic dysfunction [1–4] including obstructive bladder dysfunction (OBD). It is known that grape products contain a variety of antioxidant compounds including resveratrol, quercetin, procyanidins, flavonoids, and phenolics along with others [1]. However, it is believed by many investigators that resveratrol is the primary active ingredient responsible for grapes' antioxidant properties [5–7] and as such is being advertised as the major antioxidant in grapes while being sold in pharmacies as an over the counter (OTC) antioxidant supplement. Interestingly, virtually all of the resveratrol preparations available OTC have a variety of other ingredients including many types of additional antioxidants which would certainly make the product's statements about the resveratrol in these specific products suspect. This is why we used resveratrol purchased from Sigma Chemical Company to ensure its purity.

In a previous study, we compared the ability of a whole grape suspension with pure resveratrol in their ability to protect the bladder from in vitro oxidative stress mediated by hydrogen peroxide (H_2O_2) [8]. The results demonstrated that (1) Chemically, resveratrol at 1 mg/ml has about 20

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times the antioxidant capacity of the grape suspension at 1 mg/ml. (2) The grape suspension had significant protective effects on the contractile response to field stimulation at all concentrations of H_2O_2 while the resveratrol had no effect. (3) Citrate synthase (CS) activity of the muscle and mucosa were significantly protected by the grape suspension but not by resveratrol. One of the reviewers suggested we expand these studies to include additional important enzymes involved with bladder function and dysfunction. This formed the basis of the current study.

In this regard, there are four critical enzymes involved with bladder function which are extremely sensitive to oxidative stress caused by partial bladder outlet obstruction (PBOO) (which is an animal model for OBD [9–13]. They include: CS as a biomarker for mitochondria [14, 15], choline acetyl transferase (ChAT) which is a biomarker for the synthesis of acetylcholine (Ach) in the cholinergic synapses within the bladder smooth muscle [10, 15], plasma calcium ATPase (Ca^{2+}ATPase) as a biomarker for calcium movement through the plasma membrane and sarco-endoplasmic reticular calcium ATPase (SERCA) as a biomarker for sarcoplasmic movement of calcium from the cytosol into storage sites within the sarcoplasmic reticulum (SR) [15–17]. These enzymes have proven to be sensitive to free radical damage from obstructive bladder dysfunction (OBD)/partial bladder outlet obstruction (PBOO) and bilateral ischemia/reperfusion [12, 13, 18, 19].

Our main objective of this current study is to expand our original study [8] to include ChAT, Ca^{2+}ATPase, and SERCA. In our previous study, we compared the total antioxidant activity of the grape suspension vs resveratrol using the CUPRAC assay showing that resveratrol was significantly more potent than the grape suspension at the same concentration (1 mg/ml [8]. In the current study, we directly compared the effect of the grape suspension with resveratrol on the peroxidation of bladder muscle and mucosa by H_2O_2 by quantitating the effect on malondialdehyde formation (MDA).

OBD is a condition secondary to benign prostatic hyperplasia (BPH) which is the progressive enlargement of the prostate in man during aging [1, 2]. BPH involves hyperplasia of prostate cells which ultimately results in an increase in size and mass of the prostate leading to the progressive partial obstruction of the urethra which in turn interferes with the regular flow of urine. This causes symptoms such as urine hesitancy, frequent, and painful urination and an increased risk of urinary infections [20–22]. As stated above, this disease is a progressive issue in aging men with more than 80 % of men over the age of 50 requiring medical attention due to OBD [20–22].

One of the leading etiological factors in OBD is the relation between ischemia followed by reperfusion [12, 13, 23–25]. Ischemia is the restriction of blood supply to tissues resulting in the decrease of oxygen and glucose needed for cellular metabolism. Restoration of blood supply to ischemic tissues leads to further damage known as reperfusion injury. Reintroduction of blood flow brings oxygen back to the tissues causing a greater production of free radicals [reactive oxygen species (ROS) and reactive nitrogen species (RNS)] that damage cells [8]. Therefore, reperfusion injury causes oxidative stress in addition to restoring normal blood flow to the tissue.

Materials and methods

All protocols and experiments were approved by the Institutional Animal Care and Use Committee of the Stratton VAMC, Albany, NY.

Grape & Resveratrol Suspensions [1, 2, 26]

For these experiments, a standardized freeze-dried grape suspension was used to prepare the suspension and was kindly supplied by the California Table Grape Commission. The grape powder is a composite of whole red, green and blue-black California grapes seeded and seedless varieties in a freeze-dried powder form. It was created using Good Manufacturing Practices and precautions to preserve the integrity of the biologically active compounds found in fresh grapes. As with fresh grapes, the grape powder is known to contain anthocyanins, catechins, resveratrol, flavonols (including quercetin), flavans, and simple phenolics as well as sugars. This grape powder has 7 μM/kg powder resveratrol. The composition has been published previously [1]. Resveratrol was also tested in powdered form and purchased from Sigma Chemical Company, St. Louis, MO. The control for the grape suspension is a sugar suspension made of equal parts sucrose and fructose which gives the same carbohydrate content as the grapes but has no significant antioxidant activity.

Animal model

Four adult male New Zealand white rabbits were anesthetized with pentobarbital (25 mg/kg) and the bladder exposed through a midline incision. Each bladder was then removed and sectioned between body and base at the level of the ureteral orifices. The bladder was opened longitudinally and six full thickness isolated strips were taken (1 × 0.3 mm) and mounted in individual baths containing oxygenated Tyrodes solution (15 ml) at 37 °C for contractile studies. The contractile studies were published previously [8]. The balance of the bladder was separated by blunt dissection into muscle and mucosal compartments and each compartment frozen in liquid nitrogen and stored

at −80 °C for biochemical evaluation. The concentrations of both resveratrol and the grape suspension were 1 mg/ml within the bath. The concentration of resveratrol in the grape suspension is given by the manufacturer (California Table Grape Commission) as 7 μM/kg (1.6 μg/g grape powder). In preliminary studies, we utilized this concentration of resveratrol within the baths and observed no significant effects on any of the enzymes tested; and thus we utilized the higher concentration in the current study.

Since all of the enzymes studied have different sensitivities to H_2O_2, we performed preliminary studies to determine the range of H_2O_2 that inhibited the enzyme activity between approximately 10 % inhibition to 100 %.

Malondialdehyde (MDA) assay

MDA is a reactive species that occurs naturally through lipid peroxidation and is often used as a bio-marker for oxidative stress. Lipid peroxidation refers to the oxidative degradation of lipids and is a process in which free radicals "steal" electrons from the lipids in cell membranes resulting in cell damage. MDA levels of the tissue and experimental strips were quantified using a thiobarbituric acid (TBA)-based assay [27–29].

Choline acetyl transferase (ChAT) assay

Each frozen bladder sample (180 mg) was homogenized at 50 mg/ml in 20 mM EDTA (pH 7.6). Each homogenate was then diluted with Triton-X 100 to give a final concentration of 1 % Triton-X. The samples were centrifuged at 20,000×*g* for 30 min. The supernatant was removed and pellet discarded. Thereafter, 50 μl aliquots of each supernatant plus 100 μl of a reaction mixture were added to 7 ml scintillation vials and incubated in a water bath at 37 °C for 30 min. The reaction mixture consisted of: 0.04 mM acetyl-CoA; 8 mM choline; 50 mM sodium phosphate; 300 mM sodium chloride; 96 nM physostigmine + 0.50 μCi ^{3}H-acetyl-CoA. The reactions were stopped with 0.4 ml of acetonitrile containing 5 mg/ml tetraphenylboron. Once the reactions had stopped, 3 ml of Insta-Fluor Plus scintillation fluid was slowly added to each vial. The vials were shaken lightly and let stand for 1 h to allow the phases to separate. ^{3}H-acetylcholine was extracted into the toluene phase while radioactive acetyl-CoA remained in the aqueous phase (and thus did not add to the DPM). Activity (DPM) was counted in each vial via the scintillation counter.

Ca^{2+}ATPase and SERCA assays

40 mg of tissue (10 mg/ml) were homogenized in 50 mM TRIS buffer-pH 7.4. The sample was then centrifuged at 800 g for 10 min. The supernatant was saved and the pellet discarded. Each sample had (two) 2 tubes for each condition. The conditions were sample plus thapsigargin (10 μM), sample minus thapsigargin, control with no homogenate, and control with no ATP. All sample tubes contained: 375 μl sample, 50 μl $CaCl_2$, 50 μl EDTA, (±) 5 μl thapsigargin, 25 μl ATP, and (±) grape suspension or (±) resveratrol. Sample and control tubes were incubated at 37 °C for 40 min. At the end of the incubation, 0.5 ml trichloroacetic acid (TCA) was added to stop the reaction after which the tubes were vortexed. 0.5 ml ferrous sulfate molybdate was then added to all tubes, and the phosphate levels were measured at 650 nm.

The values for SERCA were determined by subtracting the values of sample with thapsigargin from the values of sample without thapsigargin. This was done to differentiate between the enzyme activity of plasma Ca^{2+}ATPase and SERCA. Thapsigargin is a non-competitive inhibitor of SERCA [30], thus total ATPase activity—activity in the presence of thapsigargin = SERCA activity.

Statistical analysis

Each set of data was analysed individually. One-way analysis of variance was used followed by the TUKEY test for individual differences among the three groups (control, grape, resveratrol). $p < 0.05$ was required for statistical significance.

Results

Antioxidant activity: MDA

Figure 1a, b display the muscle and mucosal tissue following 30 min incubation in the presence and absence of increasing concentrations of H_2O_2 (0.0, 0.05, 0.16, 0.5 %) and either the grape suspension, resveratrol, or nothing (Control). In the absence of H_2O_2, both muscle and mucosal MDA were significantly reduced by pre-incubation with resveratrol. The mucosa was only mildly reduced by incubation in the grape suspension.

The results in the presence H_2O_2 demonstrated clearly that as the concentration of H_2O_2 increased so did the levels of MDA which indicated an increase in oxidative stress (control tissue). Following treatment with resveratrol, MDA concentrations of both muscle and especially mucosa were significantly decreased at all H_2O_2 concentrations. Treatment with the grape suspension mildly reduced the MDA concentration only at the 0.16 % H_2O_2 in the mucosa (Fig. 1b).

Choline acetyltransferase (ChAT) activity

The ChAT activity of the muscle was 116 ± 18 μM Ach/mg tissue, whereas the activity of the mucosa was

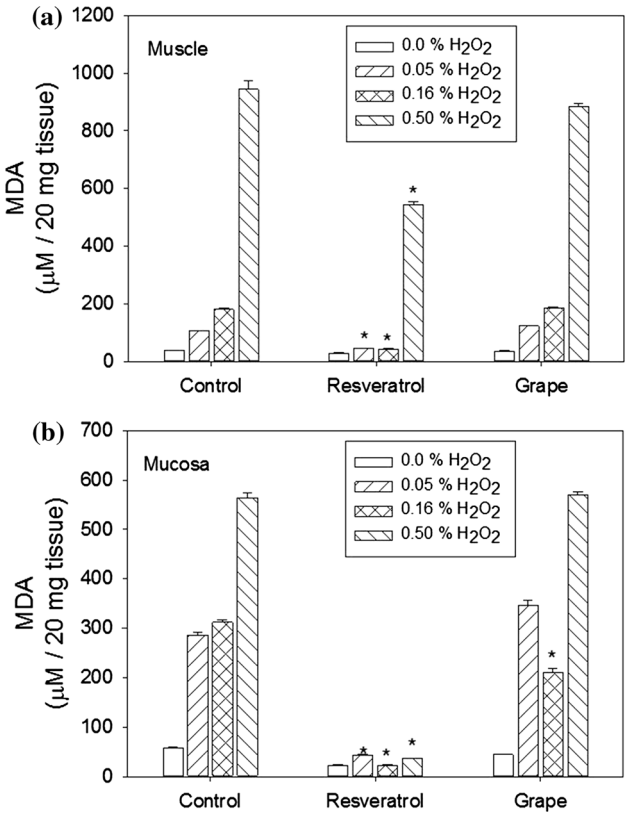

Fig. 1 The effect of H_2O_2 (0, 0.05, 0.16, 0.5 %) on MDA formation in bladder muscle (**a**) and mucosa (**b**) following exposure either resveratrol or grape suspension. *Each bar* is the mean of four individual rabbits. *Significantly different from control (nor grape or resveratrol), $p < 0.05$

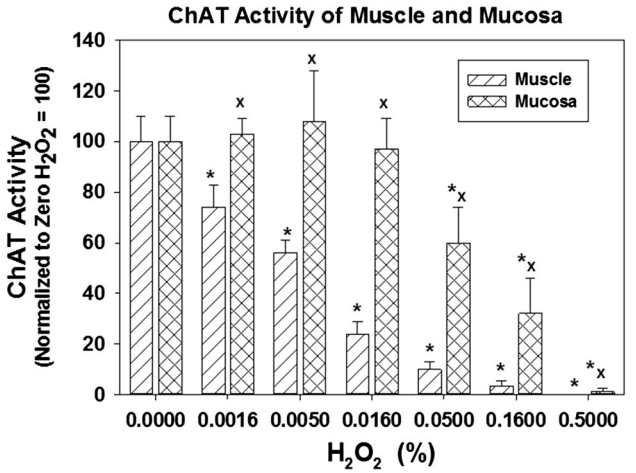

Fig. 2 The effect of H_2O_2 on ChAT activity of the bladder muscle and mucosa. *Each bar* is the mean of four individual rabbits. *Significantly different from 0 H_2O_2; *x* significantly different from muscle, $p < 0.05$

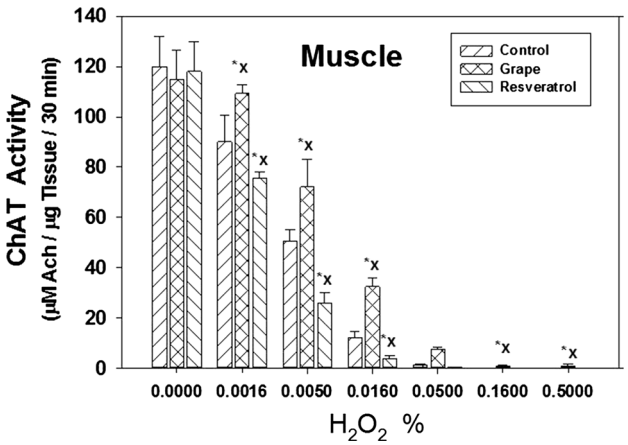

Fig. 3 The effect of the grape suspension or resveratrol on the effect of H_2O_2 on ChAT activity of bladder muscle. *Significantly different from 0 H_2O_2; *x* significantly different from grape, $p < 0.05$

significantly lower at 18 ± 6 μM Ach/mg tissue. Figure 2 displays the effect of H_2O_2 on ChAT activity of the muscle and mucosa. The bladder muscle was significantly more sensitive to H_2O_2 than the mucosa at all concentrations of H_2O_2. Because of the low ChAT activity and relatively weak response to H_2O_2, the results with both the grape suspension and resveratrol were very variable and not publishable.

Figure 3 displays the effect of H_2O_2 on ChAT activity of the muscle in the presence and absence of the grape suspension and resveratrol. Neither the grape suspension nor resveratrol had any significant effect on ChAT activity in the absence of H_2O_2. At all H_2O_2 concentrations, the grape suspension showed protective effects, whereas the resveratrol enhanced the effect of H_2O_2. We have no explanation for the resveratrol effects on ChAT activity.

Figure 4 displays calcium ATPase and SERCA activities for muscle and mucosa. The muscle and mucosa have approximately the same calcium ATPase activity; whereas the SERCA activity is ~10 % of the calcium ATPase activity, and is significantly greater in the bladder muscle than in the bladder mucosa. This is related to the contractile function in the muscle and significantly greater concentration of sarcoplasmic reticulum in the muscle than the mucosa [12, 13].

Figure 5a, b display the effect of H_2O_2 on calcium ATPase of the muscle (A) and mucosa (B). The effect of H_2O_2 on SERCA activity is displayed in Fig. 6a, b. Both calcium ATPase and SERCA were the least sensitive enzymes to H_2O_2 of the enzymes or contractility tested in either the current or previous study [8]. Neither the grape suspension nor resveratrol had any effect on the response to H_2O_2 at any concentration for the muscle for both calcium ATPase and SERCA. Both the grape suspension and resveratrol had minor protective effects on the response to 1.5 % H_2O_2 for the mucosa for both calcium ATPase and SERCA.

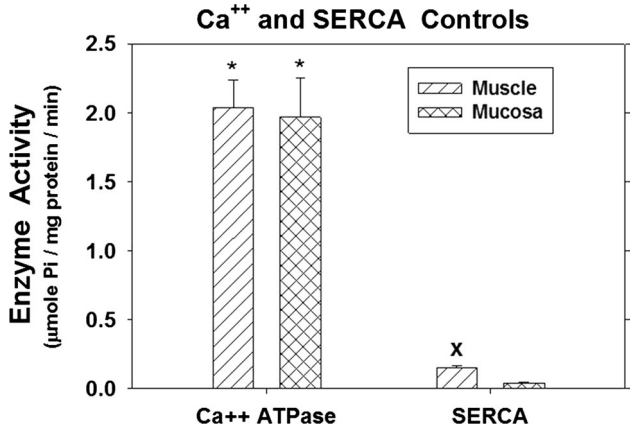

Fig. 4 Calcium ATPase and SERCA activities in bladder muscle and mucosa (no H_2O_2). *Each bar* is the mean of four individual rabbits. *Significantly different from SERCA; *x* significantly different from Mucosa, $p < 0.05$

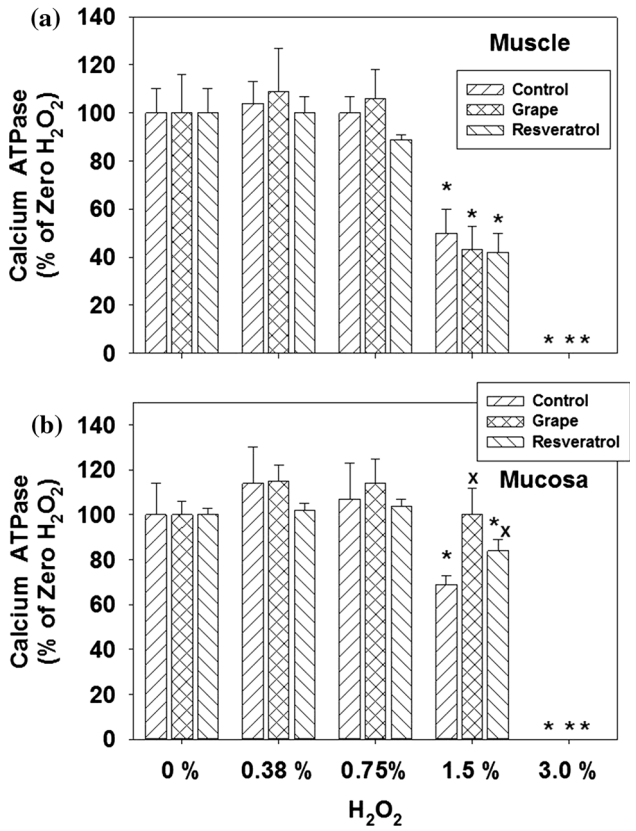

Fig. 5 Calcium ATPase activities in bladder muscle (**a**) and mucosa (**b**) following exposure to H_2O_2 and either resveratrol, grape suspension, or neither. *Each bar* is the mean of four individual rabbits. *Significantly different from zero H_2O_2; *x* significantly different from control, $p < 0.05$

It is interesting that ChAT (muscle) and CS were the most sensitive systems to H_2O_2 both of which are directly involved with mitochondria [18, 19, 31–36]. Mitochondria are very sensitive to oxidative stress [37–39]. Contractile

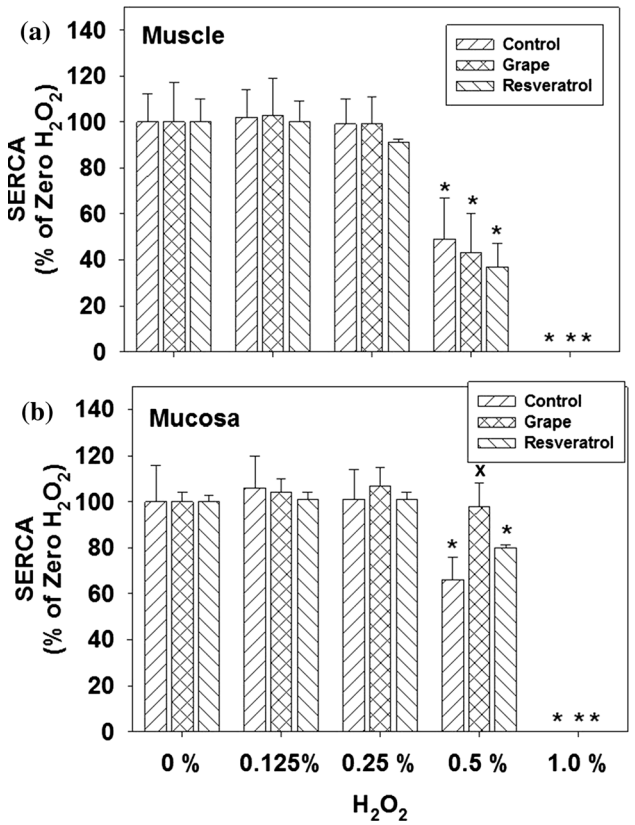

Fig. 6 SERCA activities in bladder muscle (**a**) and mucosa (**b**) following exposure to H_2O_2 and either resveratrol, grape suspension, or neither. *Each bar* is the mean of four individual rabbits. *Significantly different from zero H_2O_2; *x* significantly different from control, $p < 0.05$

studies were intermediate, while calcium ATPase and SERCA were the least sensitive to H_2O_2.

Discussion

It has been established from previous studies that the antioxidant properties that grapes possess are characteristics which can reduce the levels of free radicals and oxidative damage in protecting the urinary bladder from both obstructive and ischemic injury [1, 2, 9, 26]. It has also been confirmed that pretreating bladder tissue with grape suspension and exposing this treatment to increasing concentrations of H_2O_2 reduces the level of damage caused by the peroxide to both the contractile response to field stimulation and mitochondrial CS activity [8, 9].

Field stimulation contracts the bladder smooth muscle via the release of Ach from cholinergic nerve terminals, which diffuses across the synaptic cleft to the cholinergic receptor on the bladder smooth muscle cells. Mitochondria accumulate in high concentrations in cholinergic nerve terminals [40] and are directly involved in neural transmission [41–43].

Although grapes have been recognized for their protective effects on bladder tissue, many investigators believe that resveratrol is the primary active ingredient responsible for grapes' antioxidant properties [5]. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a fat-soluble phytoalexin that has gained recognition as an effective antioxidant and anti-aging nutraceutical [44]. It has also been recognized for a number of health benefits that include the direct scavenging of ROS, the inhibition of xanthine oxidase and the activation of intracellular pathways that improve metabolism and induce mitochondrial biogenesis [45]. Resveratrol has also been proven effective in scavenging oxidants and inhibiting low-density lipoprotein oxidation at high doses in in vitro studies [46, 47]. However, there is controversial evidence to support the theory that resveratrol supplementation can be effective in the treatment of a variety of systems in vivo [48–51].

In our lab, we have already determined that the pure grape suspension has significant in vivo protective effects on the response of rabbit urinary bladder to oxidative stress of partial outlet obstruction and in vivo ischemia/reperfusion [1, 2, 26]. Previous in vitro studies on the contractile response of isolated rabbit bladder strips to field stimulation (FS) in the presence of increasing concentrations of H_2O_2 demonstrated that pure resveratrol showed no effects while the grape suspension was significantly protective [8]. It was also demonstrated that CS activities of the muscle and mucosa were significantly protected by the grape suspension but not by resveratrol [8] which demonstrates that the grape suspension protects the mitochondria to a significantly greater degree than resveratrol against H_2O_2 oxidation.

Interestingly, the results obtained on the three enzymes (ChAT, Ca^{2+}ATPase, and SERCA), all thought to be directly related to OBD were different than that of the CS and contractility studies [8]. Whereas ChAT activity of the muscle was significantly protected by the grape suspension, resveratrol was not protective.

Resveratrol had significantly greater antioxidant activity than the grape suspension in the CUPRAC test for total antioxidant activity [8]; and also significantly greater antioxidant activity in the current study on MDA response to H_2O_2. These studies demonstrated that resveratrol had a significantly greater chemical antioxidant activity when compared to the grape suspension for both control and H_2O_2 exposed tissues. However, when evaluating several physiological and biochemical systems [8], it was found that the grape suspension showed higher antioxidant activity in protecting the bladder tissue from oxidative damage when compared to resveratrol [8]. This means that in a chemical assay resveratrol possess a higher antioxidant activity than the grape suspension, whereas in the physiological and biochemical studies the grape suspension was significantly more potent than resveratrol.

In the current studies, we clearly demonstrated that the enzyme ChAT was the enzyme most sensitive to H_2O_2 damage. This meant that the synthesis of Ach was decreased which in turn resulted in lower muscarinic receptor stimulation which in turn would lead to a decrease in the levels of intracellular Ca^{2+} necessary for contraction [40–42]. Pre-incubation with the grape suspension showed significant protective effects against H_2O_2 damage when compared to the resveratrol which showed low protective activity though not as great as with the CS enzyme from our previous study [8]. In regard to resveratrol activity on the ChAT enzyme of the muscle, not only did resveratrol not show any protection against the peroxide damage, it seemed to increase the oxidative effects of H_2O_2 on the tissue when compared to the control. This indicates that the enzyme is very sensitive to H_2O_2 and the presence of resveratrol is increasing this sensitivity. In other words, these results imply that taking resveratrol to improve bladder smooth muscle contraction in terms of neuronal activity may be a poor choice because not only is it not having any positive effect, it is intensifying the oxidative effects of H_2O_2 and ultimately increasing oxidative stress.

Throughout the study, oxidative stress was measured by administering increasing concentrations of H_2O_2 in vitro and this was chosen over a rabbit model of in vivo bilateral ischemia/reperfusion because in a live animal it would be difficult to know if the damage caused by ischemia/reperfusion was caused by the generation of H_2O_2 or free radicals. Therefore, to have a more controlled environment, the use of frozen tissue seemed more appropriate and damage from H_2O_2 was more easily controlled and quantitated.

Hydrogen peroxide (H_2O_2), a common oxygen radical and by-product of oxidative metabolisms, is known to cause substantial cellular and intracellular damage which potentially leads to oxidative stress even at the smallest concentrations [9, 52–55]. Two enzymes responsible for antioxidant defense in nearly all cells exposed to oxygen are superoxide dismutase (SOD) and catalase. SOD catalyzes the formation of oxygen and hydrogen peroxide (H_2O_2). The enzyme catalase is then responsible for reacting with the hydrogen peroxide species to ultimately form water and oxygen [9]. Partial outlet obstruction and in vivo models of ischemia have pronounced damaging effects on the ratio of SOD and catalase which in turn results in a significant increase in the production of H_2O_2 and further oxidative damage [9, 54, 56, 57].

It was also observed from this and previous studies from our lab [8] that the sensitivity to H_2O_2 varied among the enzymes examined. The current in vitro studies demonstrated that ChAT was the enzyme most sensitive to oxidative stress, CS was the second most sensitive, and SERCA and Ca^{2+}ATPase the least sensitive to the direct

Table 1 Shows sensitivity of the different systems to H_2O_2

Most Sensitive to H_2O_2 ↓ Least Sensitive to H_2O_2	System	Initial H_2O_2 % concentration which induced oxidative stress	
		Muscle	Mucosa
	ChAT	0.0016	0.05
	Citrate synthase[a,**]	0.005	0.01
	Contractile study[a,**]	0.1	
	Calcium ATPase	1.5	1.5
	SERCA	1.5	1.5

** Data published previously to provide direct comparisons with the data presented in the current study

[a] Ref. [8]

effect of H_2O_2 (Table 1). However, treatment with the grape suspension and resveratrol varied with each.

When compared to resveratrol, the use of the grape suspension was more effective in protecting the rabbit bladder tissue and enzyme activities from oxidative damage by H_2O_2. In accordance with previous studies [1, 8, 9], we believe that it is the combination of antioxidants found in the grape suspension working in synergy rather than an individual component working alone that produces the protective effect. Therefore, it would seem more beneficial for consumers to buy a bunch of grapes rather than a bottle of resveratrol.

In examining the results for the Ca^{2+}ATPase and SERCA, it was observed that a concentration of 1.5 % H_2O_2 was needed to initially create a damaging oxidative effect on tissues. This is a much higher concentration used when compared to the previously tested CS and currently tested ChAT muscle enzymes. This perhaps indicates that these enzymes are not principal enzymes involved in or affected by oxidative injury caused by H_2O_2. Not only were these enzymes less sensitive to the damaging effects of H_2O_2, but grape and resveratrol did not show much protection at the concentrations at which the tissues responded to H_2O_2. Grapes, however, showed a bit more protection than resveratrol but it was not significant. The results also showed that there was a significantly greater enzyme activity of Ca^{2+}ATPase within the muscle and mucosal tissue when compared to SERCA activity. This is mostly due to the greater surface area the plasma membrane has to house more Ca^{2+}ATPase as compared to SR in which SERCA resides. SERCA was found at significantly greater concentrations in the muscle tissue than the mucosa which corresponds with the fact that the SR is the organelle responsible for the storage of calcium.

The ChAT activity of the bladder muscle was both significantly more active and significantly more sensitive to H_2O_2 than the bladder mucosa. It is clear that the bladder mucosa has a significant effect on the muscle through a variety of neurogenic signals including Ach [58–62]. Thus, the function of ChAT in the mucosa is significantly different than the function in the muscle, and is probably the reason for the significantly different response to H_2O_2.

Conclusion

Similar to our previous study, resveratrol has significantly greater antioxidant activity than the grape suspension when tested chemically (MDA studies) both in the presence and absence of H_2O_2. Also consistent with the previous demonstrations that the grape suspension was significantly more protective of contractility and CS than resveratrol against H_2O_2 oxidation [8], the current study demonstrated that the grape suspension was also significantly more protective than resveratrol for the enzyme ChAT. Since both the mitochondrial enzyme CS and the synaptic enzyme ChAT are both directly related to bladder smooth muscle contraction, it would make sense for contractile activity to also be protected by the grape suspension.

The enzymes Calcium ATPase and SERCA were the least sensitive to H_2O_2 and the lack of protection by either the grape suspension or resveratrol was surprising based on the sensitivity of both enzymes to ischemia/reperfusion and PBOO [16, 63, 64]. These results clearly demonstrate that although PBOO and ischemia/reperfusion both generate free radicals and H_2O_2, the two forms of oxidative stress have significantly different properties.

In summary, these studies support the idea that it is the combination of antioxidants in the grape suspension that provides greater protection against H_2O_2 oxidation than resveratrol by itself.

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