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# PARTIAL OUTLET OBSTRUCTION REDUCES THE TOTAL ANTIOXIDANT CAPACITY OF BLADDER TISSUE.

Hypothesis / aims of study

There is increasing evidence that free radicals play a role in the development of bladder dysfunction [1]. Antioxidants protect against these radicals and are therefore important agents in maintaining good health. The human body has its own endogenous antioxidant system consisting of antioxidant enzymes (e.g. superoxide dismutase, catalase and glutathione peroxidase) and small molecule antioxidants (e.g. gluthathione, uric acid, melatonin and bilirubin). The TEAC (Trolox Equivalent Antioxidant Capacity) test has been widely used to measure the total antioxidant status in biological material. A high TEAC value indicates a high antioxidant capacity, which indirectly suggests a low level of oxidative stress. The aim of this pilot study is to determine the TEAC level of urinary bladders exposed to a partial outlet obstruction model.

Study design, materials and methods

A total of eleven male guinea pigs (Hartley strain; 3-4 weeks old) weighing 250-300mg were used. Four animals underwent a sham operation and in seven animals a silver jeweler's jump ring (2.2 mm internal diameter) was placed loosely around the proximal urethra to induce gradually an obstruction. Four weeks after the obstruction operation, filling and emptying characteristics of the bladder were studied during approximately 6 repetitive fillings under urethane (1.2gr/kg) anesthesia. At the end of the procedure the animals were sacrificed (high dose of barbiturate) and bladders were rapidly removed and weighed on an electronic scale. Each bladder was divided in two pieces; one part was used for contractility study the other part was used for tissue preparation. *Tissue preparation*: Bladder tissue was homogenized for 15 seconds in phosphate buffer with an Ultrathurax and supernatant was stored at –70C for biochemical analysis. The standard TEAC assay has been used [2]. Briefly this assay assesses the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical in 6 minutes. *Contractility study:* Three to four detrusor strips from each bladder were mounted in separate 6 ml organ baths containing Krebs-buffer solution which was aerated continuously with 5% $CO_2$ –95% $O_2$, at 37$^{o}$C. Mechanical responses were recorded using an isometric force transducer. Measurements were started after an equilibration period of 30 minutes with an initial tension of 2g. After the equilibration time the strips were stimulated with electrical field stimulation (32Hz), ATP (1mM) and subjected to cumulative concentration-response curves for acetylcholine ($10^{-8}$-$10^{-5}$ M) and potassium (20-100mM). Differences between mean values were statistically analyzed. All maximal contractile responses in one strip were normalized by dividing by the corresponding EFS 32Hz value. Student's t-test and one factor ANOVA were used to determine the statistical significance to 0.05 levels.

Results

Partial outlet obstruction resulted in a significant increase in bladder weight per body weight in 4 animals (2.4mg/g $\pm$ 0.34) compared to the sham operated animals (0.92 mg/g $\pm$ 0.07) ($P<0.05$). In three animals the obstruction did not result in an increase in bladder weight (0.95mg/g $\pm$ 0.02) that could be attributed to an error in the feeding of the animals. Therefore the obstruction was concluded not to be successful so we excluded these three animals from further analysis. The maximal contractile normalized responses for potassium (3.37 $\pm$ 0.20) and acetylcholine (1.93 $\pm$ 0.14) were significant higher in the obstructed group compared to the sham operated animals, 2.1 $\pm$ 0.34 and 0.53 $\pm$ 0.02 respectively ($p<0.05$). The TEAC values of obstructed bladders (56µM/mg protein $\pm$ 4.9) were significantly lower than the sham operated animals (81µM /mg protein $\pm$ 5.0).

Interpretation of results

In this study we have demonstrated that the total antioxidant status of bladders exposed to a successful outlet obstruction model decreases. This indicates that there is a disbalance between radical formation and protection probably due to ischemia-reperfusion in the bladder wall. The consequence of the low amount of antioxidants in the bladder tissue results in a high susceptibility for oxidative stress induced nerve membrane injury. This can explain the higher normalized contractions of potassium and acetylcholine stimulations in the obstructed guinea pigs what is probably due to relatively more nerve damage.

Concluding message

Partial outlet obstruction reduces the antioxidant capacity of bladder tissue. Exogenously administered antioxidants may play an important role in the prevention against outlet obstruction induced bladder dysfunction.

References

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