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COMPARATIVE BLADDER WALL TISSUE LEVELS OF RESINIFERATOXIN

Aims of Study

Intravesical instillation of resiniferatoxin (RTX) for treatment of hyperspastic bladders is becoming increasingly widespread. However, many aspects of this procedure are not fully elucidated: the optimal concentration; the optimal volume; and bladder wall tissue levels of RTX following instillation. This study addresses the last issue, comparing bladder wall tissue levels of RTX following (a) passive diffusion (PD) and (b) application of electric current (EMDA).

Methods

At a nearby abattoir pigs weighing 150 ± 18 Kg were sacrified with their standard technique [electronarcosis and incising the great veins and arteries of the neck] and 15-20 min later bladders were rapidly excised, placed in a cell culture medium (1 x DMEM, HyClone Europe, Cramlington, UK) at 4° C and transported to the laboratory. RTX 100 nM concentration in 100 ml 0.9% NaCl solution was placed in the donor compartment and NaCl 0.9% solution in the receptor compartment of a 2 chamber cell. Sections of viable pig bladder wall (~ 2.6 g) were fixed in a window between the 2 compartments with the urothelium facing the RTX solution.
In a series of 10 duplicate experiments the bladder wall was exposed to the RTX solution for 30 minutes with (EMDA), and without (PD), application of electric current (25 mA) to the RTX solution.
At the end of 30 minutes, tissue samples were removed from the cell and homogenized at room temperature for 1 min in 5.0 mM phosphate buffer pH 6.9 (1:3, w/v) using a homogenizer Blendor. The homogenate was then centrifuged at 10000 g for 20 min at 4°C. The resulting supernatant was loaded (1 ml) onto previously conditioned C18 cartridge. After washing the cartridge with water and methanol at different concentrations we eluted the RTX fraction in 90% methanol, then 100 µL of the eluate was injected into the HPLC system.

Results

Tissue levels measured for the 10 duplicate experiments are displayed in the following Table.

Experiment	Passive Diffusion	EMDA
1	0.197	0.336
2	0.581	1.925
3	0.175	2.359
4	0.212	0.481
5	0.321	0.624
6	0.141	0.486
7	0.133	0.297
8	0.144	1.094
9	0.122	0.737
10	0.097	0.599
Mean ± SEM	0.212 ± 0.05	0.894 ± 0.22
CV (%)	67.86%	78.57%
p-value	0.0076	

Conclusions

It is possible to measure tissue levels of RTX with correct preparation of samples and a HPLC column. As anticipated, application of electric current accelerated transport rates of RTX. It is noteworthy that there is a large coefficient of variation (CV) with both techniques of

administration. This is one possible explanation for the wide variability of both efficacy and duration of effect reported in clinical studies.