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EFFECTS INCREASING CONCENTRATIONS OF RESINIFERATOXIN ON THE MUCOSA OT THE FEMALE RAT BLADDER.
PRELIMINARY RESULTS OF A HISTOPATHOLOGICAL AND IMUNOHISTOCHEMICAL PLACEBO-CONTROLLED STUDY.

Hypothesis / aims of study

In recent years much attention has been drawn to the importance of the C-afferent fibbers in the pathophysiology of the bladder hyperactivity. Spinal cord injured animals were demonstrated to develop C fibber-dependent bladder hyperactivity that can be attenuated by intravesical or systemical treatment with capsaicin or resiniferatoxin (RTX). These substances, collectively referred as vaniloids, share a common molecular radical, which causes the desensitisation of C-fibbers, resulting in blocking of the pathological arising reflex. RTX is an ultrapotent analogue of capsaicin naturally occurring in the latex of a cactus-like plant named Euphorbia resinifera. Its high molecular weight renders RTX soluble only in high concentrations of ethanol. Hundred per cent ethanol is utilised for initial dilution and stocking of RTX that is then rediluted to a final solution of saline and 10% ethanol immediately before bladder instillation. Little is known about the acute effects of increasing RTX concentrations as well as the effect of ethanol “per se” on the structure of the bladder wall.
The present study was conducted to clarify the effects of three different RTX concentrations, 10% ethanol and 100% ethanol when compared to the instillation of saline solution, on the integrity and possible morphologic changes of the bladder wall of female rats.

Study design, materials and methods

Thirty-four American Whistar female rats were used in the experiment. The animals were anaesthetised with ether and a lower abdominal midline incision was made to expose the bladder and urethra. An epidural catheter was introduced in the bladder through the urethra and the residual urine was evacuated. The bladders were filled with one of seven different solutions, which remained in contact with the mucosa for 30 minutes. After that period the bladders were emptied, surgically removed and fixed in 10% formalin. Each group of animals received either saline solution, saline solution with 10 % ethanol, 100% ethanol (3 rats), RTX 50 nM in saline and 10% ethanol, RTX 150 nM in saline and 10% ethanol or RTX 300 nM in saline and 10% ethanol. In the seventh group of five animals the urethra was catheterised without the abdominal incision, a solution of RTX 300 nM in saline and 10% ethanol was left in contact with the bladder mucosa for 30 minutes and then evacuated through the catheter. These five animals were left alive for seven days and then re-anaesthetised and submitted to the bladder removal. One of the specimens of the last group (delayed bladder removal group) was found to be infected and was withdrawn from further histological analyses. All the specimens were embedded in paraffin, stained with haematoxylin-eosin and periodic acid-Schif (PAS) reagent and then submitted to histopathological evaluation. The histological parameters evaluated were integrity of the urothelial surface, presence or absence of oedema in the laminae propria, signs of inflammation and vascular congestion in the bladder wall. PAS staining allowed us to evaluate the effects of the solutions on the mucin coating of the bladder mucosa. The specimens were also submitted to immunohistochemistry using the antibody PGP (NOVOCASTRA) in 1:40 dilution to evaluate the density of nerve terminals. Only one senior pathologist (KL) who was blind to the solutions utilised in each group evaluated the slides. Statistical analyses were made with the Fisher’s exact test.

Results

Most of the bladders showed erosion of the urothelial surface and disappearance of the urothelial mucin coating. No inflammatory signs were identified in the bladder walls in any of the different groups fact that was attributed to the short period of contact of the solutions with the bladder mucosa. The density of nerve terminals was similar in all the groups. The occurrence of oedema, vascular congestion, urothelial erosion and loss of the mucin coating of the mucosa showed no statistical differences when comparing bladders infused with saline with those submitted to increasing concentrations of RTX ($p=0.28$; $p=0.52$;$p=1.00$; and $p=0.63$

respectively). Although there were no statistically significant differences among groups in the occurrence of any of the aforementioned pathological parameters surprisingly it appears to be a tendency to occur more urothelial erosion and oedema of the laminae propria in the groups submitted to instillation of saline and ethanol solutions when compared to those submitted to RTX-containing solutions. When each group was compared individually against the others there was a statistically significant increase in the ocurrence of oedema of the laminae propria in the groups submitted to the instillation of 10% ethanol and 100% ethanol ($p < 0.001$). The group submitted to the instillation of 100% ethanol showed intense urothelial erosion, oedema and vascular congestion of the mucosa. The results are summarised in table 1.

Interpretation of results

The effects of the instillation of increasing concentrations of RTX when compared to 10% ethanol and saline on the urothelial surface and the bladder wall of female rat bladders were superficial erosion, disappearance of the urothelial mucin coating and edema of the laminae propria. Rats submitted to the instillation of RTX-free solutions appeared to develop more urothelial erosion and oedema than those submitted to the instillation of RTX containing solutions. The small number of specimens evaluated prevents us to draw any definitive conclusion from the data.

Concluding message

Hundred per cent and 10% ethanol solutions used in this study promoted more changes in the bladder wall than the RTX containing solutions. In some way the RTX seems to offer protection to the loss of mucin coating and to the development of urothelial erosion at least immediately after instillation.

Table 1

	Urothelial erosion (%) *	Loss of mucin coating (%) *	Density of nerve terminals **
Saline	80	40	2.4
Saline + 10% ethanol	100	60	1.8
100% ethanol	100	100	0.67
RTX 50nM	60	0	2.0
RTX 150 nM	40	100	1.6
RTX 300 nM	80	100	1,0
RTX 300 nM (delayed removal group)	50	25	2.25

* % Of specimens presenting the alteration

** Number of nerve terminals in high power field microscopic examination/number of specimens

References

A light- and electron-microscopic histophatological study of human bladder mucosa after intravesical resiniferatoxin application. BJU International (2001) 88, 355-360.