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LIPOSOME PLATFORM FOR INTRAVESICAL SEQUENCE-SPECIFIC GENE-SILENCING OF NGF IN BLADDER

Hypothesis / aims of study

Sequence-specific gene-silencing mechanism is a promising approach to develop new targeted and localized therapeutic approaches with fewer side effects, as well as greater long-term efficacy. Drug development of this approach for the intravesical route has been hampered by inefficient intracellular delivery and cellular uptake of the oligonucleotides (ODN). The aim of this study was to examine the feasibility of cationic liposomes for bladder uptake of oligonucleotides.

Study design, materials and methods

Adult female SD rats were anesthetized with isoflurane and their bladders were catheterized with 24-gauge angiocatheters (Becton Dickinson) to instill 0.5ml of either saline or liposomal ODN targeted against rat NGF (6μM) for 30min. ODN with sequence of 5'GCCCGAGACGCCTCCCGA3` was complexed with cationic liposomes by incubation at room temperature for 30min in the molar ratio of ODN to lipid of 1:10. The efficacy of antisense treatments was assessed 24h after instillation; baseline cystometrograms (CMG) were performed on rats using saline infusion followed by 0.125% acetic acid (AA) infusion to induce bladder irritation.

Results

Examination of bladder sections by confocal microscope revealed bright red fluorescence from TYE 563. Bladder uptake accumulation was best seen at 24 h, while intensity of fluorescence was greater at 8h. Baseline CMG under saline infusion was indistinct between two groups and instillation of antisense ODN had no effect on baseline CMG as evident from mean Intercontraction interval (ICI) of 18.44±2.52min in the vehicle treated group and 18.29±1.62 min in the antisense treated group. The AA induced bladder overactivity was noticed in vehicle-treated rats as evident from mean percent reduction of (49.71± 9.68%) in ICI from baseline values. Pretreatment of antisense ODN encapsulated in liposomes targeting NGF blocked the acetic NGF over-expression and suppressed the amount of NGF available in bladder following acetic acid induced irritation with only 14.17±3.71% reduction in mean ICI from baseline.

Interpretation of results

Localization of fluorescence in urothelium demonstrates successful bladder uptake and retention in target cells presumably due to binding with target mRNA. Successful delivery of antisense targeting NGF blunts the rapid rise of NGF following acetic acid irritation.

Concluding message

Intravesical route can allow selective exposure of high concentration of antisense ODN to the NGF producing cells in urothelium and avoid systemic side effects from genetic manipulation of NGF expression and the safety concerns noted with systemic administration of monoclonal human NGF antibodies (tanezumab) such as paresthesia, hypoesthesia and arthralgia.

References

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