

Effect of Supplemental Intracameral Lidocaine on Visual Sensations and Fear Experienced during Cataract Surgery under Topical Anaesthesia – Results of a Placebo-controlled, Double-blind, Randomised Trial

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Aim: To determine whether supplemental intracameral lidocaine reduces the visual sensations and the resultant fear experienced by patients during cataract surgery under topical anaesthesia.

Methods: Five hundred and six cataract patients undergoing routine phacoemulsification under topical anaesthesia were randomised to receive a supplemental injection of either 0.5 mL of 1% intracameral lidocaine or balanced salt solution (BSS). The injected fluid was left in the anterior chamber for 1 minute prior to continuous circular capsulorhexis. The patients were then interviewed after their surgery by a masked interviewer using a standardised questionnaire about their visual experience and their reaction to it. The surgeon, patient and interviewer were masked to the randomisation.

Results: There were no significant differences in demographics between the lidocaine (277 patients, 54.7%) and BSS (229 patients, 45.3%) groups. Five hundred patients (98.8%) experienced at least light perception during the surgery. There was no statistically significant difference in the proportion of patients who experienced various visual sensations intraoperatively between the 2 groups. The intraoperative visual experiences were frightening in 6.5% of patients in the lidocaine group and 4.8% in the BSS group ($P = 0.448$). On a visual analogue scale of 1 to 10, the mean grade of fear in the lidocaine group was 2.7 compared to 4.6 in the BSS group ($P = 0.032$).

Conclusion: Intracameral lidocaine at the concentration, volume and duration used in this study did not reduce the visual sensations experienced by patients during phacoemulsification compared to BSS. However, patients given intracameral lidocaine were less fearful of their visual experience compared to those given BSS.

TPA-induced Transmembrane Protein (TTMP), a Newly Described Protein, Localises to the Endoplasmic Reticulum and has Growth Retardation Effects in Pancreatic Cancer Cells

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Aim: To determine the intracellular location and functional activity of the newly described TPA-induced transmembrane protein (TTMP).

Methods: Immunofluorescence was used to determine the intracellular location of TTMP. HeLa cells were transiently transfected with TTMP-pcDNA3.1 expression vector carrying the V5 epitope. These cells were subsequently fixed, permeabilised and then incubated with V5 antibody as well as endoplasmic reticulum (ER)-specific protein disulphide isomerase antibody (Santa Cruz). Secondary antibodies with different immunofluorescence were used to identify the primary antibodies and co-localisation was demonstrated when the different wavelengths were merged. Functional studies of cell growth were carried out using cell-counting experiments, cell cycle analysis and immunoblotting studies of phosphorylation status of retinoblastoma

protein in stably transfected populations of TTMP and control vector CD18 cells.

Results: Co-immunofluorescence studies showed that TTMP was localised to the endoplasmic reticulum. Cell proliferation studies showed that CD18 pancreatic cancer cells that have been stably transfected with TTMP grew at a slower rate when compared to native CD18 as well as empty vector transfected CD18 cells. Furthermore, expression of TTMP induces a G1 phase arrest in stably transfected CD18 cells, as demonstrated by cell cycle analysis. Expression of TTMP also induces hypophosphorylation of retinoblastoma protein as seen on Western blotting studies.

Conclusion: We have demonstrated that TTMP was localised to the endoplasmic reticulum. This in vitro evidence concurs with the in silico prediction of its intracellular localisation. We have also demonstrated that TTMP causes growth retardation effects in pancreatic cancer cells. This may indicate the potential role of TTMP as a novel tumour suppressor gene.

Interleukin-15 (IL-15) in Systemic Lupus Erythematosus: Its Association with Active Lupus Arthritis and Nephritis, and Correlation with Disease Activity Markers

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Aim: We sought to determine the serum IL-15 levels in a prospective large systemic lupus erythematosus (SLE) cohort and to assess the relationship with disease activity and organ manifestations.

Methods: A total of 1901 serum specimens were collected from 891 patients (female:male ratio of 10.8:1, 79.6% Chinese) from our centre, who fulfilled the 1991 revised American College of Rheumatology (ACR) classification criteria for SLE. The mean age was 40.2 ± 13.0 (mean \pm standard deviation) years; the mean disease duration, 111 ± 98 months. Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and categorised into inactive (≤ 4), mild ($>4-8$), moderate ($>8-12$) and severe (>12). The serum IL-15 levels were determined using enzyme-linked immunosorbent assay (ELISA). Controls were randomly selected, unrelated, healthy subjects.

Results: The median SLEDAI score was 4 [interquartile range (IQR): 2 to 6]; 70.7% inactive, 16.7% mild, 7.7% moderate and 4.9% severe activity. Coincident active haematological, renal, mucocutaneous, and neurologic involvement were noted among 47.3%, 23.0%, 15.0%, and 2.5% of patients respectively. IL-15 levels were elevated in all patients compared to the healthy controls (42.4, IQR: 4.9 pg/mL to 151.5 pg/mL versus 8.8, IQR: 5.9 pg/mL to 12.4 pg/mL, respectively; $P = 0.0002$), and correlated weakly with the SLEDAI scores ($r = 0.08$, $P = 0.007$). Significant correlations were also found with elevations of anti-dsDNA antibodies and hypocomplementaemia (all with $P < 0.005$), but not erythrocyte sedimentation rate (ESR) or C-reactive protein levels. Active arthritis ($P = 0.0002$) and renal disease ($P = 0.0001$) were associated with elevated IL-15 levels but not haematologic, cutaneous or neurologic involvement.

Conclusion: Serum IL-15 level is markedly increased in SLE, correlates with the disease activity and is associated with active arthritis and renal disease.

Nonviral Cytokine Gene Therapy for Bladder Cancer in an Orthotopic Mouse Model

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Aim: The purpose is to assess cytokine gene transfection in tumour cells and its therapeutic efficacy in an orthotopic mouse bladder cancer model after liposome-mediated gene transfer. In addition, we aim to evaluate the local immune response induced by cytokine gene delivery.

Methods: Orthotopic tumour was established with 1 x 10⁵ MB49 cells in C57BL/6 mice using an electrocautery technique. In situ gene transfer to bladder tumours was accomplished via intravesical instillation of plasmid DNA/DOTAP/methyl-beta-cyclodextrin solubilised cholesterol after a single 2-hour transfection. Using the orthotopic model, mice with confirmed tumours were given liposome-mediated GM-CSF gene therapy twice a week for 3 weeks intravesically. The growth of bladder tumour was monitored and the local immune response was evaluated by immunohistochemistry and ELISA.

Results: The treatment group showed dramatically decreased tumour incidence and improved survival as compared to the control group. Cytokine analysis revealed that GM-CSF was produced after in vivo transfection both in bladder tissue and in urine. Furthermore, GM-CSF gene therapy induced the production of other cytokines and chemokines as well as immune cell infiltration.

Conclusion: We demonstrated in the orthotopic mouse bladder cancer model that successful inhibition of tumour cell growth could be obtained with cytokine gene therapy. The results suggest that our liposome transfection system appears to be a promising method for gene therapy of bladder cancer in vivo.

Suppression of Laminin-5 Expression Leads to Increased Motility, Tumorigenicity and Invasion

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Aim: Laminin-5 (Ln-5) is a major basement membrane component expressed in several human carcinomas and hypothesised to contribute to tumour invasion. This study was undertaken to understand the role of Ln-5 in oral squamous cell carcinoma.

Methods: We stably delivered small interfering RNAs (siRNAs) directed against the Ln-5 $\gamma(\gamma)2$ chain into JHU-SCC-022(022), a non-invasive oral squamous cell carcinoma (OSCC) cell line which secrete Ln-5. Control cells were obtained by delivering siRNAs against a random sequence into the same cell line.

Results: Lysates from $\gamma(\gamma)2$ siRNA cells [022-si $\gamma(\gamma)2$] had nearly undetectable levels of the $\gamma(\gamma)2$ chain while the $\alpha(\alpha)3$ and $\beta(\beta)3$ subunits of Ln-5 remained unchanged compared to parental and control. In conditioned medium from 022-si $\gamma(\gamma)2$ cells, the $\gamma(\gamma)2$ chain and the Ln-5 heterotrimer were barely detectable, similar to an invasive OSCC cell line. Conditioned medium from 022-si $\gamma(\gamma)2$ contained less $\alpha(\alpha)3$ and $\beta(\beta)3$ subunits than both parental and control. Although proliferation and adhesive properties of the 022-si $\gamma(\gamma)2$ cells remained similar to parental and control cells, 022-si $\gamma(\gamma)2$ cells showed increased detachment and a fibroblastic morphology similar to invasive cells. Moreover, migration, in vivo invasion, and in vivo tumorigenicity were enhanced in 022-si $\gamma(\gamma)2$ cells.

Conclusion: Our results suggest that the Ln-5 $\gamma(\gamma)2$ chain regulates the secretion of the $\alpha(\alpha)3$ and $\beta(\beta)3$ subunits. More importantly, suppression of Ln-5 results in a phenotype that is representative of invasive tumour cells.