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PDGFRα+ CELLS IN RAT AND RABBIT COLON: OPTIMISING METHODOLOGY FOR IMMUNOHISTOCHEMISTRY

Arwa Al-Robeye[1], Hanan Elkuwaila[2], Jim Deuchars[2], Jonathan Sutcliffe[3]
[1]Faculty of Medicine, University of Leeds, Leeds, UK, [2]Faculty of Biological Science, University of Leeds, Leeds, UK, [3]Department of Paediatric Surgery, Leeds General Infirmary, Leeds, UK

Aim of the Study: Outcomes in Hirschsprung Disease (HD) may be compromised by dysmotility. "Interstitial Cells" (IC), including cKit+ and the recently recognised PDGFRα cells, are pivotal in smooth muscle motility control. PDGFRα cells may play a role in HD as they affect smooth muscle relaxation, but have not been investigated. Nine papers have examined cKit cells in HD, yet experimental technique (including use of fixative and antibody) and results differ. Our aim was to identify an optimal methodology to fix tissues to allow this cell group to be examined in the colon.

Methods: Anaesthetised rabbit and rats were euthanized (UK Home Office Licence 40/3126), colons removed and rinsed in phosphate buffered saline. Segments were fixed in four different fixatives: acetic ethanol, paraformaldehyde, zinc and Zamboni's. Samples were cryoprotected in sucrose and then sectioned using a cryostat in a cross-sectional orientation. Sections were blocked using donkey serum to minimise non-specific binding of antibodies, followed by incubation with antibodies against cKit (Miltenyi Biotec) and PDGFRα (R&D Systems). Antibody binding was detected with the biotinylated secondary antibodies and visualised with streptavidin Alexx^{555}. Staining was analysed using a confocal microscope.

Main Results: Both cKit+ and PDGFRα+ ICs were identified in the muscular layers of the rabbit and rat colon. Tissue fixed using acetic ethanol or zinc revealed qualitatively better labelling of cells compared to those fixed with paraformaldehyde or Zamboni's. The cKit antibody raised in mouse enabled labelling of the rabbit tissue since the DAKO gold-standard is raised in rabbit. Sections labelled using the biotinylated method exhibited clearer staining compared to the direct secondary approach.

Conclusion: Acetic ethanol and zinc fixatives resulted in enhanced staining for both cKit and PDGFRα positive cells in comparison to paraformaldehyde and formalin. This will facilitate optimisation of immunohistochemistry for human tissues.