A New Method for Quantitative Detection of Degranulation in Mast Cells

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Mast cells function as key effector cells that contribute to anaphylactoid reactions via the release of preformed granules. As degranulation is a dynamic procession, conventional in vitro detection methods for degranulation marker (β-Hexosaminidase or Histamine) as well as Scanning Electron Microscope, undergo fixed treatment before detection. This process would lead to the loss of biological live information. Moreover, Color-effect of Herbal Medicine would also cause measurement error in colorimetric method. Therefore, a sensitive and precise live cell quantitative detection of degranulation in mast cell is pressed for in vitro detection.

In this study, the change of intracellular calcium \([\text{Ca}^{2+}]_i\) of RBL-2H3 cells (rat basophilic leukemia cell line) stimulated by Compound 48/80, was observed by laser scanning confocal microscopy (FV-1000, Olympus, Japan). Cells were loaded with Fluo-4 for Calcium and screened 10 sec interval for continuously 25 min at 488nm with 100×(oil) object lens. The result showed that following stimulation with compound 48/80 (final concentration from 10 to \(1.6\times10^{-1}\ \mu\text{g/ml}\)), intracellular calcium concentration was significantly increased 75 second after stimulation (2 times baseline) compared with controls and lasted for 500 second gradually descended to the baseline (Figure 1). The Parallel detection for \(\beta\)-hexosaminidase release in the supernatant was measured by colorimetric method showed striking coherence with that of \([\text{Ca}^{2+}]_i\) (Figure 2).

The results showed that \([\text{Ca}^{2+}]_i\) is a sensitive evaluation method at early stage of degranulation, which has the potential to be an early, sensitive, dynamic in vitro diagnostic approach of degranulation.

Reference: