Determination of cell viability using acridine orange/propidium iodide dual-spectrofluorometry assay

ABSTRACT

In vitro cell viability tests are usually done using 3-(4,5 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) method. A new spectrofluorometry method was developed using acridine orange (AO) and propidium iodide (PI) using multi-label microplate reader. Nine biogenic amines (BAs) [histamine (HIM), putrescine (PUT), cadaverine (CAD), 2-phenylethylamine (PHM), tyramine (TYM), tryptamine (TPM), spermine (SPM), spermidine (SPD) and agmatine (AGM)] were exposed to RAW 264.7 macrophage in singles at 37°C with 5% carbon dioxide supplementation for 18–24 hours and cell viability was determined using MTS method and AO/PI developed method using dual-spectrofluorometry. Based on MTS assay, SPM and SPD were found to be cytotoxic and it was supported by AO/PI assay. The precedence of disintegration in the nucleus rather than mitochondria upon cell non-viability was also supported by transmission electron microscopy (TEM). The results showed that AO/PI method could be used as an alternative method to determine cytotoxicity besides usual usage in confocal microscopy.

Keyword: Acridine orange; Propidium iodide; Dual-spectrofluorometry; Cell viability