Radiation Induced Apoptosis in Peripheral Blood Mononuclear Cells by Acridine Orange Staining Technique

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Introduction

Apoptosis (programmed cell death) is a normal event that occurs in tissues and organisms and is not associated with inflammation (1). It is an important and inevitable event in the remodeling of tissues during development and aging. Aberrant induction of apoptosis has been implicated in many age related disease processes, such as cancer, autoimmunity and central nervous system degeneration. Ionizing radiation is also known to interact with cells, producing a variety of molecular lesions as well as oxidative damage to DNA, resulting in base modifications and strand breaks (2). In certain cell types, radiation is known to trigger a different model of cell death known as apoptosis (3,4). It is conceivable that effective diagnostic strategies may be designed on the basis of genetic and biochemical events involved in apoptosis. Therefore, simple and rapidly detectable methods to accurately characterize and quantify apoptotic cells have become important in further understanding apoptosis.

Commonly used methods for the estimation of apoptosis are based on characteristic biochemical and morphological changes in apoptotic cells, analysis of DNA fragmentation and quantification assays, in situ DNA-labeling methods (5), and flow cytometry (6). However, gel electrophoresis to demonstrate the pattern of the DNA ladder usually involves a DNA isolation procedure from volumes of cells, and obtained results cannot be quantified. Apoptosis-related protein quantification for estimation of apoptosis in cell lysates also requires a large number of apoptotic cells, and is relatively insensitive for the detection of low levels of apoptotic events. Although sensitive, these methods are associated with a number of artifacts, as the labeling of DNA strand break and loss of frail apoptotic cells during processing is common. On the other hand, morphological estimation for apoptosis is based on methodologies with cell characteristics such as chromatin condensation, formation of apoptotic bodies, and cytoplasm shrinkage (1,7,8).

In this report, we describe a method to accurately and rapidly quantify apoptotic cells from peripheral blood mononuclear cells. Using the fluorescent DNA binding dye Acridine Orange (9), herein is described a method of improving the sensitivity of the apoptosis whereby live polymorphonuclear cells may be distinguished from apoptotic and necrotic cells on a single cell basis. We have compared the results of the Wright-Giemsa staining method with widely used assays for apoptosis using peripheral blood mononuclear cells undergoing radiation-induced apoptosis. Exposing cells and animals to known apoptosis-inducing means such as carcinogens and ionizing radiation was validated by this technique.