Abstract

Background: Histidine-rich glycoprotein (HRG) treatment ameliorated the survival rate of septic mice by suppressing excess immunothrombus formation. Although such findings suggested that HRG may be one of the most useful drugs for sepsis, obtaining a stable experimental system to standardize the HRG drug product is difficult to achieve using neutrophils isolated from volunteers. This is due to the short survival time and individual differences of human neutrophils. In the present study, we determined whether the differentiated neutrophil-like cell lines exhibited similar responses to HRG compared to human purified neutrophils. Method: All-trans retinoic acid (ATRA) was employed to induce the differentiation of the human myeloid leukemia cell lines, HL-60 and NB-4. Thereafter, the cells were treated with Hank's balanced salt solution, human serum albumin, or HRG. The effects of HRG on these cells were evaluated according to cell shape, microcapillary passage, ROS production, neutrophil extracellular traps (NETs) formation, the expression of activated CD11b, and cell viability. Result: HRG maintained the round shape of differentiated neutrophil-like cells, decreased the time required by cells to pass through the microcapillaries, and inhibited ROS production, NETs formation, and the expression of activated CD11b on the cell surface. Moreover, the cells could survive longer in the presence of HRG than the control. Conclusion: The ATRA-induced differentiated cell lines could be used as alternatives to neutrophils to investigate the effects of HRG on neutrophils. This method can thus be used as an essential

standardization test in pharmaceutical development.