duced platelet aggregation (27.7 ± 7.2, 34.9 ± 6.8, and 80.3 ± 3.7% [n = 6], respectively) in washed human platelets which reached a maximal inhibitory effect at about 60 min (Fig. 1B). These results indicate that LPS not only dose-dependently but also time-dependently inhibited agonist-induced human platelet aggregation.

Effect of LPS on LDH Released from Platelet Cytosol

In this study, LPS (100-500 μg/mL) slightly and significantly increased the LDH activity for a incubation 10-min with human platelets as compared with resting platelets (resting 18.7 ± 2.6 units vs. LPS, 28.3 ± 2.9 [100 μg/mL], 27.3 ± 2.8 [200 μg/mL], and 31.0 ± 3.2 [500 μg/mL] units [n = 6], respectively) (Fig. 2). Furthermore, LPS (200 μg/mL) also slightly and significantly increased LDH activity of human platelets for the indicated incubation times (10, 30, and 60 min) (27.3 ± 2.8, 29.0 ± 2.3, and 31.3 ± 3.4 units [n = 6], respectively) as compared with resting platelets (Fig. 3). The release of LDH from sonicated platelets as a maximal value was about 135.9 ± 14.5 (n = 6). These results indicate that LPS can slightly induce LDH release in human platelets.

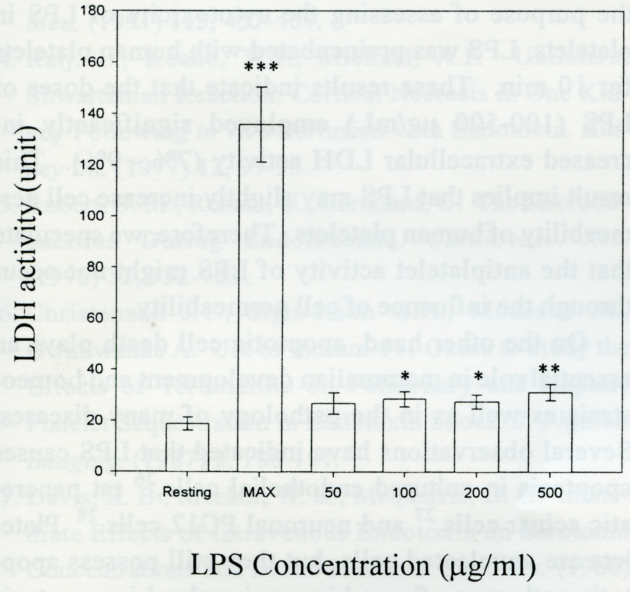

Fig. 2. Dose-response effect of LPS on LDH released from platelet cytosol. Platelets were preincubated with various concentrations of LPS (50, 100, 200, and 500 μg/mL) for 10 min, and supernatant (100 μL) of platelet suspensions was then added to a cuvette containing 2.4 mL phosphate buffer, 0.2 mg β-NADH, and 100 μL sodium pyruvate (22.7 mM). The activity of LDH was calculated from the absorbance change at 334 nm. MAX indicates as the maximal value of LDH activity of sonicated platelets. Data are presented as means ± S.E.M. (n = 6). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ as compared with the resting group.

Effect of LPS on Apoptosis in HL-60 Cell Lines

In this study, 10,000 cells were stained with propidium iodide and counted per experimental group. In the absence of LPS, the intensity of the fluorescence was 198.6 ± 6.1 (n = 4) (Fig. 4A). As shown in Fig. 4B, sonicated cells were measured by DNA fragmentation (negative control, 15.7 ± 3.8 [n = 4]). LPS (10 and 100 μg/mL) did not significantly change the fluo-

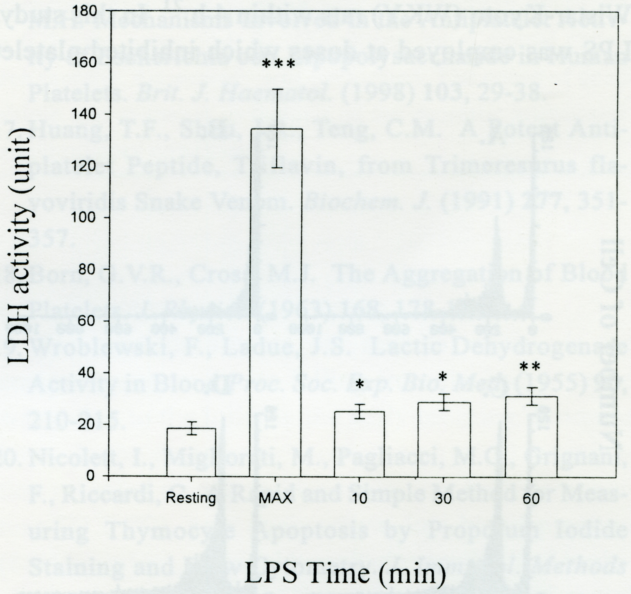

Fig. 3. Time-response effect of LPS on LDH released from platelet cytosol. Platelets were incubated with LPS (200 μg/mL) for 10, 30, and 60 min, respectively, and supernatant (100 μL) of platelet suspensions was then added to a cuvette containing 2.4 mL phosphate buffer, 0.2 mg β-NADH and 100 μL sodium pyruvate (22.7 mM). The activity of LDH was calculated from the absorbance change at 334 nm. MAX indicates as the maximal value of LDH activity of sonicated platelets. Data are presented as means ± S.E.M. (n = 6). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ as compared with the resting group.