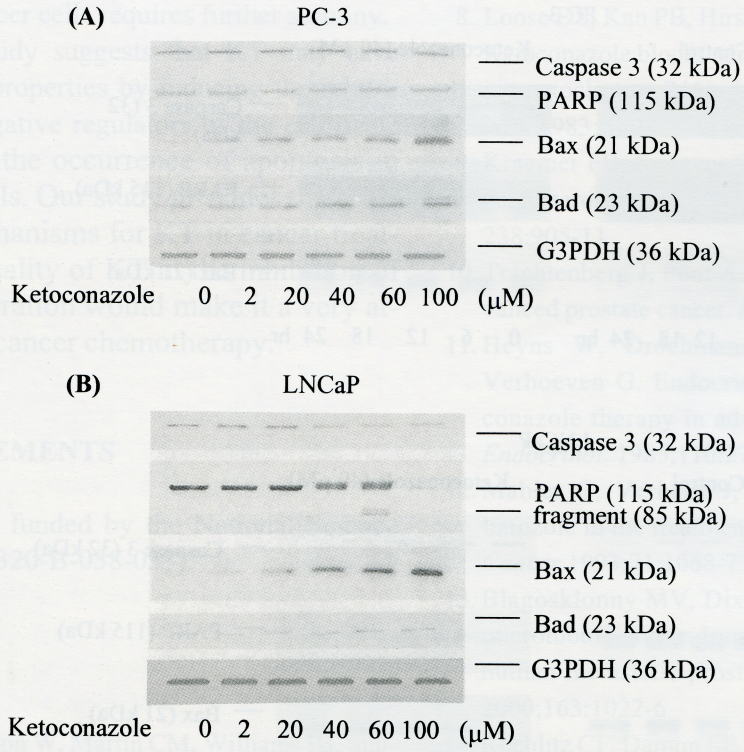

Fig. 3. KT-induced caspase 3 activation and apoptosis-related proteins in PC-3 and LNCaP cells. Human prostate cancer cells, (A) PC-3 and (B) LNCaP, were treated with various concentrations of KT (2-100 M) for 24 h. Proteins were isolated from cells treated with KT. Aliquots of 40 g of protein extracts were loaded onto SDS-PAGE, and Western blot analyses were performed. Intracellular responses of caspase 3 activation, PARP degradation, and Bax and Bad protein expressions were determined. An antibody specific to the GAPDH was used for determination of the protein loading control.

easily induced in LNCaP cells compared to PC-3 cells. We demonstrate that the Bax protein was induced at 6 h after 40-μM of KT treatment in LNCaP cells (Fig. 4B). In contrast, the induction of Bax protein was not observed in PC-3 cells (Fig. 4A).

DISCUSSION

Ketoconazole (KT) is an orally active broad-spectrum antifungal drug especially useful in patients with histoplasmosis or nonmeningeal cryptococcosis.[23] Recent studies have demonstrated that KT is an active agent against various malignant cell lines *in vitro*[14] and *in vivo*.[10-12,16,17] A previous report on humans showed that serum levels of KT after administration of single doses of 200 and 400 mg were 3.6 g/mL and 6.5 μg/mL, respectively.[24] Heyns et. al. demonstrated that 9 patients with prostate cancer treated with a high dose of KT (400 mg every 8 h) were detected as having serum levels of KT in a range of 2.0-9.1 μg/mL (means of 12 measurements recorded during 1 year of continuous treatment).[11] Different human cancer cell lines treated with KT showed similar IC 50 values of from 7.3 to 10.0 μg/mL.[14] Since a goal of this study is to develop a rational therapy against human cancer, we do not emphasize the effects of KT at concentrations exceeding the clinically achievable range of 10 to 20 μg/mL.[25] Our recent study demonstrated that the minimal dose of KT inducing apoptosis in various human cancer cell lines was 5 μM (2.6 μg/mL).[19] We have also demonstrated that G0/G1 arrest in human COLO 205 cells was clearly induced by KT at a concentration of 1 μM (0.52 μg/mL).[18] Our *in vitro*[18,19] and *in vivo* results indicate that some other mechanisms such as apoptosis induction might be also involved in suppres-