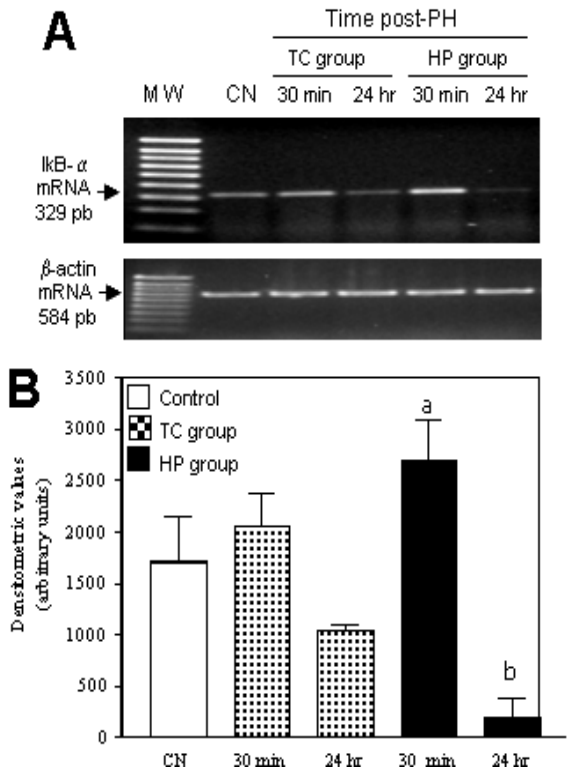

Figure 4

Expression of IkappaB-α mRNA in hepatocarcinogenesis and liver regeneration. Expression levels of IkappaB-α mRNA in early stages of hepatocarcinogenesis and during liver regeneration by semiquantitative RT-PCR. A: Representative agarose gels. B: Densitometric analysis of the IkappaB-α RNAm bands was quantified and normalized according to α-actin mRNA and expressed as mean ± S.D. (n = 3) aP < 0.05 against control, bP < 0.05 compared 24 hr post-HPx in TC against HP group by Student's t test.

paB-β appeared from 7 d after DEN administration, remained low at 24 h after the last dose with 2-AAF, in rats treated with DEN plus 3 doses of 2-AAF, and reached its lowest concentration 60 min after PH. In comparison, in liver regeneration there were no changes in cytosolic protein levels at any of the time analyzed. From the literature it is quite clear that IkappaB-β, has a slower degradation than IkappaB-α, which agrees with our results. Previous reports have shown that IL-1 induced a slower IkappaB-β degradation than IkappaB-α in glial cells, accompanied with a persistent long term activation of NF-kappaB [18]. Furthermore, IkappaB-β has been shown to be a less-efficient substrate for the IkappaB-kinase (IKK) when compared with IkappaB-α, therefore, it has been proposed that the IkappaB-β has a slower rate of phosphorylation kinetic [19]. It has been suggested that IkappaB-β phosphorylation may be a critical stage in the regulation of inhibition activity [20,21]. In a hyperphosphorylated state, IkappaB-β is degrades by means of prior ubiquitination, but the inhibitory activity is displayed [22,23] in a phosphorylated form and in the case of hypophosphorylate, the domain of nuclear localization of Rel A/p65 is not masked, permitting NF-kappaB/IkappaB-β complexes to enter the nucleus and support the persistent NF-kappaB activation [24].

Another point of consideration in our study was the IkappaB-α mRNA diminution at 24 h after PH, which is less pronounced in the hepatocarcinogenesis group in contrast to the group which was only hepatectomized. It has been suggested that upon activation of NF-kappaB, the newly synthesized IkappaB-α attenuates the NF-kappaB nuclear translocation, entering the nucleus and displacing NF-kappaB bound to DNA [11]. In an attempt to explain this phenomenon, we hypothesized the coexistence of inactive binary complexes IkappaB-α/NF-kappaB and active NF-kappaB complexes activation in the nucleus.

Theses studies and our results point a major IkappaB-β role in persistent NF-kappaB activation. The presence of the two IkappaB molecules should allow greater flexibility in the Rel transcription factor regulation, since both inhibitors respond differentially to proliferating stimuli [25]. These capacities of IkappaB's have been tested; IkappaB-α is a 10 times more efficient inhibitor of NF-kappaB than IkappaB-β, a capacity which results from the ability to remove NF-kappaB from DNA [26]. Possibly, IkappaB-α corresponds to stress situations, while the NF-kappaB persistent activation is an obliged response to the chronic inflammation condition, infection, differentiation and carcinogenesis. It has been shown that p65/IkappaB-β complexes are associated with kappaB-Ras proteins [27]. These ternary complexes respond differentially to extracellular signals, due to the incapacity of IKK to phosphorylate IkappaB-β in presence of k-Ras, sice kappaB-Ras masks the exposed p65 nuclear localization signal (NLS) [28]. It's important to point out that binary IkappaB-β/NF-kappaB complexes remain entirely in the cytosol, although IkappaB-α complexes wait around close to the nucleus, a difference which may confer more efficient activity [29]. In order to explain the different inhibition capacities of IkappaB, a novel computational program has been developed to predict temporal control to NF-kappaB activation based on coordinate degradation of both IkappaB. This model indicates a certain responsibility on the part of IkappaB-α for the negative feedback which leads to a transitory NF-kappaB activation, while IkappaB-β works to reduce the oscillatory potential of the system and to stabilize the NF-kappaB response during prolonged stimuli [30].