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Ethanol reduces p38 kinase activation and cyclin D1 protein expression after partial hepatectomy in rats

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***Background/Aims*: Chronic ethanol consumption inhibits liver regeneration. We examined the effects of chronic ethanol consumption on two mitogen-activated protein kinases in relation to induction of cell cycle proteins after partial hepatectomy (PH).**

***Methods*: Male Wistar rats were ethanol-fed (EF) or pair-fed (PF) for 16 weeks before PH. Hepatic activation of extracellular signal regulated kinase (ERK)1/2, p38 kinase and expression of cyclinD1, cyclin-dependent kinase-4 (cdk4) and proliferating cell nuclear antigen (PCNA) were studied.**

***Results*: In PF rats, PH-induced p38 activation was evident at 2 h and was maximal at 12 h. There was a close temporal relationship between p38 activation, cyclin D1 and PCNA expression. Alcohol exposure reduced p38 activation, cyclin D1 and PCNA, each by ~50%. ERK1/2 activation occurred during the first 2 h post-PH in both EF and PF rats, and there was no later increase in PF rats. In vivo inhibition of p38 suppressed PCNA expression whereas the effect of ERK1/2 inhibition was inconsistent.**

***Conclusions*: p38 kinase activation is linked temporally with cyclin D1 expression after PH and appears to exert cell cycle control in the adult liver. p38 signaling also appears to be a target for the inhibitory effect of chronic alcohol on liver regeneration.**

***Keywords*: Ethanol; Liver regeneration; MAPK; p38 kinase; Cyclin D1; PCNA**

1. Introduction

Delayed clinical recovery is a feature of alcoholic liver disease and results, in part, from impaired liver regeneration. Both acute and chronic ethanol administration to rats inhibits liver regeneration [1–3]. This appears to be due to a direct toxic effect of ethanol because it can be produced in the absence of severe liver disease [3], and is not found in dietary models of steatohepatitis [4].

Previous studies have shown that chronic ethanol exposure produces several abnormalities in mitogenic signaling pathways pertinent to control of hepatocyte proliferation, such as alteration of the hepatic response to tumor necrosis factor (TNF) which is required to prime hepatocytes for replicative competence [5–7], diminished phosphorylation of insulin receptor substrate-1 (IRS-1) [8,9], and impaired autophosphorylation of the epidermal growth factor receptor (EGFR) [10,11]. One of the downstream consequences of these effects is reduced activation of ras-linked mitogen-activated protein kinase (MAPK) and stress-activated protein kinases (SAPK) [11]. Despite these results, understanding about which of these signaling pathways are central to the toxic effect of alcohol

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Abbreviations: EF, ethanol-fed; EGFR, epidermal growth factor receptor; ERK, extracellular signal regulated kinase; IRS-1, insulin receptor substrate-1; MAPK, mitogen-activated protein kinase; PCNA, proliferating cell nuclear antigen; PF, pair-fed; PH, partial hepatectomy; SAPK, stress activated protein kinase.

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on liver regeneration is incomplete, largely because it is not yet known how various triggers and intracellular signaling molecules interact to regulate hepatocyte cell cycle progression in the normal liver after PH.

MAPK/SAPK cascades mediate several, and often opposing physiological responses, including cell proliferation/growth, differentiation, oncogenic transformation and apoptosis. Among these kinases, ERK1/2 is an important pro-mitogenic pathway in hepatocellular carcinoma cells and other cell types [12,13], but inhibition of proliferation has also been described [14,15]. ERK1/2 has not been demonstrated to exert pro-proliferative effects in the liver in vivo, but some studies attributed growth factor-induced proliferative responses in cultured hepatocytes to ERK1/2 activation [16–18]. Others have found that prolonged activation of ERK1/2 reduces DNA synthesis by increasing expression of the cyclin-dependent kinase inhibitor, p21 [19,20].

P38 kinases are also activated by both cytokine and mitogenic receptors and may be involved in transmitting anti-proliferative or pro-apoptotic signals [21,22]. p38 has also been proposed to play a role in hepatocyte proliferation [20,23]. For example, we observed that activation of p38 correlated with cell cycle entry following preconditioning against hepatic ischemia-reperfusion injury [24,25], and Chen et al. [20] noted a disproportionate reduction of p38 kinase activation after mitogen exposure to hepatocytes from chronic EF rats. A critical target of pro-proliferative cell signaling pathways is regulation of the cyclin D1 kinase, a holoenzyme complex formed by cyclin D1 and cyclin-dependent kinases-4 (cdk4) and cdk6. Cyclin D1 regulates catalytic activity of this complex; its up-regulation is sufficient to promote progression of hepatocytes from G_o through the G_1 restriction point and into S-phase of the cell cycle [26–29].

In this study, we first sought to clarify whether p38 and ERK1/2 pathways are altered by chronic ethanol exposure during liver regeneration after PH, and then correlated the time course of such activation with cyclin D1 expression, cdk4 and PCNA expression. We then tested the importance of p38 and ERK1/2 on cell cycle control after PH in control rats, using doses of chemical inhibitors that, at the doses used, have specific effects on individual protein kinase pathways.

2. Materials and methods

2.1. Animal model of chronic ethanol consumption

Male Wistar rats (180–220 g) were fed commercial rat pellets. Ethanol was added to the drinking water in gradually increasing concentration (to 40% at 6 weeks) for 16 weeks [3,30]. Controls were pair-fed (PF) the same diet as ethanol-fed (EF) rats, except that dextrose was isocalorically substituted for ethanol. This study was approved by the Animal Ethics Committee of the Western Sydney Area Health Service.

2.2. Partial hepatectomy and administration of inhibitors for MAPK and p38

EF and PF rats were subjected to two-thirds PH. In brief, rats were anesthetized with ether and the median and left lateral lobes of the liver were ligated and excised. Animals were allowed to recover and were permitted food and water ad libitum until the time of experiments. They were then re-anesthetized with ether, sacrificed by exsanguination, and the liver remnant harvested for isolation of total RNA and preparation of whole liver homogenate.

For experiments involving MAPK and p38 inhibitors, rats were injected with the following inhibitors: PD98059 (2′-amino-3′-methoxyflavone), a selective inhibitor of MAP kinase kinase (MEK), and SB203580 [4-(4′-fluorophenyl)-2-(4′-methylsulfinylphenyl)-5-(4′-pyridyl)-imidazole], a highly specific inhibitor of p38 (both purchased from Calbiochem, San Diego CA). Each inhibitor was dissolved in DMSO and administered intraperitoneally (ip). In preliminary studies, doses of kinase inhibitors ranging from 0.1 to >10 mg/kg were studied; at the dose of 0.25 mg/kg inhibition of the pathways of interest was observed, without effects on other kinases. For this reason, all further studies were conducted with this dose. Two further injections (same dose) were administered 6 and 12 h after PH. Rats were sacrificed 24 h after PH, liver homogenates prepared and levels of ERK1/2 and p38 phosphoproteins determined (see below). Remnant liver was collected and processed for determination of PCNA expression by immunohistochemistry and Western blot analysis (see below).

2.3. Preparation of liver homogenates

Liver was homogenized in ice-cold lysis buffer (50 mM Hepes, pH 7.4; 150 mM NaCl; 0.1% Triton X-100; 10% glycerol; 1.5 mM $MgCl_2$; 1 mM EGTA; 10 μg/ml aprotinin; 10 μg/ml leupeptin; 1 mM NaF; 200 μM Na_3VO4, 1 mM PMSF and 1 mM dithiothreitol). Homogenates were clarified by centrifugation at $10{,}000\times g$ for 10 min at 4 °C, and protein concentration was estimated by the Bio-Rad Bradford protein assay kit (BioRad, Hercules, CA, USA). Samples were stored at −80 °C until use.

2.4. Separation of proteins and immunoquantitation by Western blot analysis

Proteins (20 μg) were separated by 12% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes (Millipore, Bedford, MA). Cyclin D1 and PCNA were detected with specific primary antibodies from Santa Cruz Biotechnology (Santa Cruz, CA). ERK1/2 and p38 and their corresponding phosphoproteins were detected with primary antibodies from Cell Signalling Technology (Beverly, MA). Membranes were first blocked with 3% bovine serum albumin (for ERK1/2 and p38) or 1% skim milk (for cyclin D1 and PCNA), then incubated with primary antibodies overnight at 4 °C. Immunoblots were incubated with appropriate peroxidase-conjugated secondary antibodies (Santa Cruz, CA) (1 h, room temperature), followed by chemiluminescent detection with SuperSignal West Pico Chemiluminescent Substrate (Pierce Perbio, Rockford, IL). Bands corresponding to the known molecular masses of the proteins of interest were semi-quantified by scanning laser densitometry. Results were expressed relative to the time 0 values for PF control liver, unless otherwise indicated.

2.5. Determination of mRNA by quantitative RT-PCR

Total RNA was isolated from remnant liver by TRIzol reagent (Lifetechnologies, Rockville, MA) and stored at −70 °C. One-step RT-PCR was carried out by using 1 μg of total RNA in the Thermoscript One-Step system (Lifetechnologies, Rockville, MA). Primer sequences for rat cyclin D1 mRNA were: 5′-ATTGAAGCCCTTCTGGAGTCAAGCC-3′ (forward), 5′-TCTATTTTTGTAGCACCCCCCCGTC-3′ (reverse). GAPDH mRNA was amplified as a control gene, using primers with the sequences: 5′-AACGACCCCTTCATTGAC-3′ (forward) and 5′-TCCACGACATACTCAGCAC-3′ (reverse). The PCR products of cyclin D1 and GAPDH were separated on 2% agarose gels and stained with 0.5 μg/mL ethidium bromide. Gels were illuminated with UV light, photographed and analyzed by digital image analysis.

2.6. Detection of proliferating cell nuclear antigen (PCNA) in liver sections

Hepatic expression of PCNA was detected from formalin-fixed paraffin-embedded tissue. In brief, sections were incubated with the primary PCNA antibody, followed by incubation with a biotinylated rabbit anti-mouse IgM (1:80), washed, and incubated with avidin–biotin–horseradish peroxidase detection system (Vector Laboratories, Burlingame, CA) according to the Manufacturer's instructions. Sections were then reacted with 3,3′-diaminobenzidine (DAB) hydrochloride for 10 min, followed by counter-staining in hematoxylin. Slides were viewed under light microscopy. PCNA-positive hepatocytes were counted in five random 200× microscopic fields, and the number expressed as the percent of PCNA positive cells to the total number of cells counted.

2.7. Expression of data and statistical analysis

Results are expressed as mean ± SD. Analysis of variance (ANOVA) and Fisher's least significant method were used for comparisons among more than two means. A P value of <0.05 was considered to be significant.

3. Results

3.1. Effects of chronic ethanol exposure on ERK1/2 activation after PH

Hepatic expression of total ERK1/2 proteins was not affected by PH or ethanol feeding (Fig. 1(A)). In PF rats, PH stimulated an early increase in the phosphorylated ERK1/2 in both groups (Fig. 1(B) and (C)). Thereafter, levels of ERK1 and ERK2 phosphorylation declined to baseline by 2 and 4 h, respectively, (Fig. 1(B) and (C)). In PF rats, there was no further increase in the level of ERK1/2 phosphorylation during the time course of these experiments. In contrast, ERK1/2 phosphorylation increased between 6 and 12 h after PH in EF rats (Fig. 1(B) and (C)).

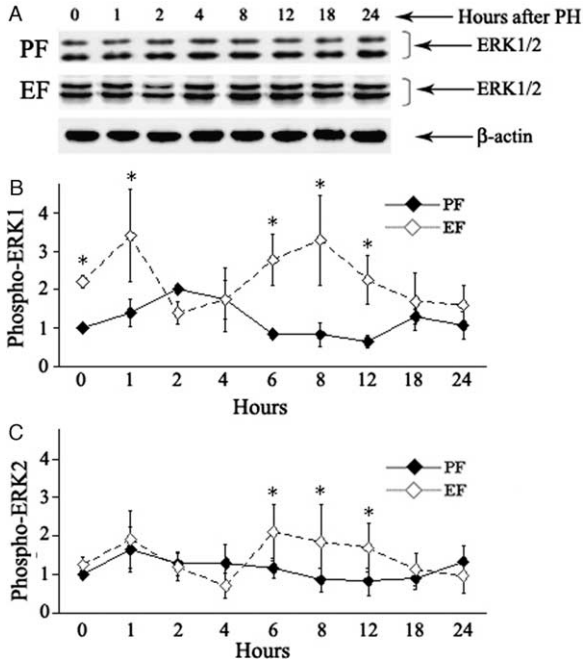

Fig. 1. ERK1/2 expression and activation in remnant liver after partial hepatectomy (PH) in pair-fed (PF) and ethanol-fed (EF) rats. (A) Total ERK1/2 expression in liver homogenates. β-actin was used to demonstrate equivalence of protein loading. Data are representative of FOUR identical experiments. (B) ERK1 and (C) ERK2 activity as determined by using specific antibodies against phosphorylated ERK proteins. Time 0 data were determined in remnant liver immediately after completion of PH. Data from four identical experiments were determined by densitometric semiquantitation of Western blot bands, and are expressed as mean ± SD in arbitrary units. *$P<0.005$, comparing EF with PF control.

3.2. Effects of chronic ethanol and PH on p38 activation

Expression of p38 total protein in remnant liver was not affected by chronic ethanol exposure or PH (Fig. 2, A)). However, levels of phospho-p38 (reflecting activation of p38 protein kinase) were substantially less in livers from EF rats compared with PF controls at time 0, and throughout the time course after PH (Fig. 2). In PF control rats there was a transient activation of p38 activity at time 0 that was not observed in EF rats (Fig. 2, A)). In separate experiments, we showed that the stress incurred by ether inhalation (used in the earlier experiments) accounted for this paradoxical 'time 0' activation of hepatic p38 kinase, and others have made similar observations [31,32]; p38 activation did not occur with intraperitoneal ketamine injection (data not shown). Following a return to lower values at 1 h after PH (Fig. 2, A), p38 kinase activity increased in both groups at 8 h post-PH, becoming maximal at 12 h, thereafter, declining to baseline by 24 h. In EF rats, p38 kinase activity remained at ~50% that of PF control liver values until 18 h after PH (Fig. 2, B).

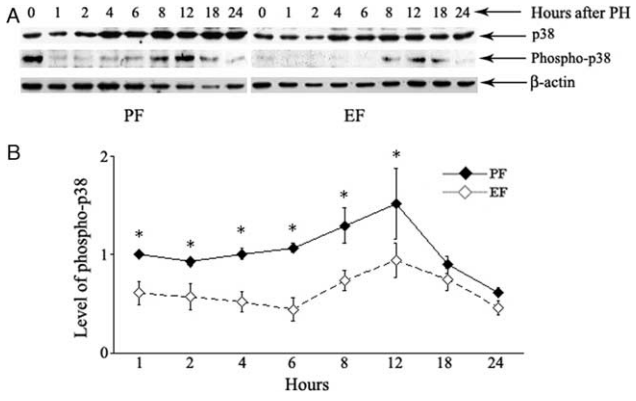

Fig. 2. p38 kinase activity in remnant liver after partial hepatectomy (PH) in pair-fed (PF) and ethanol-fed (EF) rats. Time 0 data were determined in remnant liver immediately after completion of PH. (Panel A) Representative Western blots of p38, phospho-p38 and β-actin in homogenates of remnant liver. (Panel B) Relative amount of phospho-p38 determined by semiquantitation of Western blot bands using densitometry, and expressed as mean ± SD of four individual experiments. *$P<0.01$, between EF and PF control.

3.3. Effects of chronic ethanol feeding and PH on cyclin D1 mRNA and protein levels, cdk4 and PCNA

Cyclin D1 transcripts were readily detected in PF control liver during the first 6 h after PH. At 8 h, values of cyclin D1 mRNA appeared to increase, with maximum levels observed at 18–24 h (Fig. 3(A)). In remnant liver from EF rats, cyclin D1 mRNA remained significantly lower than in controls (Fig. 3(A)). Cyclin D1 protein was not detected in liver from either group of rats during the first 6 h after PH. Levels were first detected 8 h after PH in PF control, rising to peak at 18–24 h (Fig. 3(B)). In the liver of EF animals, cyclin D1 was not detectable until 18 h, and values at 24 h remained diminished compared to PF controls (Fig. 3(B)).

The levels of hepatic cdk-4, another component of the cyclin D1 complex, remained relatively unchanged until 12 h after PH in either EF or PF control rats (Fig. 4). Thereafter, values increased in both groups; there were minimal differences in cdk-4 expression between EF and PF rats (Fig. 4).

Because cyclin D1 governs the passage of hepatocytes through the G_1 restriction point and into the cell cycle [26,28,29,33], increases in cyclin D1 protein should soon be followed by elaboration of PCNA, a protein expressed by

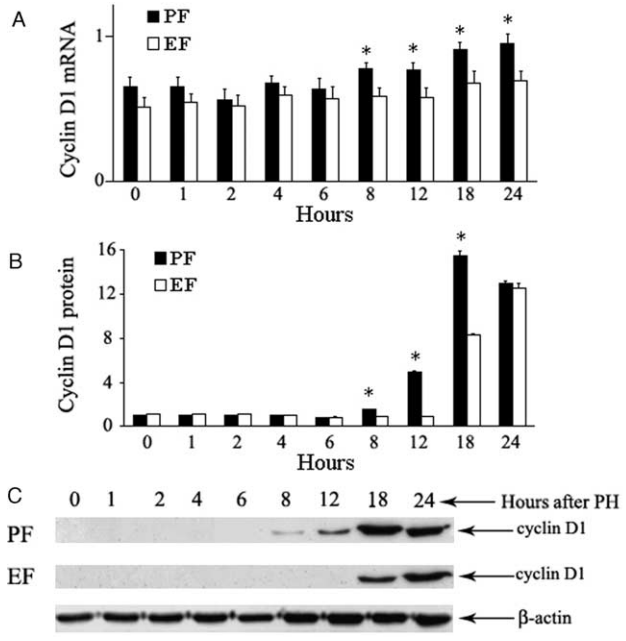

Fig. 3. Expression of cyclin D1 mRNA and protein in remnant liver after partial hepatectomy (PH) in pair-fed (PF) and ethanol-fed (EF) rats. (A) Time course of cyclin D1 mRNA, as determined by RT-PCR and expressed as the density of PCR products relative to those of GAPDH in arbitrary units. Data are mean ± SD of three identical studies. (B) Time course of cyclin D1 protein levels in remnant liver after PH, as determined by Western blot and expressed as the density of blot bands relative to those of β-actin in arbitrary units. Data are mean ± SD of three identical studies. (C) Representative Western blots of cyclin D1 in homogenates of remnant liver. β-actin was used to demonstrate equivalence of protein loading. *$P < 0.001$, between EF and PF controls.

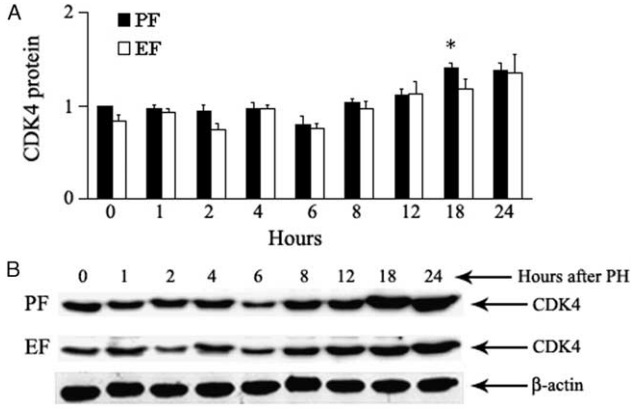

Fig. 4. Expression of cdk4 in remnant liver after partial hepatectomy (PH) in pair-fed (PF) and ethanol-fed (EF) rats. (A) Relative amount of cdk4, as determined by semiquantitation of Western blot bands using densitometry. Data are expressed as mean ± SD of four individual experiments in arbitrary units. *$P < 0.001$, between EF and PF control. (B) Representative Western blots of cdk4 in homogenates of remnant liver. β-actin was used to demonstrate equivalence of protein loading.

replicating cells in the late G1 and S phases. As shown in Fig. 5, expression of PCNA followed soon after cyclin D1 in PF control liver but was delayed and the level of its expression was greatly reduced by ethanol feeding.

3.4. Relationships between p38 activation, cyclin D1 and PCNA after PH, and effects of chronic ethanol consumption

The close temporal relationships between p38 kinase activation, cyclin D1 protein and PCNA expression after PH in PF control liver are depicted in Fig. 6. These relationships also show that chronic alcohol exposure produced similar reductions (~50%) in p38 kinase activity, cyclin D1 protein and PCNA levels.

3.5. Effects of p38 and ERK1/2 inhibitors on cell cycle entry after PH in control rats

In order to test whether the close temporal relationship between the p38 kinase activation and the expression of cyclin D1 and PCNA after PH was attributable to a direct effect of p38 on cell cycle control, we injected rats with relatively selective chemical inhibitors of p38 and ERK1/2

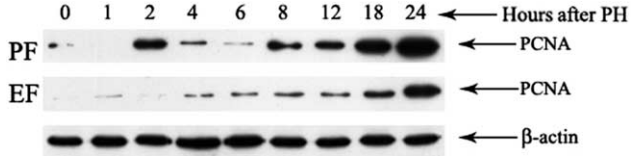

Fig. 5. Expression of PCNA in remnant liver after partial hepatectomy (PH) in pair-fed (PF) and ethanol-fed (EF) rats. Time 0 data were determined in remnant liver immediately after completion of PH. PCNA expression was determined by Western blot using specific antibody against PCNA protein. β-actin was used to demonstrate equivalence of protein loading. Data are representative of four identical experiments.

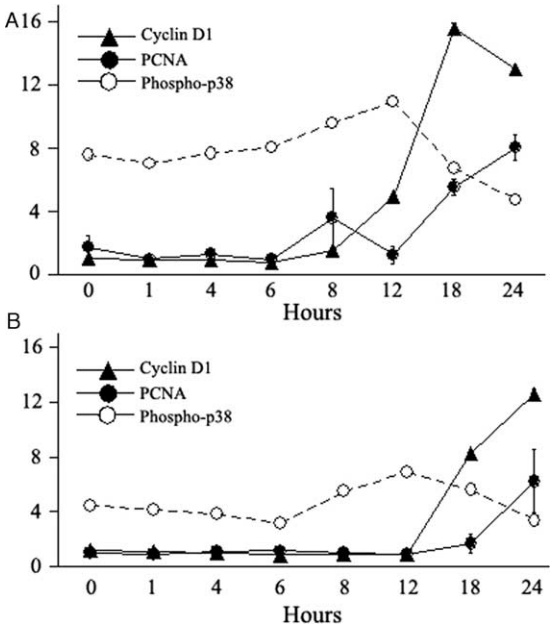

Fig. 6. Temporal relationship between cyclin D1, PCNA, and pospho-p38 MAPK activation following PH in PF (A) and EF (B) rats. Expression levels of proteins were determined by Western blot and expressed as the density of blot bands relative to those of β-actin in arbitrary units. Data are mean ± SD of three identical studies.

activation, and determined the hepatic expression of cyclin D1 and PCNA. Inhibition of p38 by SB203580 or ERK1/2 by PD98059 both appeared to decrease cyclin D1 expression and significantly reduced PCNA expression (Fig. 7). Expression of PCNA after PH was also studied by immunohistochemistry; although both SB203580 and PD98059 appeared to suppress PCNA expression, only the effect of SB203580 was significant (Fig. 7(C)–(F)).

4. Discussion

The first important finding of this study is that, in remnant liver after PH, the inhibitory effect of ethanol on activation of p38 kinase is commensurate with and temporally related to delayed and reduced expression of cyclin D1 and cell cycle activation. The latter was indicated by PCNA expression, a late G_1 through S marker. The second key finding was that in vivo administration of a specific p38 kinase inhibitor reduced PCNA expression after PH. Thus, while inhibition of ERK1/2 activation also appeared to have some effect, these results suggest that p38 kinase is a critical signaling pathway for inducing liver regeneration.

In different cell types under varying experimental conditions, p38 kinase exerts pro-proliferative, anti-proliferative, pro-apoptotic or anti-apoptotic functions [21–25,34]. Until now, p38 has not been a focus for the regulatory control of liver regeneration in adult liver [22]. However, the following evidence supports such a role: (1) p38 kinase is implicated in TNF-mediated regulation of IL-6 synthesis [22,35], the process required to confer hepatocytes in G_0 with replicative competence [6,7,36,37]. (2) $G_{\alpha i2}$ and insulin-like-growth-factor receptor signaling through IRS-1 both activate p38 [38], and both are decreased in rat liver by chronic ethanol exposure which inhibits liver regeneration [9,39]. (3) Inhibition of p38 kinase with SB203580 reduces mitogenic stimulation of DNA synthesis in primary hepatocytes [20]. (4) Activation of p38 correlates best with cyclin D1 induction and PCNA expression after ischemic preconditioning or low-dose TNF injection to mice subsequently subjected to ischemia-reperfusion injury [24,25].

The time course of p38 activation between 8 and 24 h after PH has not previously been studied. However, it is at just this time that induction of cyclin D1 occurs, particularly accumulation of cyclin D1 protein [26,27,29,33]. Although inhibitory effects of p38 activation on cyclin D1 transcription have been demonstrated in some cell types [22,40], others have shown that p38 kinase regulates stability of cyclin D1 protein [41]. Previous research by others and that of our group showed that p38 may be readily stimulated by various forms of oxidative stress [23–25,42]. The presence of oxidative stress in remnant liver after PH, therefore, provides one potential mechanism for p38 activation in this context [43,44]. Another possibility is that p38 is activated through certain cell surface receptors, such as TNF, hepatocyte growth factor (HGF) and EGF receptors [20]. A plausible candidate mitogen at 8–12 h after PH is transforming growth factor-alpha (TGFα), which is synthesized by the liver between 4 and 18 h after PH [45]; TGFα produces prolonged stimulation of MAPK pathways through the EGFR [16].

While the time course of ERK1/2 activation after PH and the effects of alcohol established in the present study seem inappropriate for direct roles in regulating cyclin D1, this pathway could influence regulation of immediate early response genes [46], and proteins that oppose hepatocellular proliferation. ERK1/2 is the archetypical pro-proliferative MAPK in hepatoma cells and other rapidly turning over cells [47]. However, the temporal sequence of ERK1/2 activation at 1–2 h after PH noted in the present experiments does not support a direct role for this MAPK as an important pro-proliferative driver in adult liver. Further, the effects of chronic ethanol exposure, which caused a late activation of ERK1/2 but inhibited liver regeneration, are inconsistent with a predominantly pro-proliferative role for ERK1/2. Experiments with PD98059, a putatively selective ERK1/2 inhibitor at the doses used (higher doses also inhibit p38 kinase), reduced PCNA on Western blots but did not have a significant effect on PCNA immunohistochemistry. This discrepancy could be due to a combination of technical difficulties, including non-specific staining of some cells thought to be PCNA positive, and relatively small numbers. However, these results are inconclusive for a pro-proliferative effect of ERK1/2 MAPK on cell cycle

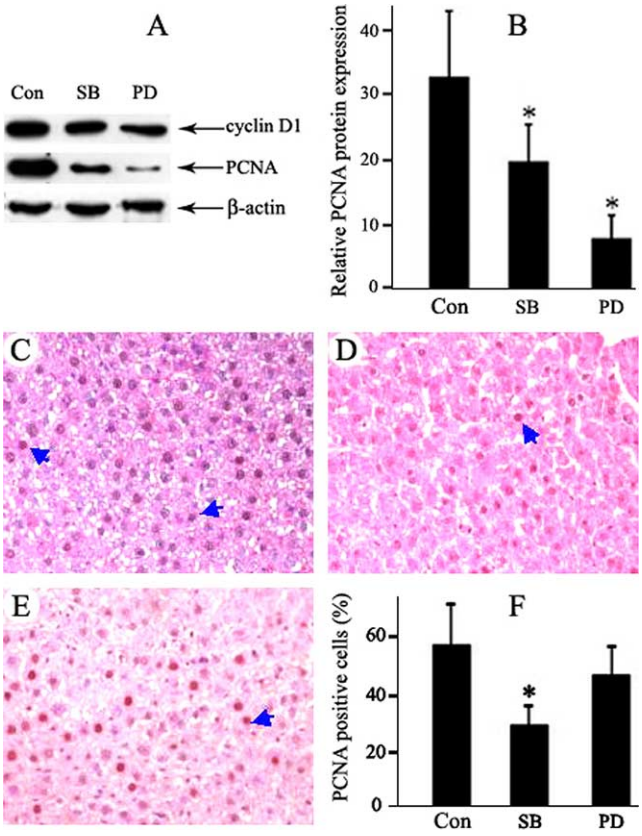

Fig. 7. Effects of inhitors of p38 kinase and ERK1/2 activation n expression of cyclin D1 and PCNA in remnant liver after partial hepatectomy (PH). Rats were injected with SB203580 (SB, p38 inhibitor), or PD98059 (PD, inhibitor of ERK1/2 activation) before being subjected to PH, as detailed in the Section 2. Liver tissues were collected and protein extracted for Western blot analysis for cyclin D1 and PCNA. (A) Representative Western blots, representative of 4 identical experiments. (B) Relative PCNA protein expression in homogenates of remnant liver from vehicle control rats (Con) and rats injected with SB 203580 (SB, p38 inhibitor), or PD 98059 (PD, inhibitor of ERK1/2). The results are expressed as density of blot bands relative to those of β-actin in arbitrary units. Data are mean ± SD of four individual experiments. *$P < 0.001$, between rats treated with inhibitors and vehicle-treated controls. (C–F) Effect of p38 and ERK1/2 inhibition on cell cycle entry as indicated by PCNA immunostaining of hepatocytes nuclei in control rats at 24 h after PH. Animals were injected with SB203580 (SB, p38 inhibitor), or PD98059 (PD, inhibitor of ERK1/2), or vehicle control (Con) prior to PH, and liver sections were processed for immunohistochemical staining for PCNA, as all detailed in Section 2. (C) Liver section from a rat injected with vehicle (Con). (D) Liver section from a rat injected with SB. (E) Liver section from a rat injected with PD. Results are representative sections from one of three replicate experiments. (F) Number of the PCNA-positive hepatocytes. Cells were counted in five random microscopic fields at 200× magnification, and expressed as percentage of PCNA positive cells to the total number of cells counted. Data are mean ± SD. *$P < 0.001$, between rats treated with inhibitors and vehicle-treated controls.

induction after PH. On the other hand, the increase at 6–12 h after PH in EF but not PF rat liver could be explained by ERK1/2 opposing liver cell proliferation, for example by up-regulation of p21 [19,34].

The present data advance understanding about how toxic effects of alcohol impact on the adequacy of liver regeneration through inhibition of the p38 and ERK1/2 signaling pathways activated by cytokine and mitogenic receptors. Attempts to understand the mechanism of how alcohol produces effects on intracellular signaling pathways may clarify the mechanism of how alcohol impairs cell proliferation. Meanwhile, correction of reduced p38 kinase activity carries potential to restore cyclin D1-mediated regenerative capacity to the liver of individuals chronically exposed to alcohol.

In summary, the ERK1/2 pathway is unlikely to be responsible for direct induction of cyclin D1 after PH. In contrast, activation of p38 kinase after PH temporally correlated with cyclin D1 and PCNA protein expression. Further, chronic ethanol consumption proportionately reduced p38 activity, cyclin D1 protein and PCNA expression, but produced changes in ERK1/2 activation, including a later increase, that cannot explain the decrease in cell cycle induction. Intervention studies with a p38 kinase inhibitor in vivo clearly implicated involvement of p38 kinase in cell cycle induction after PH; inhibition of ERK1/2 activation produced ambiguous results. Thus, p38 kinase is a crucial signaling pathway that controls cyclin D1 protein expression and cell cycle progression during liver regeneration. It also appears to be a critical locus of the inhibitory effect of chronic alcohol exposure on liver regeneration, and is therefore a potential target to reverse this inhibition.

Acknowledgements

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