Disrupted NF-κB activation after partial hepatectomy does not impair hepatocyte proliferation in rats

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INTRODUCTION

The mechanisms initiating the regenerative response after partial hepatectomy (PH) remain controversial. Several lines of evidence point to the critical role of the activation of nuclear factor kappa B (NF-κB)\cite{5-8}. This is one of the earliest events constantly detectable in the liver after PH and the previous findings have advocated NF-κB as a critical effector of the cascade initiating the regenerative process\cite{9}. Some authors also emphasize an anti-apoptotic role of NF-κB during the regenerative process\cite{9}. Mice lacking TNF receptor type 1 (TNF-R1) showed deficient NF-κB binding, low interleukin-6 (IL-6) production, decreased signal transducer and activator of transcription 3 (Stat3) activation and low levels of hepatocyte DNA replication after PH\cite{6-8}. Based on this and similar observations, it has been proposed that activation of the NF-κB pathway is a critical step to usher cells into the cell cycle in response to PH. This process might also require the sequential activation of TNF/NF-κB/IL-6 and Stat3. However, recent studies using mice lacking the common signal transducer of all IL-6 family members gp130 showed only minor effects on the cell cycle and on the peak of DNA synthesis after PH despite an abolished acute phase response and inhibition of Stat3\cite{9}. Moreover, recent studies in a model of transgenic mice, with specific inhibition of NF-κB at the hepatocyte level, did not impair DNA synthesis and did not increase liver apoptosis\cite{9}. In order to clarify the importance of the activation of NF-κB in the liver regeneration process, we have evaluated the consequences of NF-κB inhibition on downstream activation of Stat3 and DNA synthesis after PH. Numerous exogenous and endogenous stimuli are capable of inducing NF-κB activity\cite{10}, but the pathways leading to NF-κB activation are complex. One of them involves oxidative stress with elevated level of reactive oxygen species\cite{11,12,13}. We have therefore administered the antioxidant pyrrolidine dithiocarbamate (PDTC), a compound with metal chelator properties that has been used as a reversible inhibitor of NF-κB in vitro and in vivo\cite{14,15,16}. Increased production of TNF-α is a potential activator of NF-κB after PH\cite{17}, although the source of TNF-α after

Abstract

AIM: To analyze the effects of NF-κB inhibition by antioxidant pyrrolidine dithiocarbamate (PDTC) or TNF inhibitor pentoxifylline (PTX) on liver regeneration after partial hepatectomy (PH).

METHODS: Saline, PDTC or PTX were injected 1 h before PH and rats were killed at 0.5 and 24 h after PH. Several control groups were used for comparison (injection control groups).

RESULTS: Compared to saline injected controls, NF-κB activation was absent 0.5 h after PH in rats treated with PDTC or PTX. At 24 h after PH, DNA synthesis and PCNA expression were identical in treated and control rats and thus occurred irrespectively of the status of NF-κB activation at 0.5 h. Signal transducer and activator of transcription 3 (Stat3) activation was observed already 0.5 h after PH in saline, PDTC or PTX group and was similar to Stat3 activation in response to injection without PH.

CONCLUSION: These data strongly suggest that (1) NF-κB p65/p50 DNA binding produced in response to PH is not a signal necessary to initiate the liver regeneration, (2) Stat3 activation is a stress response unrelated to the activation of NF-κB. In conclusion, NF-κB activation is not critically required for the process of liver regeneration after PH.

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PH has not been entirely elucidated. To reduce TNF-α production, we administered pentoxifylline (PTX), a methylxanthine derivative, which has been demonstrated to suppress LPS-induced TNF-α production. It may also modulate the expression of other cytokines like IL-6 and IL-1.

MATERIALS AND METHODS

Animals
Male Wistar rats (220-270 g body weight) were obtained from the Rat Breeding Facilities of the Catholic University of Louvain Medical School, Brussels, Belgium. All animals were kept in a temperature- and humidity-controlled environment in a 12 h light-dark cycle. At all times, they were allowed free access to water and standard food pellet diet (Usine d’Alimentation Rationnelle, Villemoisson-sur-Orges, France). The animals were handled according to the guidelines established by the Catholic University of Louvain.

Surgical procedures and experimental design
All animal manipulations were carried out under light ether anesthesia at room air between 9:00 a.m. and 12:00 noon with the use of a clean, but non-sterile technique. PH consisted in mid-ventral laparotomy and resection of the left lateral and median lobes (70% of the liver), according to Higgins and Anderson. Saline, PDTC (100 mg/kg) and PTX (100 mg/kg) were injected intraperitoneally (i.p.) to the rats 1 h before PH. The rats were killed under ether anesthesia by exsanguination after the puncture of the abdominal aorta and transection of the inferior vena cava in the thoracic cavity, at 0.5 h after PH, i.e. 1 h 30 min after the injection of the active compound, and 24 h after PH. Several additional control groups were used for comparison: (1) rats administered saline, PDTC or PTX without PH and killed 1 h after the injection (injection control group), (2) naïve rats that received neither injection nor PH (true controls). The livers were removed, lobes were rapidly weighed, snap frozen in liquid nitrogen and stored at -80 °C. A minimum of three rats was killed in each group at each of the indicated time points.

Preparation of nuclear extracts and electrophoretic mobility shift assays (EMSA)
Nuclear extracts were prepared as previously described. Protein content was determined using a bicinchoninic acid (BCA) protein assay with serum albumin as a standard (Pierce Chemical, Rockford, IL, USA).

Six to ten micrograms of nuclear proteins were pre-incubated for 10 min at room temperature with 2 μg poly (dl-dc) in the following binding buffers: NF-κB (20 mmol/L Hepes, 60 mmol/L KCl, 5 mmol/L MgCl₂, 0.2 mmol/L EDTA, 0.5 mmol/L PMSF, 0.5 mmol/L DTT, glycerol 10%, Nonidet P40 1%); Stat3 (10 mmol/L Hepes, 50 mmol/L NaCl, 1 mmol/L EDTA, glycerol 10%). Double stranded oligonucleotides were ³²P end-labeled with γ³²P ATP and added to the extracts (10⁷ cpm). The mixtures were incubated for 30 min at room temperature and then electrophoresed (200 V, 2 h) on a 5% polyacrylamide gel in a 1× TBE buffer (25 mmol/L Tris-HCl, 25 mmol/L boric acid, 0.5 mmol/L EDTA). To confirm the identity of the protein/DNA complex, supershift analysis was performed: 4 μL of specific antibody (1 μg/μL) was added to the samples after 30 min of incubation with the labeled probe and incubated for a further 30 min. Polyclonal antibodies against NF-κB components p50 and p65, but also p52, c-rel, and rel-B, and anti-Stat3 were purchased from Santa Cruz (CA, USA). The following probes were used: chromatography-purified double stranded oligonucleotides from the class I major histocompatibility complex enhancer element H2kB; TCGAGGGCTGGGATTCCCC CATCTCT (NF-κB) and from the serum-inducible factor binding element in the c-fos promoter; CCAGCATTTCCCGTAAATCCTCCAG; (Stat3). A rabbit reticulocyte (Promega Benelux, Leiden, Netherlands) and an EGF-stimulated A431 cell nuclear extract (Santa Cruz Biotechnology) were used as standards for NF-κB and Stat3, respectively. Gels were dried and exposed to a Kodak Biomax MS film (NEN™ Life Science Products, Inc., Boston, MA, USA) for 16 to 24 h.

Thymidine incorporation
One hour before being killed, 50 μCi of [³²H]-Thymidine (Amersham, Buckinghamshire, UK) was administrated into the femoral vein under light ether anesthesia. At the time of killing (24 h after PH), livers were removed, weighed and rapidly snap frozen in liquid nitrogen and stored at -80 °C. Hepatic DNA synthesis was evaluated by measuring the incorporation of [³²H]-Thymidine into the DNA. Total hepatic DNA was extracted as previously described, and incorporation of radioactive nucleotide was measured in a liquid scintillation counter (Wallac 1409, Turku, Finland). Its value is expressed as disintegrations per minute (dpm) per μg of DNA. All samples were analyzed in duplicate.

PCNA labeling index
Sections from formalin fixed liver tissue (5 μm thick) were air-dried at 37 °C overnight and dewaxed. Slides were incubated for 30 min in H₂O; 0.3% to inhibit endogenous peroxidases, then in TBS containing 10% normal goat serum (NGS, APP Products Ltd, West Midlands, UK) to block non-specific binding sites. Slides were incubated in a monoclonal primary anti-PCNA mouse antibody (PC10, Dako, Denmark; 1:100, overnight), and after washing, with the secondary antibody (anti-mouse antibody; Boehringer, Mannheim, Germany; 1:500; 30 min) followed by streptavidin peroxidase (Boehringer, Mannheim, Germany; 1:1 000; 30 min). Peroxidase activity was revealed by immersion of the slides for 10 min in a 3,3-diaminobenzidine hypochloride solution (DAB 50 mg/100 mL, pH 7.4, Amersham, Cardiff, UK) supplemented with 0.02% H₂O₂. All slides were treated simultaneously to ensure homogeneity of the technique. PCNA labeling index was obtained by examining 3-5 high-power fields in three rats per group. Each field was divided into four zones,
zone 1 being closest to the centrolobular vein and zone 4 corresponding to the periportal areas. Labeling index was defined as the ratio between marked cells and total counted cells.

The same experimented pathologist examined all the slides carefully to detect evidence of necrosis or apoptosis.

**Statistical analysis**

Results were expressed as mean±SE. The statistical differences between the groups were tested using the one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls multiple comparison tests. Statistical significance was admitted for a $P$ value of <0.05.

**RESULTS**

**Effect of saline, PDTC, and PTX on NF-κB binding activity (Figure 1A)**

NF-κB p65/p50 DNA binding activity was not detected in the liver of untreated rats (lane 1) nor in the livers from non-hepatectomized rats 1 h after i.p. injection of saline (lane 2), PDTC (lane 4) or PTX (lane 6). NF-κB was strongly activated 0.5 h after PH preceded by saline injection (lane 3), similarly to activation observed 0.5 h after PH alone\[24\]. Supershift analysis confirmed the binding of the heterodimer p65/p50 NF-κB complex (lanes 8-10). Injection of PDTC or PTX 1 h before PH prevented the occurrence of the expected p65/p50 DNA binding complex at 0.5 h after PH, since the upper complex was not observed on EMSA (lanes 5 and 7). However, two distinct faster-migrating complexes have been found after PTX (lane 7). The first one, also present after saline or PDTC injection, was partially supershifted by p50 antibody; the second one totally disappeared with p65 antibody. Supershift assays performed with p52, c-rel and rel-B had no influence on this lower complex (Figure 1B).

**Effect of PDTC and PTX on hepatocyte proliferation 24 h after PH (Figures 2 and 3)**

Hepatocyte proliferation was followed by PH peaks at 24 h. Pretreatment of rats with PDTC or PTX 1 h prior to PH had no effect on liver regeneration. Thymidine incorporation was indeed similarly elevated in all the groups (Figure 2). In untreated rats, 90% of hepatocytes was strongly activated 0.5 h after PH preceded by saline injection (lane 3), similarly to activation observed 0.5 h after PH alone\[24\]. Supershift analysis confirmed the binding of the heterodimer p65/p50 NF-κB complex (lanes 8-10). Injection of PDTC or PTX 1 h before PH prevented the occurrence of the expected p65/p50 DNA binding complex at 0.5 h after PH, since the upper complex was not observed on EMSA (lanes 5 and 7). However, two distinct faster-migrating complexes have been found after PTX (lane 7). The first one, also present after saline or PDTC injection, was partially supershifted by p50 antibody; the second one totally disappeared with p65 antibody. Supershift assays performed with p52, rel B, and c-rel had no influence on this lower complex (Figure 1B).
is responsible for the transcriptional activity of NF-κB
NF-κB p65 subunit, important for liver development
transcription of a large set of immediate-early genes
to the nucleus as soon as 0.5 h after PH
inflammatory and cellular growth control
factor NF-κB. The inducible NF-κB factor regulates
following hepatic cell mass reduction, a key role seems
Stat3
transcription factors molecules such as NF-κB and
inflammatory cytokines like TNF-α or IL-6
PH induces an early cellular response involving pro-
activation after PH in non-injected rats at this time point.
Indeed, as already reported by us and others, PH in non-
untreated rats (lane 1), but a Stat3 DNA binding complex
in the livers from non-hepatectomized rats receiving saline 1 h before being killed (lane 2), PDTC (lane 3)
or PTX (lane 4). PH was associated with high Stat3 activity
at 0.5 h in animals pretreated with saline (lane 5), PDTC
(lane 6) or PTX (lane 7), contrasting with very low Stat3
activation after PH in non-injected rats at this time point.
Effect of saline, PDTC and PTX on Stat3 DNA binding activity
Figure 3
Free probe

Figure 4

nuclei expressed PCNA 24 h after HP. Pretreatment
with PDTC or PTX did not modify the proportion of
PCNA positive nuclei in agreement with the thymidine
incorporation (Figure 3). No evidence of liver necrosis
or massive apoptosis was observed by histological
examination.

Effect of injection of saline, PDTC and PTX on Stat3
binding activity (Figure 4)
Stat3 DNA binding activity was not detected in the liver of
untreated rats (lane 1), but a Stat3 DNA binding complex
was present in the livers from non-hepatectomized rats
receiving saline 1 h before being killed (lane 2), PDTC (lane 3)
or PTX (lane 4). PH was associated with high Stat3 activity
at 0.5 h in animals pretreated with saline (lane 5), PDTC
(lane 6) or PTX (lane 7), contrasting with very low Stat3
activation after PH in non-injected rats at this time point.
Indeed, as already reported by us and others, PH in non-
injected rats was followed by slight Stat3 activation at 0.5 h
after PH, peaked at 3 h and was still detected till 8 h[24,26].
Identity of Stat3 was confirmed by supershift analysis with
a specific Stat3 antibody (lane 8).

DISCUSSION
PH induces an early cellular response involving pro-
inflammatory cytokines like TNF-α or IL-6[37-39] and
transcription factors molecules such as NF-κB and
Stat3[3,30]. In the initiation of proliferating response
following hepatic cell mass reduction, a key role seems
to be attributed to the activation of the transcription factor NF-κB. The inducible NF-κB factor regulates
the expression of numerous genes involved in immune,
inflammatory and cellular growth control[11,31,32]. Migrating
to the nucleus as soon as 0.5 h after PH[3], it induces the
transcription of a large set of immediate-early genes[33]. The
NF-κB p65 subunit, important for liver development[34],
is responsible for the transcriptional activity of NF-κB[35]
and also for its anti-apoptotic effects, preventing the
cytotoxic effect of TNF-α and cell death[36,37]. Convergent
data suggest that this mediator collaborates with other
acute phase gene products in order to protect the cells
during the regenerative process[5,38-40]. The most active
form of NF-κB is a heterodimer consisting of subunits
p50 or NF-κB1 and p65 also called RelA, which contains
the transactivation domain necessary for the induction
of target genes[41,42]. This active form has been identified
after PH as the post-hepatectomy factor[22,38]. Although not
consistently reported, the activation of NF-κB after PH
has been supposed to be related to an increased expression
of TNF-α, not consistently reported, and/or to oxidative stress[43]. However, various other non specific
stimuli are able to induce this activation[44]. Although the
importance of the TNF-α pathway has been outlined[22,38],
recent studies analyzing liver regeneration in different types
of knockout mice suggest that a complete regenerative
response may occur in the absence of early factors such as
TNF-α, IL-6 or Stat3[43,44]. In order to investigate the
role of NF-κB activation in determining the progression
into the cell cycle and liver regeneration, we analyzed the
effect of NF-κB inhibition on hepatocyte proliferation
after PH in normal rat with otherwise normal cytokine
expression, regulation and signaling capabilities. Two
strategies have been used to prevent NF-κB activation.
First, we administered an antioxidant molecule (PDTC) to
reduce the oxidative stress, a known stimulus of NF-κB
activation. Second, we aimed at decreasing the influence
of TNF-α by reducing its production by the use of PTX.
PTX and PDTC effectively prevented the activation of
NF-κB p65/p50 complex observed 0.5 h after PH in non-
treated and saline-treated animals. Despite this absence
of NF-κB activation, the hepatocytes responded to the
proliferation stimulus: PCNA expression as well as DNA
synthesis analyzed 24 h post-PH were normal compared
to a classical PH. At that time we did not observe any
evidence of liver necrosis or massive apoptosis compared
to livers from untreated animals (data not shown). This last
observation is in contrast with the results obtained when
NF-κB is inhibited by an adenoaviral vector expressing a
mutated form of IκB-α[45]. The inhibition of NF-κB using
this adenoaviral vector led to massive apoptosis but also
failed to interfere with hepatocyte proliferation. This
massive apoptosis could be induced by the adenoaviral itself which causes increase of TNF levels and apoptosis
before PH. More recently, it has been advocated that
the activation of NF-κB in hepatocytes is not needed to
induce liver regeneration after PH[10]. The authors also
postulated that a preserved activation in non-parenchymal
cells could be sufficient to drive both proliferative and anti-
apoptotic effects of NF-κB during liver regeneration.
This last assumption cannot be found in our experiments, since
the inhibition of NF-κB was not targeted to a specific cell
type in the liver and, in principal, the substances used in
our experiments do act on all liver cell types including the
non-parenchymal cells.

We observed that PTX induced another DNA binding
complex after PH, migrating faster, and partially supershifted by the p65 antibody. The functional role of such a complex is not elucidated but may constitute a compensatory response of the Rel family of transcription factors to substitute the usual factor. In a recent study using p50\(^{-/-}\) mice\(^{[46]}\), PH was associated with a normal regenerative response. P65 protein expression was elevated but without p65/p50 DNA binding activity suggesting that p65 could be part of other complexes, allowing normal regeneration. Considering our data and those obtained in p50 knockout mice, the p65/p50 DNA binding after PH does not seem to be absolutely required for proper liver regeneration. PTX is a potent inhibitor of TNF production which inhibited NF-κB activation but without reducing liver regeneration. These data further reinforce the absence of a link between NF-κB activation and hepatocyte proliferation. However, they do not bring any information concerning a role for TNF-α in the initiation of liver regeneration, since the expression of this factor was not investigated in the study. In contrast to the data demonstrating the quasi absence of Stat3 activation during the first hour after PH\(^{[12,13]}\), an early activation of Stat3 was observed as soon as 0.5 h after PH preceded by saline, PDTC and PTX. This suggested that Stat3 was already induced in our model by the first stimulus, i.e. the injection and the required animal handling. This hypothesis was confirmed by the increased Stat3 DNA binding obtained after a single injection of saline, PDTC and PTX. It seems therefore that Stat3 is a component of the acute-phase response induced by stress procedures as minimal as the i.p. injection. This rather non-specific origin for Stat3 activation does not exclude that this factor may play an important role in liver regeneration as documented in studies conducted in IL-6 and TNFR-1 knockout mice in which the restoration of the Stat3 DNA binding following IL-6 supplementation corrected the impaired liver regeneration process\(^{[6,7]}\). It seems thus that Stat3 activation, which is a part of the priming process as described by Bucher et al\(^{[40]}\), can be produced by a great variety of stimuli and not necessarily by the PH itself.

As demonstrated previously, sham operation also induced activation of NF-κB and Stat3\(^{[23]}\). PH preceded by a sham operation was associated with a second activation of these transcription factors\(^{[49]}\). In this work, the i.p. injection is a minor stress compared to a sham operation. However, the i.p. injection procedure produced by itself an activation of Stat3 that persisted at the time of PH and probably for a longer period, possibly masking the effects induced by this second intervention. By contrast, a NF-κB response was clearly observed after PH when only saline was injected. When drugs able to inhibit NF-κB activation were injected, this response was indeed suppressed but the proliferation indices were not affected. An activation of liver NF-κB seems thus unnecessary to initiate liver regeneration in those conditions. In conclusion, and in complement to those of others using a transgenic model, our data strongly suggest that an increase of liver NF-κB p65/p50 DNA binding as an immediate response to PH is not essential for inducing liver cell proliferation.

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REFERENCES

1. Akerman P, Cote P, Yang SQ, McClain C, Nelson S, Bagby GJ, Diehl AM. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. Am J Physiol 1992; 263: G579-G585
2. Kirillova I, Chaisson M, Fausto N. Tumor necrosis factor induces DNA replication in hepatic cells through nuclear factor kappaB activation. Cell Growth Differ 1999; 10: 819-828
3. Cressman DE, Greenbaum LE, Haber BA, Taub R. Rapid activation of post-hepatectomy factor/nuclear factor kappa B in hepatocytes, a primary response in the regenerating liver. J Biol Chem 1994; 269: 30429-30435
4. FitzGerald MJ, Webber EM, Donovan JR, Fausto N. Rapid DNA binding by nuclear factor kappa B in hepatocytes at the start of liver regeneration. Cell Growth Differ 1995; 6: 417-427
5. Iimuro Y, Nishiura T, Hellerbrand C, Behrens K, Schoonhoven R, Grisham JW, Brenner DA. NFκB prevents apoptosis and liver dysfunction during liver regeneration. J Clin Invest 1998; 101: 802-811
6. Yamada Y, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci USA 1997; 94: 1441-1446
7. Yamada Y, Fausto N. Deficient liver regeneration after carbon tetrachloride injury in mice lacking type 1 but not type 2 tumor necrosis factor receptor. Am J Pathol 1998; 152: 1577-1589
8. Yamada Y, Webber EM, Kirillova I, Peschon JJ, Fausto N. Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: requirement for type 1 but not type 2 receptor. Hepatology 1998; 28: 959-970
9. Wuestefeld T, Klein C, Streetz KL, Betz U, Lauber J, Buer J, Manns MP, Muller W, Trautwein C. Interleukin-6/glycoprotein 130-dependent pathways are protective during liver regeneration. J Biol Chem 2003; 278: 11281-11288
10. Chaisson ML, Brooking JT, Ladiges W, Tsai S, Fausto N. Hepatocyte-specific inhibition of NF-kappaB leads to apoptosis after TNF treatment, but not after partial hepatectomy. J Clin Invest 2002; 110: 190-202
11. Baldwin AS. The NF-kappaB and I kappaB proteins: new discoveries and insights. Annu Rev Immunol 1996; 14: 649-683
12. Schreck R, Rieber P, Baueuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J 1999; 18: 2247-2258
13. Bowie A, O'Neill LA. Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries. Biochem Pharmacol 2000; 59: 13-23
14. Brennan P, O'Neill LA. Effects of oxidants and antioxidants on nuclear factor kappa B activation in three different cell lines: evidence against a universal hypothesis involving oxygen radicals. Biochem Biophys Acta 1995; 1260: 167-175
15. Schreck R, Meier B, Mannel DN, Dröge W, Baeuerle PA. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. J Exp Med 1992; 175: 1181-1194
16. Pinkus R, Weiner LM, Daniel V. Role of oxidants and antioxidants in the induction of AP-1, NF-kappaB, and glutatione S-transferase gene expression. J Biol Chem 1996; 271: 13422-13429
17. Lauzurica P, Martinez-Martinez S, Marazuela M, Gómez del Arco P, Martinez C, Sánchez-Madrid F, Redondo JM. Pyrrolidine dithiocarbamate protects mice from lethal shock induced by LPS or TNF-alpha. Eur J Immunol 1999; 29:
of the stress response.

Mol Cell Biol c-Rel-p50) and PHF, a novel kappa B site-binding complex.

Bravo R, Taub R. Rapid induction in regenerating liver of and target gene transcription during liver regeneration.

pro-inflammatory cytokines..

of growth factors and cytokines in hepatic regeneration.

1995; Stat3 transcription complex in liver regeneration.

of liver regeneration by positron emission tomography.

factors associated with liver regeneration in different rat surgical models.

Horsmans Y. Expression of presumed specific early and late genes during sepsis in rats.

M, Obled C. Cytokine modulation by PX differently affects specific acute phase proteins during sepsis in rats.

Rowlands BJ, Boston VE. Modulation of TNF alpha and IL-6 transcription.

JA. Pentoxifylline suppression of tumor necrosis factor gene expression on lipopolysaccharide (LPS) fever, plasma interleukin 6 (IL 6), and tumor necrosis factor (TNF) in the rat. Cytokine 1990; 2: 300-306

Doherty GM, Jensen JC, Alexander HR, Buresh CM, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription. Surgery 1991; 110: 192-198

Refsum SE, Halliday MJ, Campbell G, McCaig M, Rowlands BJ, Boston VE. Modulation of TNF alpha and IL-6 in a periportalitis model using pentoxifylline. J Pediatr Surg 1996; 31: 928-930

Voisin L, Breuillé D, Ruot B, Rallière C, Rambourdin F, Dalle M, Obled C. Cytokine modulation by PX differently affects specific acute phase proteins during sepsis in rats. Am J Physiol 1998; 275. R1412-R1419

Nelson JL, Alexander JW, Mao JX, Vohs T, Ogle CK. Effect of growth factors and cytokines in hepatic regeneration.

Pentoxifylline suppression of tumor necrosis factor in the rat. Cytokine 1990; 2: 300-306

Doherty GM, Jensen JC, Alexander HR, Buresh CM, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription. Surgery 1991; 110: 192-198

the presence of a novel complex of NF-kappaB component of NF-kappa B.

Nature 1995; 275: G173-G183

Leu JJ, Crisoe MA, Leu JP, Cibello G, Taub R. Interleukin-6-deficient STAT3 and AP-1 amplify hepocyte nuclear factor 1-mediated transactivation of hepatic genes, an adaptive response to liver injury. Mol Cell Biol 2001; 21: 414-424

Grilli M, Chiu JJ, Lenardo MJ. NF-kappa B and Rel: participants in a multiformal transcriptional regulatory system. Int Rev Cytol 1993; 143: 1-62

Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998; 16: 225-260

Sakamoto T, Liu Z, Murase N, Ezure T, Yokomuro S, Poli V, Demetris AJ. Mitosis and apoptosis in the liver of interleukin-6-deficient mice after partial hepatectomy. Hepatology 1999; 29: 403-411

Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 1994; 12: 141-179

Fujita J, Marino MW, Wada H, Junghbluth AA, Mackrell PJ, Rivadeneira DE, Stapleton PP, Daly JM. Effect of TNF gene deletion on liver regeneration after partial hepatectomy in mice. Surgery 2001; 129: 48-54

DeAngelis RA, Kovalovich K, Cressman DE, Taub R. Normal liver regeneration in p50/nuclear factor kappaB1 knockout mice. Hepatology 2001; 33: 915-924

Cressman DE, Greenbaum LE, DeAngelis RA, Cibello G, Furth EE, Poli V, Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 1996; 274: 1379-1383

Bucher NL, Scheck TR, Moolten FL. An experimental view of hepatic regeneration. Johns Hopkins Med J 1969; 125: 250-257

Laurent S, Stärkel P, Starkel P, Leclercq IA, Lambotte L, Maiter D, Horsmans Y. Molecular events associated with accelerated proliferative response in rat livers when partial hepatectomy is preceded by a sham operation. Eur J Clin Invest 2005; 35: 140-147

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