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ORIGINAL ARTICLE

Stimulation of oval cell and hepatocyte proliferation by exogenous bombesin and neurotensin in partially hepatectomized rats

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Abstract

AIM: To investigate the effect of the neuropeptides bombesin (BBS) and neurotensin (NT) on oval cell proliferation in partially hepatectomized rats not pretreated with a known hepatocyte inhibitor.

METHODS: Seventy male Wistar rats were randomly divided into five groups: Ⅰ = controls, Ⅱ = sham operated, Ⅲ = partial hepatectomy 70% (PHx), Ⅳ = PHx + BBS (30 μg/kg per day), Ⅴ = PHx + NT (300 μg/kg per day). Forty eight hours after liver resection, portal endotoxin levels and hepatic glutathione redox state were determined. α-fetoprotein (AFP) mRNA (*in situ* hybridisation), cytokeratin-19 and Ki67 antigen expression (immunohistochemistry) and apoptosis (TUNEL) were evaluated on liver tissue samples. Cells with morphological features of oval cells that were cytokeratin-19 (+) and AFP mRNA (+) were scored in morphometric analysis and their proliferation was recorded. In addition, the proliferation and apoptotic rates of hepatocytes were determined.

RESULTS: In the control and sham operated groups, oval cells were significantly less compared to groups Ⅲ, Ⅳ and Ⅴ ($P < 0.001$). The neuropeptides BBS and NT significantly increased the proliferation of oval cells compared to group Ⅲ ($P < 0.001$). In addition, BBS and NT induced a significant increase of hepatocyte proliferation ($P < 0.001$), whereas it decreased their apoptotic activity ($P < 0.001$) compared to group Ⅲ. BBS and NT significantly decreased portal endotoxemia ($P < 0.001$) and increased the hepatic GSH: GSSG ratio ($P < 0.05$ and $P < 0.001$, respectively) compared to group Ⅲ.

CONCLUSION: BBS and NT stimulated oval cell proliferation in a model of liver regeneration, without use of concomitant suppression of hepatocyte proliferation as oval cell activation stimuli, and improved the hepatocyte regenerative response. This peptides-induced combined stimulation of oval cell and hepatocyte proliferation might serve as a possible treatment modality for several liver diseases.

Key words: Liver regeneration; Partial hepatectomy; Hepatic progenitor cells; Oval cells; Apoptosis; Proliferation; Oxidative stress

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INTRODUCTION

The efficiency of the regenerative response of human liver is of major clinical importance for patients' outcome in a number of diverse clinical conditions. When liver damage occurs, it is always followed by regeneration of the organ, which is mainly mediated by proliferation of the non-damaged mature hepatocytes[1,2]. When proliferation of the mature hepatocytes is suppressed, the majority of regeneration is carried out by oval cells, which have the capacity to differentiate into biliary epithelial cells or hepatocytes replacing the lost liver parenchyma[2-5]. Rat oval cells are frequently referred to as equivalent to hepatic progenitor cells in humans[3].

The hepatocyte is the most efficient cell for liver repopulation after injury; however, oval cells participate, possibly as an amplifying transit compartment for hepatocyte differentiation, in processes in which hepatocytes do not respond quickly enough or are unable to respond to proliferative stimuli[6]. Therefore, improving the efficiency of the regenerative response of liver progenitor cells might have a substantial clinical impact, especially in cases of coexisting inhibition of mature hepatocyte proliferation, such as in viral hepatitis[7], chemical toxicity[8] and obstructive cholestasis[9]. For therapeutic application, a non-toxic activation of this stem cell compartment would have been required. Up until now, most experimental trials of pharmaceutical expansion of oval cell compartment have been conducted in models of mature hepatocyte proliferation inhibition[10-12]. Recent studies by our group have demonstrated that oval cells may also proliferate in a model of experimental liver cirrhosis, even in the absence of pretreatment with a known hepatocyte inhibitor[13].

Bombesin (BBS) and neurotensin (NT) are neuropeptides with a wide spectrum of actions on the gut-liver axis, influencing bile acid secretion, enterohepatic circulation, intestinal motility, blood flow, secretion, nutrient absorption and immune response[14-19]. These agents activate diverse intracellular signals in hepatocytes, including induction of mitogenic, antioxidant and metabolic responses[20-23], and confer protection against liver injury and oxidative stress[21,22,24,25]. In our previous studies, we have shown that these neuropeptides reduce hepatic oxidative stress after partial hepatectomy (PHx) and improve the regenerative response of the cholestatic liver in rats[24,26].

This study was undertaken to investigate the possible effectiveness of BBS and NT as a pharmacological intervention for induction of oval cell proliferation in a widely applied experimental model of liver regeneration (PHx) without pretreatment with a known hepatocyte inhibitor.

MATERIALS AND METHODS

Animals

Seventy male albino Wistar rats, weighing 250-320 g, were used in the study. They were housed in stainless-steel cages, three rats per cage, under controlled temperature (23℃) and humidity conditions, with 12 h dark/light cycles, and maintained on standard laboratory diet with tap water ad libitum throughout the experiment, except for an overnight fast before surgery.

The experiments were carried out according to international standards on animal welfare (86/609/EEC) and to the guidelines of the Ethics Committee of Patras University Hospital. The study was approved by the local ethics committee.

Experimental design

Animals were divided randomly into five groups: group Ⅰ (n = 10): non-operated controls; group Ⅱ (n = 15): sham operated; group Ⅲ (n = 15): PHx (70%); group Ⅳ (n = 15): PHx and BBS administration; group Ⅴ (n = 15): PHx and NT administration.

Starting on day 0, the animals of groups Ⅳ and Ⅴ were treated daily with BBS (10 μg/kg, subcutaneously, three times a day) and NT (300 μg/kg, intraperitoneally, once a day) respectively, while the animals of groups Ⅰ, Ⅱ and Ⅲ were divided to receive daily either three subcutaneous or one intraperitoneal injection of 0.5 mL normal saline. Previous studies have shown that the route of saline administration does not affect the results[24]. On the 8th day, animals from groups Ⅲ, Ⅳ and Ⅴ underwent laparotomy and PHx (almost 70%) as described by Higgins and Andersson[27], while animals in group Ⅱ underwent laparotomy and mobilization of the liver. The abdominal incision was closed in two layers with chromic 4-0 cat gut and 4-0 silk. All surgical procedures were performed under strict sterile conditions, using light ether anesthesia. Administration of BBS, NT and normal saline was continued for 48 h after surgery. On the 10th day, all animals were operated (group Ⅰ) or reoperated on (groups Ⅱ, Ⅲ, Ⅳ and Ⅴ), again under strict sterile conditions. Samples were obtained according to the experimental protocol, after which the rats were sacrificed by exsanguination.

Peptides preparation

A stock solution of BBS (Sigma Chemical Co., St. Louis, Missouri, United States) was prepared by first dissolving the amount of peptide needed for the study in 1 mL sterile water containing 0.1% (w/v) bovine serum albumin and then diluted with normal saline containing 1%

(w/v) bovine serum albumin to a concentration of 3.5 μg BBS/0.1 mL. This solution was divided into equal aliquots of 0.1 mL that were stored in plastic tubes at -20℃. At the time of administration, a volume corresponding to a dose of 10 μg BBS/kg body weight was taken from each aliquot and was further diluted with sterile saline to a final volume of 0.5 mL that was injected subcutaneously three times daily. Selection of dose and route of administration was based on previous reports[24].

A stock solution of NT (Sigma Chemical Co., St. Louis, Missouri, United States) was prepared by first dissolving the amount of peptide needed for the study in 1 mL sterile water containing 0.1% (w/v) bovine serum albumin and then diluted with normal saline containing 0.1% (w/v) bovine serum albumin to a concentration of 100 μg NT/0.1 mL. This solution was divided into equal aliquots of 0.1 mL that were stored in glass vials at -20℃. At the time of administration, a volume corresponding to a dose of 300 μg NT/kg body weight was taken from each aliquot and was further diluted with sterile saline to a final volume of 0.5 mL that was injected intraperitoneally once daily. Selection of dose and route of administration was based on previous reports[24].

Portal endotoxin measurements

For the determination of endotoxin concentrations in the portal vein, a laparotomy was performed in all groups, the portal vein was punctured and samples of 1 mL of blood were obtained. Endotoxin concentration was determined by the Limulus Amebocyte Lysate test (LAL, QCL-1000, Lonza, Walkersville, MD, United States) according to the manufacturer's instructions.

Determination of glutathione redox state

After laparotomy, a tissue sample of the liver of each animal was excised, washed in 9 g/L of NaCl and homogenized in sodium phosphate buffer 10 mmol/L, pH = 7.2 (containing 1 mmol/L ethylenediaminetetraacetic acid and 1 mmol/L butylated hydroxyanisole in 0.15% ethanol) by liquid nitrogen for the determination of glutathione redox state. Reduced glutathione was determined spectrophotometrically using Elman's reagent (DTNB) and oxidized glutathione (GSSG) was quantitated by a standard enzymic assay, as described previously[24].

Pathological analysis

***In situ* hybridization for α-fetoprotein expression in paraffin sections:** For the detection of α-fetoprotein (AFP) mRNA (oval cell phenotype), a standard non-radioactive *in situ* hybridization method (ISH) was performed on paraffin sections, as described elsewhere[13]. The Hybridization/Detection Complete System (MBI, Rockville, MD) and the digoxigenin (DIG)-labeled riboprobe for AFP subunit-1 in a 10-fold dilution in hybridization solution were used. Paraffin sections of embryonic rat liver tissue were used as a positive control. To confirm that the positive stain was specific, the slides were processed in an identical way and hybridized with probes known to be complementary to sequences in the test sections (rat genomic DNA probes) (positive control probes). These probes (biotynilated oligonucleotide probes) were similar in length and GC content to AFP probe. For negative control purposes, the slides were processed in the same way but hybridized with heterologous probes. The latter were not complementary to any sequence in the test tissues. These negative control probes were similar in length and GC content to AFP probe.

Immunohistochemistry for the detection of CK19 and Ki67 proteins in paraffin sections: The detection of CK19 protein expression (oval cell phenotype)[5,13] and Ki67 expression (proliferation marker) relied on immunohistochemistry based on a streptavidin biotin peroxidase method (ImmunoCruz™ Staining systems sc-2053; Santa Cruz Biotechnology, Santa Cruz, CA). Briefly, 4-μm thick sections were dewaxed in xylene and hydrated through graded concentrations of alcohol. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 15 min. Sections were then processed in a microwave oven twice for 5 min each time at high power, and subsequently stained with anti-CK19 [goat polyclonal (sc-33119) (Santa Cruz Biotechnology, Santa Cruz, CA) in a dilution of 1:150] and anti-Ki67 [goat polyclonal antibody (M-19) (sc-7846) (Santa Cruz Biotechnology, Santa Cruz, CA) in a dilution of 1:100]. All incubations were performed for 30 min at room temperature. Between the steps, sections were washed in TBS. Diaminobenzidine (Sigma Fast DAB tablets-D-4293, St Louis, MO) was used as the chromogen. Cytoplasmic staining for CK19 and nuclear staining for Ki67 were considered as positive. For negative control purposes, the same streptavidin-biotin technique was used on tissue sections in which 1% BSA in PBS was substituted for the primary antibody.

***In situ* labeling of fragmented DNA for the detection of apoptotic cells:** On paraffin sections, a standard terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick-end labeling (TUNEL) method was employed to detect the fragmented nuclear DNA associated with apoptosis[9]. For this purpose, the *In Situ* Cell Death Detection Kit, POD (Roche, United States) was used according to the manufacturer's instructions. After standard deparaffinization, hydration, incubation with proteinase K and blocking of endogenous peroxidase, tissue sections were incubated: (1) with TdT and DIG-dUTP (TUNEL reaction mixture) at 37℃ for 60 min; and (2) with peroxidase converter anti-fluorescein antibody at 37℃ for 30 min. Diaminobenzidine (Sigma Fast DAB tablets, D-4293, Sigma St. Louis, MO, United States) was used as the chromogen. For physiological positive controls, sections of rat small intestine were subjected to the same procedure. For negative controls, some slides were incubated with label solution that did not contain TdT.

Morphometric analysis: (1) Oval cell measurement: morphometric analysis for the evaluation of oval cell

Table 1 Portal Endotoxin concentrations and hepatic glutathione redox state (mean ± SD)

Markers	Control (Ⅰ) (*n* = 10)	Sham (Ⅱ) (*n* = 15)	PHx (Ⅲ) (*n* = 15)	PHx + BBS (Ⅳ) (*n* = 15)	PHx + NT (Ⅴ) (*n* = 15)
Endotoxin (EU/mL)	0.41 ± 0.07	0.40 ± 0.10	2.45 ± 0.62[a]	1.36 ± 0.53[b]	1.59 ± 0.44[b]
GSH: GSSG	11.55 ± 3.40	12.02 ± 2.85	20.43 ± 4.64[a]	25.88 ± 5.27[c]	28.69 ± 5.94[b]

[a]$P < 0.001$ *vs* sham; [b]$P < 0.001$ *vs* partial hepatectomy (PHx); [c]$P < 0.05$ *vs* PHx. BBS: Bombesin; NT: Neurotensin. GSH: Reduced glutathione hormone; GSSG: Oxidized glutathione.

presence was performed as described previously[13]. Briefly, sections were screened at low power and areas with increased oval cell staining were determined. Cells were scored when they fulfilled the morphological criteria for oval cells (small cells with ovoid nuclei and scant cytoplasm) and expressed AFP mRNA, cytoplasmic protein CK19 and/or nuclear protein Ki67. Cell counts were performed manually at a × 400 magnification (high power field, HPF) using a 10 × 10-microscope grid. The number of oval cells was determined by visual inspection of five non-overlapping different fields per section. The variance in oval cell counts from section to section in the same biopsy was < 10%. The average of these scores was then taken; and (2) Evaluation of proliferation and apoptosis in hepatocytes: immunohistochemical results regarding Ki67 expression and ISH results (TUNEL+ cells) were estimated for hepatocytes. All $Ki67^+$ hepatocytes were considered proliferating cells. Regarding apoptosis, in order to avoid its overestimation by the TUNEL method, hepatocytes were considered apoptotic only if, in addition to positive TUNEL stain, they displayed the morphological features of apoptosis on light microscopy (cytoplasmic fragmentation and nuclear condensation) and were not "surrounded" by inflammatory elements. Estimation of proliferation and apoptosis in each case was performed by a stereological method. Specifically, sections from each liver biopsy were viewed through a light microscope with × 40 flat field objective. A square lattice of 100 points with a total surface area of 0.064 mm^2 was superimposed onto the tissue. Data were collected from a series of randomly selected 15 adjacent fields extending throughout the biopsy. For each field a percentage value for each parameter ($Ki67^+$ cells, apoptotic cells) was obtained by dividing the points falling on stained tissue by the total number of measured points. Also, for each field, the ratio of the obtained values (% $Ki67^+$ cells/% apoptotic cells) was calculated as a balancing index expressing net cell turnover. It should be noted that the variance in cell counts from field to field in the same section was < 10%. The average of these scores was then taken and expressed as proliferation and apoptotic indexes and proliferation/apoptosis ratio respectively for each case.

Statistical analysis

Data were analyzed using the SPSS statistical package (SPSS Inc., 2001, Release 11.0.0, United States). In groups Ⅰ, Ⅱ and Ⅲ, data obtained from subcutaneously and intraperitoneally saline-treated rats were pooled, as there was no significant difference between differentially injected animals for all parameters studied. Results are expressed as mean (SD). Comparisons among multiple groups were performed using the one-way ANOVA, followed by Bonferroni's post hoc test when variances across groups were equal or by Dunnette's T3 post hoc test when variances were not equal. Variance equality was tested by Levene statistical analysis. In all cases, differences were considered significant when $P < 0.05$.

RESULTS

Portal endotoxin concentration

Hepatectomized animals (group Ⅲ) presented significantly elevated endotoxin concentrations in portal blood compared with groups Ⅰ and Ⅱ ($P < 0.001$). Treatment with BBS or NT led to significantly lower endotoxin values in portal vein in groups Ⅳ and Ⅴ ($P < 0.001$ *vs* group Ⅲ, respectively) (Table 1).

Hepatic glutathione redox state

Evaluation of glutathione redox state showed significantly increased levels of reduced glutathione hormone (GSH):GSSG in hepatectomized rats of group Ⅲ ($P < 0.001$ *vs* groups Ⅰ and Ⅱ). Administration of BBS or NT resulted in further increase of GSH:GSSG ratio in groups Ⅳ and Ⅴ ($P < 0.05$ and $P < 0.001$ *vs* group Ⅲ, respectively) (Table 1).

Oval cell detection and proliferation

Oval cells were present in all specimens studied. In the control and sham operated groups, oval cells were significantly less compared to groups Ⅲ, Ⅳ and Ⅴ ($P < 0.001$, Table 2). In PHx rats (group Ⅲ) they were located in periportal areas and the formation of small ducts was occasionally recorded. Oval cells expressed CK19 (Figure 1A and B), AFP mRNA (Figure 1C and D) and Ki67. When rats subjected to PHx were treated with either BBS (group Ⅳ) or NT (group Ⅴ), the levels of expression of all three molecules were significantly increased compared to group Ⅲ ($P < 0.001$, Table 2).

Proliferation and apoptosis detection in hepatocytes

The proliferation index of hepatocytes was significantly higher in group Ⅲ as compared to groups Ⅰ and Ⅱ ($P < 0.001$, respectively). Administration of BBS or NT in PHx rats induced a significant increase of hepatocyte proliferation in groups Ⅳ and Ⅴ compared to group Ⅲ ($P < 0.001$, respectively, Table 3) (Figure 2A and B).

Table 2 Morphometric analysis of oval cell presence and proliferation (marker-positive oval cells per high power field) (mean ± SD)

Markers	Control (Ⅰ) (*n* = 10)	Sham (Ⅱ) (*n* = 15)	PHx (Ⅲ) (*n* = 15)	PHx + BBS (Ⅳ) (*n* = 15)	PHx + NT (Ⅴ) (*n* = 15)
CK19 protein	1.65 ± 0.31	1.63 ± 0.28	6.22 ± 0.82[a]	18.51 ± 2.31[b]	19.37 ± 3.48[b]
AFP mRNA	1.34 ± 0.25	1.31 ± 0.21	5.45 ± 0.91[a]	16.32 ± 1.81[b]	17.53 ± 4.12[b]
Ki67 protein	1.28 ± 0.14	1.26 ± 0.11	5.22 ± 0.11[a]	15.61 ± 2.54[b]	16.64 ± 3.59[b]

[a]$P < 0.001$ *vs* sham; [b]$P < 0.001$ *vs* partial hepatectomy (PHx). BBS: Bombesin; NT: Neurotensin; AFP: α-fetoprotein.

Table 3 Hepatocytes' proliferation and apoptosis (mean ± SD)

Markers	Control (Ⅰ) (*n* = 10)	Sham (Ⅱ) (*n* = 15)	PHx (Ⅲ) (*n* = 15)	PHx + BBS (Ⅳ) (*n* = 15)	PHx + NT (Ⅴ) (*n* = 15)
Proliferation index	5.95 ± 1.33	6.12 ± 1.64	18.32 ± 3.11[a]	27.25 ± 4.13[b]	25.62 ± 3.41[b]
Apoptotic index	0	0	19.31 ± 4.16[a]	9.24 ± 2.65[b]	8.97 ± 4.14[b]
Proliferation/apoptosis	-	-	0.94 ± 0.74	2.99 ± 1.55[b]	2.85 ± 0.82[b]

[a]$P < 0.001$ *vs* sham; [b]$P < 0.001$ *vs* partial hepatectomy (PHx). BBS: Bombesin; NT: Neurotensin.

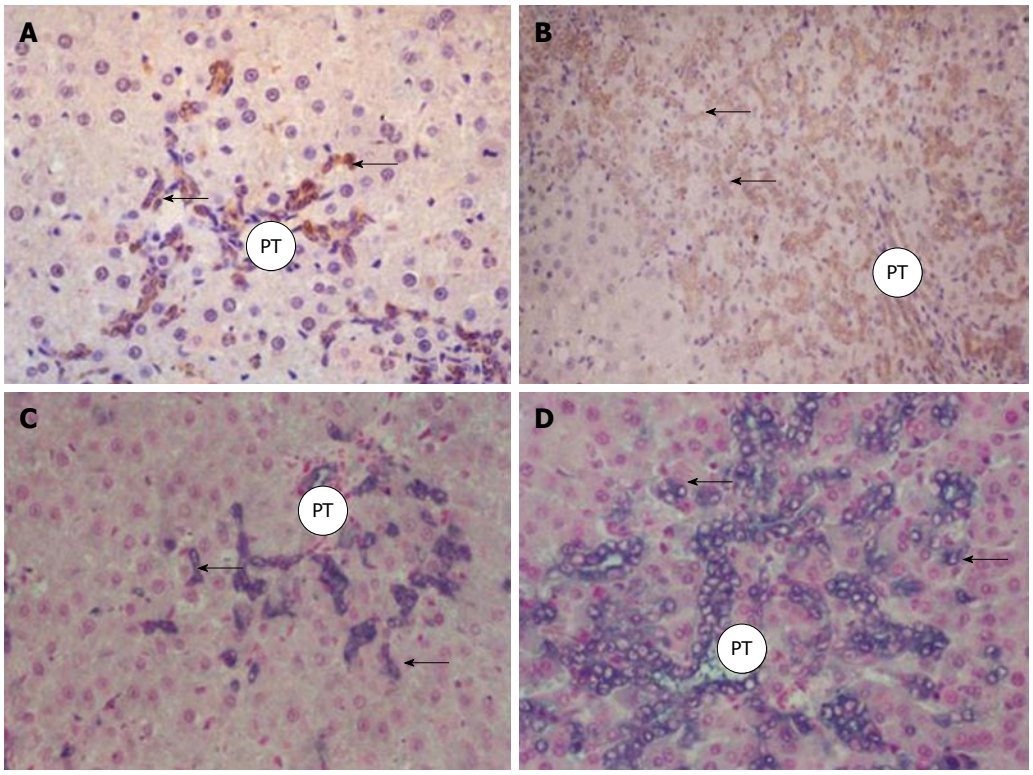

Figure 1 Microphotographs showing expression of CK19 from oval cells (arrows) in livers of group Ⅲ (A) and group Ⅳ (B) and α-fetoprotein mRNA expression from oval cells (arrows) in group Ⅲ (C) and group Ⅳ (D). In group Ⅳ there is higher oval cell presence as demonstrated by both markers [CK19: streptavidin biotin peroxidase (A) × 250, (B) × 100, α-fetoprotein mRNA: *in situ* hybridization × 250]. PT: Portal tract.

In control and sham operated rats, no apoptotic bodies were detected in hepatocytes. After PHx (group Ⅲ), increased apoptotic activity was detected in lobules, whilst administration of BBS or NT significantly decreased the apoptotic index ($P < 0.001$, for groups IV and V compared with group Ⅲ, Table 3) (Figure 2C and D). The proliferation/apoptosis ratio was significantly increased in groups Ⅳ and Ⅴ compared to group Ⅲ ($P < 0.001$, respectively).

DISCUSSION

Effective liver regeneration after extended liver resection or hepatocytic necrosis is of great clinical importance and several experimental studies have focused on the pharmacological augmentation of these process. Experi-

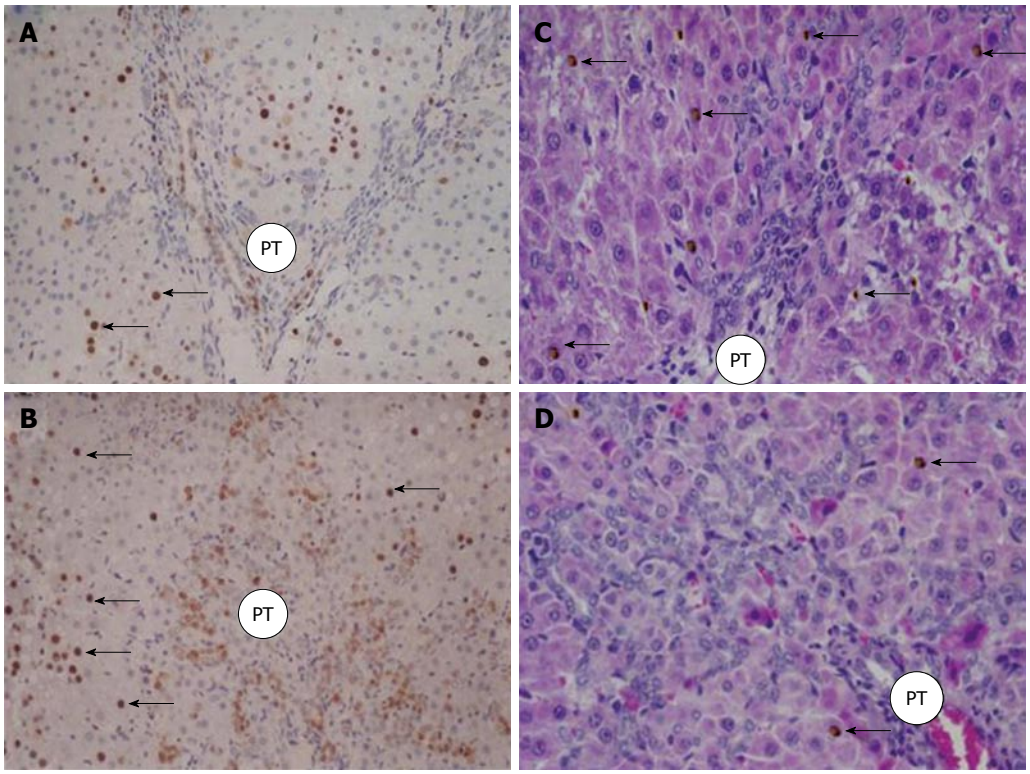

Figure 2 Microphotographs showing Ki67 (+) hepatocytes (arrows) from livers of group Ⅲ (A) and group Ⅳ (B) and TUNEL (+) hepatocytes (arrows) in group Ⅲ (C) and group Ⅳ (D). In group IV there are more Ki67 (+) hepatocytes (streptavidin biotin peroxidase × 100) and less TUNEL (+) hepatocytes (streptavidin biotin peroxidase and hematoxylin eosin × 250). PT: Portal tract.

mentally, the PHx and the carbon tetrachloride administration animal models have been widely used to simulate the clinical conditions of liver resection or hepatocytic necrosis, respectively. Previous studies have unequivocally shown that the hepatocytes are the replicating cells responsible for liver regeneration in these models and that progenitor cell activation leading to lineage generation is not observed during these processes[28]. Despite the low replicative rate of hepatocytes in the normal liver, these highly differentiated cells are not terminally differentiated and replicate in a highly regulated manner after loss of cell or tissue mass[28]. On the other hand, oval cells constitute a transit amplifying cell compartment, which is activated when hepatocyte proliferation is compromised[5,29]. The biological effect of several primary hepatocyte mitogens on hepatocytic proliferation has been extensively studied in experimental models but there are hardly any data about the influence of these compounds on oval cells[10,30,31]. Moreover, to the best of our knowledge, all *in vivo* pharmaceutical trials of oval cell compartment expansion have been conducted in conditions of mature hepatocyte proliferation inhibition, which does not allow safe conclusions on the clear effect of tested compounds on oval cell proliferation and also does not always simulate the clinical situation.

The present study evaluated the effect of the neuropeptides BBS and NT on oval cells and hepatocytes in partially hepatectomized rats, a widely applied model of liver regeneration. The experimental design did not include any treatment with known inhibitors of hepatocyte proliferation as a method for oval cell compartment activation. Therefore, we aimed at evaluating *in vivo* the potential net proliferative effect of tested peptides on oval cells for the first time. The results presented clearly demonstrate the proliferative effect of BBS and NT on oval cells in partially hepatectomized rats in conjunction with improvement of the regenerative response of mature hepatocytes evidenced by promotion of hepatocytes' proliferation and prevention of their apoptosis. Promotion of oval cell proliferation is of great clinical importance profoundly in cases of inhibition of hepatocyte proliferation as this cell type carries on the process of liver repopulation, as well as in cases of effective hepatocyte proliferation, providing a ready cell compartment to continue liver regeneration if a hepatocytic inhibitory insult arises. The presence of hepatic progenitor cells in failed livers at autopsy and in livers removed at transplantation indicates the importance of the efficiency of their regenerative response[32]. Agents that stimulate oval cell proliferation and differentiation, or transplantation of oval cells could be a potent therapeutic modality in the treatment of patients with fatal liver disease, such as fulminant hepatic failure.

In our previous studies with experimentally jaundiced rats, we have shown that BBS and NT attenuated oval cell proliferation in the cholestatic liver, inducing cell type-specific effects on oval cells, hepatocytes and cholangiocytes[26]. The findings of the present study with

promotion of oval cell proliferation suggest that oval cells' response to BBS and NT may be different depending on the type of liver injury. Taking into consideration our previous and current findings, one should assume that neuropeptides' effects on oval cells may be significant for the regeneration of a healthy liver (e.g. in living donor transplantation), but not relevant in an injured liver. However, in our previous experiments with bile duct ligated rats, BBS and NT significantly improved cholestatic liver injury, despite reduction of oval cell proliferation[26]. This finding might reflect the fact that induction of hepatocytes' regenerative response by neuropeptides' action is effective for liver repair in cholestasis, diminishing the role of oval cell participation in this process. On the other hand, in the model of liver regeneration used in the present study, the induction of hepatocytes' regenerative response by BBS and NT might be relatively insufficient for an effective liver repopulation, thus activating the transit amplifying compartment of oval cells.

The biological effects of BBS and NT on hepatic oval cells of rats subjected to PHx may result from direct receptor-mediated action[33,34]. Specific G protein-coupled receptors of BBS and NT have been previously identified in hepatocytes and cholangiocytes[33-35]. Similarly G protein-coupled receptors have been identified in oval cells as well, and their expression has been interrelated with the activation with this stem cell compartment[36]. The growth pathways that govern activation and liver differentiation of liver stem cells after PHx are quite complex and not fully elucidated, involving interplay of diverse cytokines and growth factors such as the hepatocyte growth factor (HGF) and the transforming growth factor (TGF)-β[37]. Although a very rapid increase in tumor necrosis factor-α levels after hepatectomy (possibly endotoxin-induced) is considered as the first step in activation of these growth factors[38], reduction of portal endotoxemia by BBS and NT, demonstrated in the present study, does not preclude the activation of oval cells *via* diverse HGF and TGF dependent pathways. In addition, an indirect mechanism of action of BBS and NT on oval cells in the regenerating liver could exist through the action of other gastrointestinal or systemic hormones released in response to these neuropeptides[39-44].

The present study also demonstrated that BBS and NT enhanced hepatocytes' regenerative response, attributed to increased proliferation and decreased apoptosis of mature hepatocytes and theoretically to increased transition of oval cells to mature hepatocytes. Estimation of hepatocytes' proliferation/apoptosis ratio showed that BBS and NT induced a threefold net increase in proliferating over apoptotic hepatocytes in the regenerating liver. Hepatocytes' apoptosis, which is a major factor of a defective regenerative response, could have been attenuated by neuropeptides administration, either through a direct receptor-mediated mechanism or indirectly through reduction of hepatic oxidative stress and portal endotoxemia shown in the present study, with mechanisms previously reported[24,45]. According to the present and our previous results, liver regeneration takes place under low oxidative stress conditions; however, the further attenuation of hepatic oxidative stress induced by BBS and NT might contribute to the augmentation of the hepatocytes' regenerative response[24].

In conclusion, the present study demonstrates that the neuropeptides BBS and NT exert a net proliferative effect on oval cells in a model of liver regeneration without use of concomitant suppression of hepatocyte proliferation as oval cell activation stimuli. Concurrently, these factors promote hepatocyte proliferation and prevent its apoptosis, thus improving the hepatic regenerative response. Although the results from animal studies should be transferred with much caution in clinical practice, we feel that there is an emerging need for further evaluation of our findings, as the observed pharmacological combined stimulation of hepatocyte and oval cell proliferation might serve as a possible treatment modality for several liver diseases.

COMMENTS

Background

The regenerative response of human liver is of major clinical importance for patients' outcome in a number of diverse clinical conditions. The hepatocyte is the most efficient cell for liver repopulation after injury; however, oval cells participate, possibly as an amplifying transit compartment for hepatocyte differentiation, in processes in which hepatocytes do not respond quickly enough or are unable to respond to proliferative stimuli.

Research frontiers

Pharmacological augmentation of the hepatic regenerative response in diverse types of liver injury has been the topic of intense research for several decades. Improving the efficiency of the regenerative response of liver progenitor cells might have a substantial clinical impact, especially in cases of coexisting inhibition of mature hepatocyte proliferation, such as in viral hepatitis, chemical toxicity and obstructive cholestasis. Up until now, most experimental trials of pharmaceutical expansion of oval cell compartment have been conducted in animal models of mature hepatocyte proliferation inhibition by toxic chemical compounds. However, for therapeutic application a non-toxic activation of this stem cell compartment is required.

Innovations and breakthroughs

The present study evaluated the effect of the neuropeptides bombesin (BBS) and neurotensin (NT) on oval cells and hepatocytes in partially hepatectomized rats, a widely applied model of liver regeneration. The experimental design did not include any treatment with known inhibitors of hepatocyte proliferation as a method for oval cell compartment activation. Therefore, we aimed at evaluating *in vivo* the potential net proliferative effect of the tested peptides on oval cells for the first time. The results presented clearly demonstrate the proliferative effect of BBS and NT on oval cells in partially hepatectomized rats in conjunction with improvement of the regenerative response of mature hepatocytes, evidenced by promotion of hepatocytes' proliferation and prevention of their apoptosis.

Applications

Promotion of oval cell proliferation is of great clinical importance profoundly in cases of inhibition of hepatocyte proliferation as this cell type carries on the process of liver repopulation, as well as in cases of effective hepatocyte proliferation, providing a ready cell compartment to continue liver regeneration if a hepatocytic inhibitory insult arises. BBS and NT promoting oval cell and hepatocyte proliferation could be a potent therapeutic modality in the treatment of patients with several liver diseases, such as fulminant hepatic failure.

Peer review

This is an interesting observational study. The concept is new, the results are robust and may provide potential target for clinical patient care.

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