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The Role of Hepatic Stimulator Substance (HSS) on Liver Regeneration Arrest Induced by Cadmium

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Abstract. *Background: The mechanism of cadmium-induced liver regeneration arrest in relation to hepatic stimulator substance (HSS) biological activity was investigated. Materials and Methods: In Wistar rats subjected to 65-70% partial hepatectomy, saline, cadmium, cadmium and HSS were administered. The rats were also subjected to 30-34% partial hepatectomy. Mitotic index, immunochemistry for PCNA, ^{3}[H]-thymidine incorporation into DNA and thymidine kinase activity were used as indices of liver regeneration. HSS biological activity was evaluated in all groups of rats using a bioassay. Results: Liver regeneration and HSS activity were arrested by cadmium during the first 24 h after partial hepatectomy. Both in normal and in cadmium-treated rats, the HSS activity was increased and liver regeneration coincided. HSS activity was stable in 30-34% hepatectomized rats. HSS administration was able to restore liver regeneration arrest induced by cadmium. Conclusion: The biological activity of HSS increased at the time of G1/S transition of hepatocytes in the cell cycle and no increase was observed with asynchronous G1/S transition (30-34% partial hepatectomy). The suppression of HSS biological activity by cadmium seems to represent an important factor for liver regeneration arrest induced by the metal and HSS administration is able to restore liver regeneration.*

Liver regeneration after surgical removal of a portion of liver tissue or toxic injury is a highly regulated and precisely orchestrated process involving sequential proliferation of different cell types while the organ preserves its metabolic functions (1-3). This phenomenon of tissue restoration has been fervently investigated in recent years, but fundamental aspects still remain unexplained. Scientific interest in recent years has been focused on the cell cycle progression of liver cells in the regenerating liver and on the various cell cycle checkpoints that operate on a molecular level. From this point of view, hepatocytes pass through an initiating step (priming), which is characterized by the transition from G0- to G1-phase of the cell cycle and progress in G1-phase (2, 3). Priming and progression in the early G1-phase is controlled by cytokines, including TNFα and IL-6, as well as changes in the extracellular matrix (4, 5). The next transition point represents the G1/S transition and this major cell cycle checkpoint is under the control of growth factors, cyclin D1 and its cyclin-dependent kinase playing a fundamental role (6-8). Transforming Growth Factor (TGFα), Heparin Binding Epidermal Growth Factor (HB-EGF), and mainly Hepatocyte Growth Factor (HGF), according to our current knowledge, control this checkpoint and they seem to operate through activation of Ras/mitogen-activated protein kinase (MAPK). Following activation of MAPK, cyclin D1 protein is expressed, driving hepatocytes in the S-phase of the cell cycle (9, 10).

Cadmium is unique among non-essential metals for its toxic effects on several systems of the human body. It is present in water and food, being widely distributed throughout the biosphere and accumulating due to industrialization. Cadmium is able to induce renal, hepatic and testicular injury (11, 12).

Cadmium is a known inhibitor of liver regeneration. When administered 24 h prior to partial hepatectomy, cadmium inhibits the first wave of hepatocyte proliferation which normally peaks 24 h post-operatively. This effect has not been fully explained, but it has been attributed to the inhibition of thymidine kinase which is the rate-limiting enzyme of DNA biosynthesis (13-16), though the effects of the metal on intracellular and extracellular components other than thymidine kinase can not be excluded.

Hepatic Stimulator Substance (HSS) is a liver-specific, species-non-specific growth factor that acts as a progression

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factor in hepatocytes released from the G0-phase of the cell cycle, both *in vivo* and in cell cultures (17-23). The factor has the ability to augment the mitogenic effect of other growth factors, and it has been proposed to act at the G1/S cell cycle checkpoint, given that its administration abbreviates the G1-phase by 60% in already primed hepatocytes (24). Recently, the purification and cloning of a growth factor that shares common characteristics with HSS has been reported (25-27) and the peptide purified from HSS activity was renamed Augmenter of Liver Regeneration (ALR). The discrepancy between the two names remains in the bibliography and the name HSS is still used by some authors.

In the present study, we investigated the progress of liver regeneration after 65-70% and 30-34% partial hepatectomy, both in normal rats and after acute cadmium intoxication, in relation to the biological activity of HSS.

Materials and Methods

Experimental animal model. Male Wistar rats (Hellenic Pasteur Institute Athens, Greece), weighing 180-220 g, were used in this study. The animals were kept in a temperature-controlled room (22-25˚C), under 12 h of light (08.00 h - 20.00 h) and 12 h of darkness (20.00 h - 08.00 h), with free access to a commercial pelleted diet and tap water. The animals were handled with humane care in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. All experiments were conducted between 07.00 and 09.00 a.m. with the animals under light ether anesthesia (diethyl ether per anesthesia; Codex, Carlo Erba, Milan, Italy). The experimental rats were assigned randomly into five groups as follows:

Group I: administration of normal saline 24 h prior to 65-70% partial hepatectomy.

Group II: administration of cadmium chloride ($CdCl_2$) intraperitoneally at the dose of 2.5 mg/kg bodyweight 24 h prior to 65-70% partial hepatectomy.

Group III: cadmium administration and partial hepatectomy as in group II and intraperitoneal administration of HSS at the dose of 100 mg of protein/kg bodyweight 12 h after partial hepatectomy.

Group IV: cadmium administration and partial hepatectomy as in group II, and intraperitoneal administration of HSS at the dose of 100 mg of protein/kg bodyweight 16 h after partial hepatectomy.

Group V: administration of normal saline 24 h prior to 30-34% partial hepatectomy.

Subgroups of 6-8 animals from all groups were killed at 8, 16, 20, 24, 32, 40, 48, 54, 60, 72 and 96 h after partial hepatectomy *via* cardiac puncture. One hour prior to sacrifice, the animals of all groups were injected with [^{3}H]-thymidine (Amersham Corp., Buckinghamshire, UK) at the dose of 250 μCi/kg bodyweight intraperitoneally. A standard portion of the median liver lobe was used for histological evaluation and the rest was rapidly frozen in liquid nitrogen for further determinations.

Histological evaluation. A standard portion of the liver was fixed in 4% buffered formalin for 24 h. Sections 5 μm thick were processed routinely, stained with hematoxylin-eosin (HE) and analyzed for mitoses. Mitoses were counted in 10 randomly selected high-power fields (HPF) and expressed per 1000 liver cells.

The mitotic index was also evaluated by the immunochemical detection of PCNA (monoclonal mouse anti-proliferating cell nuclear antigen, Clone PC10, 1:200; DakoCytomation, Glostrup, Denmark) and expressed per 1000 liver cells.

Biochemical determinations of liver regeneration. The rate of liver regeneration was evaluated by the rate of [^{3}H]-thymidine incorporation into hepatic DNA, the enzymatic activity of liver thymidine kinase (TK), the mitotic index in HE sections and the immunochemical detection of PCNA.

Liver thymidine kinase enzymatic activity. The enzymatic activity of TK was assayed according to the method of Kahn *et al.* (28) and triplicate aliquots of each sample were tested. The protein content of each sample was determined by the method of Lowry *et al.* (29) and the enzymatic activity was expressed as cpm/min/mg.

Rate of [^{3}H]-thymidine incorporation into hepatic DNA. The animals of all groups were injected intraperitoneally with 25 μCi/100 g bodyweight of [^{3}H]-thymidine 1 h prior to sacrifice. DNA was extracted from the tissue according to the method of Munro and Fleck (30) as modified by Kyprianidis *et al.* (31). The content of tissue DNA was estimated by the method of Richards (32). The rate of [^{3}H]-thymidine incorporation into hepatic DNA was calculated from the radioactivity measured in a liquid scintillation counter (Wallac LKB 1211, Rackbeta, Sweden) and results were expressed as cpm/min/μg of DNA.

Preparation of HSS. HSS was extracted from the rat livers of Groups I, II and V according to the method of Labrecque and Pesch (18) and Fleig *et al.* (17). Briefly, a 35% (w/v) homogenate of 1 g of liver tissue was prepared. The homogenate was incubated at 95˚C for 15 min, centrifuged at 27,000 x g for 20 min at 4˚C, added with six volumes of absolute ethanol and stirred for 2 h at 4˚C. After centrifugation at 27,000 x g for 20 min, the precipitate was dissolved in water and centrifuged again at 20,000 x g for 20 min. The supernatant was collected, lyophilized and stored at –30˚C. The HSS administered to Groups III and IV rats was prepared as described above from the liver of weanling rats and was given intraperitoneally as a normal saline solution at the dose of 100 mg protein/kg bodyweight.

HSS activity assay. The assay of HSS biological activity was performed using 80-120 g male Wistar rats subjected to 30-34% partial hepatectomy by resection of the left lateral lobe of the liver. Twelve hours after partial hepatectomy, the rats were injected intraperitoneally with HSS dissolved in normal saline at the dose of 100 mg protein/kg bodyweight. The animals were killed 24 h after partial hepatectomy, and 1 h prior to sacrifice were injected with [^{3}H]-thymidine as described above. The rate of liver regeneration in the excised livers was evaluated by the [^{3}H]-thymidine incorporation into hepatic DNA.

Statistical analysis. Data were expressed as means±SE. All observations were obtained from at least six animals. The statistical analysis of the results was performed by one-way analysis of variance and unpaired Student's *t*-test.

Results

In normal partially hepatectomized rats (Group I), liver regeneration as evaluated by [^{3}H]-thymidine incorporation

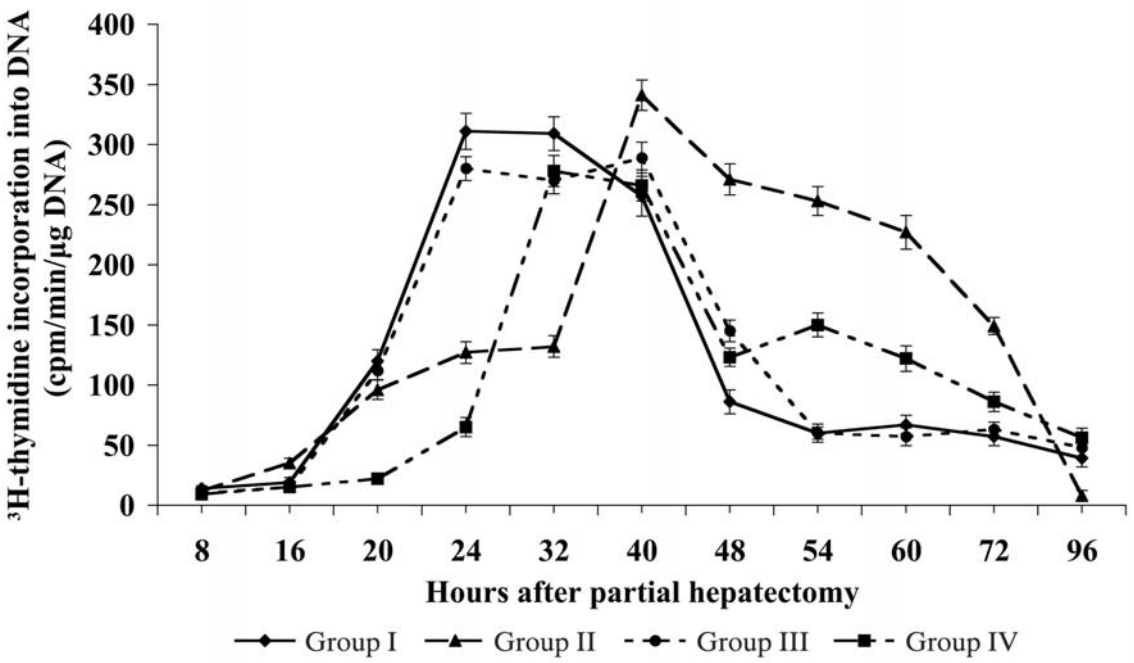


Figure 1. *Time-course of liver regeneration as evaluated by [^{3}H]-thymidine incorporation into DNA in 65-70% partially hepatectomized rats having received 24 h prior to partial hepatectomy: a) saline (Group I), b) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) (Group II), c) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 12 h after partial hepatectomy (Group III) and d) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 16 h after partial hepatectomy (Group IV). Values are expressed as means±SE. Group I vs Group II; p<0.001: 24, 32 and 48-96 h; p<0.01: 16 and 40 h. Group I vs Group III; p<0.01: 48 h; p<0.05: 32 h. Group I vs Group IV; p<0.001: 20, 24 and 54 h; p<0.01: 60 h; p<0.05: 48 and 72 h.*

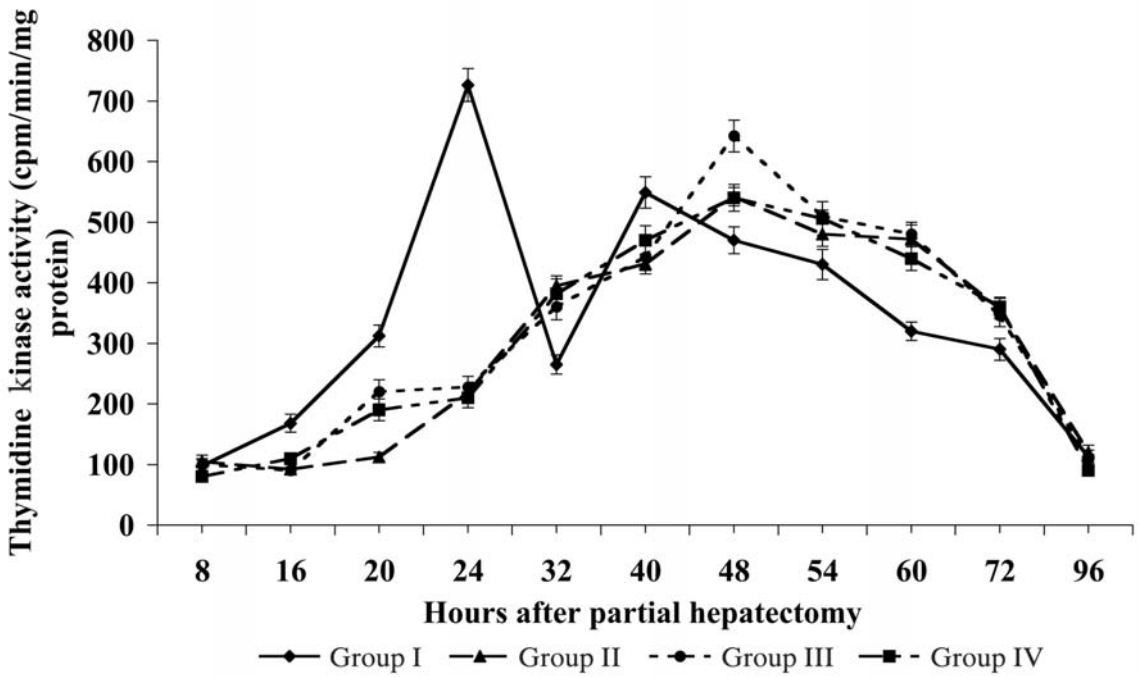


Figure 2. *Time-course of hepatic thymidine kinase (TK) enzymatic activity in 65-70% partially hepatectomized rats having received 24 h prior to partial hepatectomy: a) saline (Group I), b) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) (Group II), c) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 12 h after partial hepatectomy (Group III) and d) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 16 h after partial hepatectomy (Group IV). Values are expressed as means±SE. Group I vs Group II; p<0.001: 16-32 h and 60 h; p<0.01: 40 h; p<0.05: 48 and 72 h. Group I vs Group III; p<0.001: 16, 24, 48 and 60 h; p<0.01: 20, 32 and 40 h; p<0.05: 54 h. Group I vs Group IV; p<0.001: 20, 24 and 60 h; p<0.01: 16 and 32 h; p<0.05: 40, 48 and 72 h.*

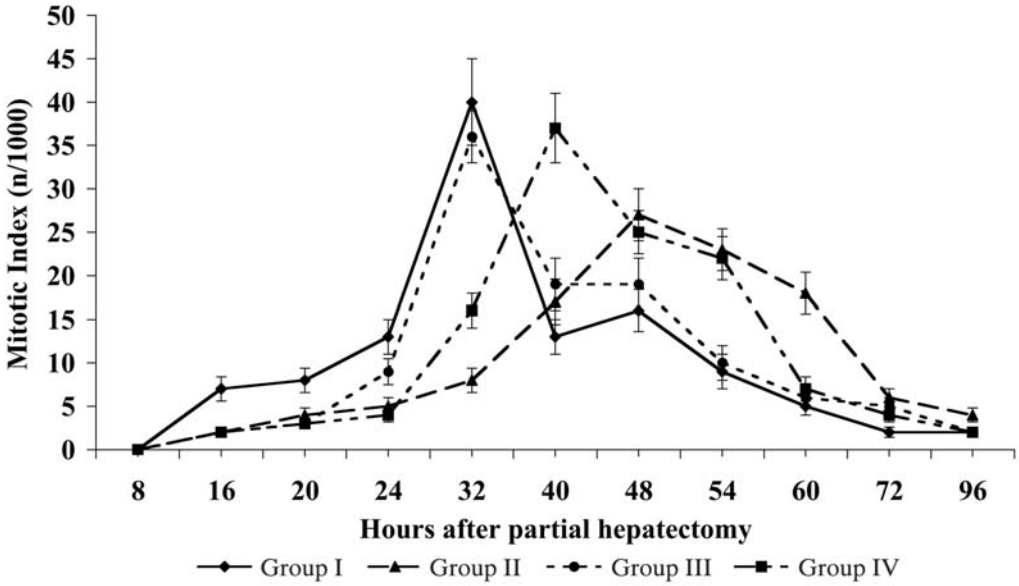


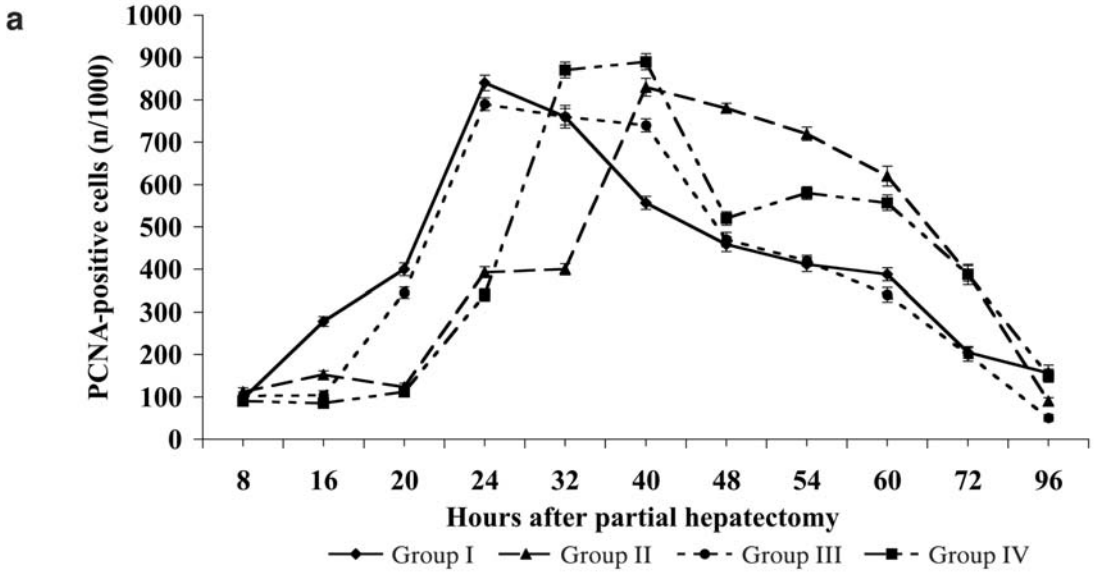
Figure 3. *Time-course of mitotic index (HE sections) in 65-70% partially hepatectomized rats having received 24 h prior to partial hepatectomy: a) saline (Group I), b) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) (Group II), c) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 12 h after partial hepatectomy (Group III) and d) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 16 h after partial hepatectomy (Group IV). Values are expressed as means±SE. Group I vs Group II; $p<0.001$: 32, 54 and 60 h; $p<0.01$: 24 and 72 h; $p<0.05$: 16, 20 and 48 h. Group I vs Group III; $p<0.01$: 20 h; $p<0.05$: 16 and 72 h. Group I vs Group IV; $p<0.001$: 40 h; $p<0.01$: 20, 32 and 54 h; $p<0.05$: 16, 24 and 48 h.*

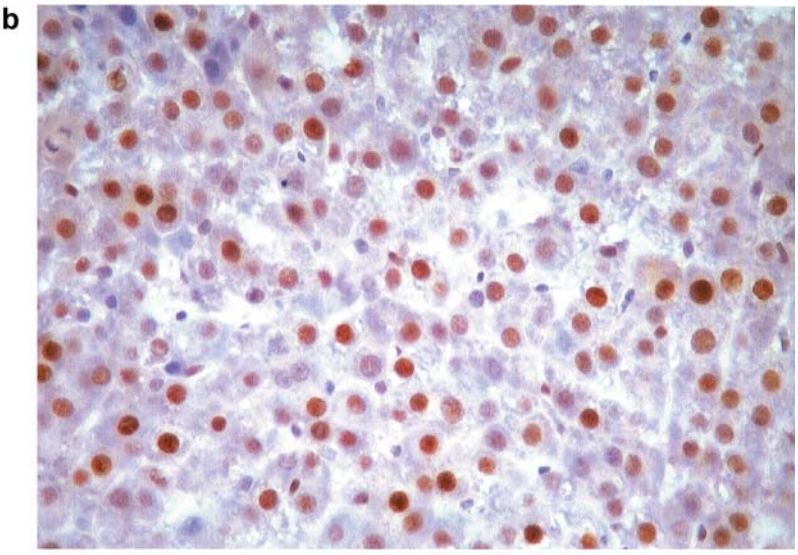
into hepatic DNA started increasing at 16 h and peaked at 24 and 32 h after partial hepatectomy, before starting to decline, with low levels being observed after 48 h (Figure1). In cadmium-treated rats (Group II), low rates were observed during the first 16 h after partial hepatectomy, the regenerative activity starting to increase at 16 h, peaked at 40 h and remained at high levels until 60 h, when it abruptly decreased thereafter (Figure 1). In Group III rats, the time profile of liver regenerative activity as evaluated by [^{3}H]-thymidine incorporation into hepatic DNA was almost identical to that of Group I, with small deviations at 24, 32 and 48 h (Figure 1). In Group IV rats, [^{3}H]-thymidine incorporation into hepatic DNA started increasing at 20 h and peaked at 32 and 40 h after partial hepatectomy, while the time profile of liver regeneration was similar to that in Groups I and III, with a lag-period of 8 h (Figure 1).

Liver TK activity peaked at 24 and 40 h in Group I. Cadmium administration in Group II rats inhibited liver TK activity for the first 20 h after partial hepatectomy, the activity then gradually increasing and remaining at relatively high levels between 32 and 72 h after partial hepatectomy. HSS administration at 12 and 16 h in Groups III and IV of rats did not alter the time profile of liver TK activity, which showed a similar time pattern to Group II (Figure 2).

The mitotic index, as evaluated in HE sections, started to increase at 24 h for Group I rats and was maximal at 32 h with a second lower peak at 48 h (Figure 3). In Group II rats, the index was low for the first 24 h after partial hepatectomy, started increasing thereafter and was maximal at 48 h, and, in general, remained at relatively high levels between 40 and 60 h after partial hepatectomy (Figure 3). In Group III rats, the mitotic index showed the same time profile as in Group I (Figure 3). The mitotic index started increasing at 24 h in Group IV and was maximal at 40 h, with high levels at 48 and 54 h after partial hepatectomy (Figure 3).

The immunochemical detection of PCNA nuclear antigen showed a sudden increase in the number of positive cells between 16 and 24 h after partial hepatectomy in normal partially hepatectomized rats (Group I); the percentage of positive cells peaked at 24 h and gradually decreased thereafter until 96 h after partial hepatectomy (Figure 4a, Figure 4b). In Group II, the number of positive cells was at low levels during the first 20 h; the percentage of PCNA-positive cells started increasing between 20 and 40 h where it was maximal and gradually decreased thereafter (Figure 4a and 4c). In Group III rats, the time-pattern of PCNA-positive cells was similar to that of Group I, with the exception of 16 and 40 h where small differences were observed (Figure 4a). In Group IV rats, a sudden increase in the number of positive cells was observed between 20 and 32 h; the number of positive cells was maximal at 32 and 40 h, while relatively high numbers of positive cells were noted thereafter and up to 72 h (Figure 4a).





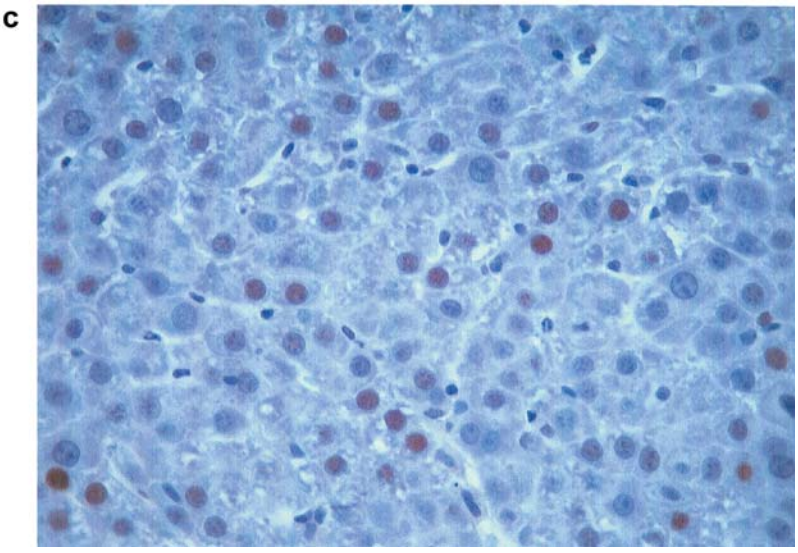


Figure 4. *A. Time-course of PCNA-positive cells in 65-70% partially hepatectomized rats having received 24 h prior to partial hepatectomy: a) saline (Group I), b) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) (Group II), c) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 12 h after partial hepatectomy (Group III) and d) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) followed by HSS administration (100 mg/kg bodyweight) 16 h after partial hepatectomy (Group IV). Values are expressed as means±SE. Group I vs Group II; $p<0.001$: 16-72 h; $p<0.01$: 96 h. Group I vs Group III; $p<0.001$: 16, 40 and 96 h; $p<0.05$: 20 h. Group I vs Group IV; $p<0.001$: 16-24, 40, 54-72 h; $p<0.01$: 32 h; $p<0.05$: 48 h. B. PCNA-positive cells at 24 h (x400) in 65-70% partially hepatectomized rats having received saline 24 h prior to partial hepatectomy (Group I). C. PCNA-positive cells at 24 h (x400) in 65-70% partially hepatectomized rats having received cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) 24 h prior to partially hepatectomy (Group II).*

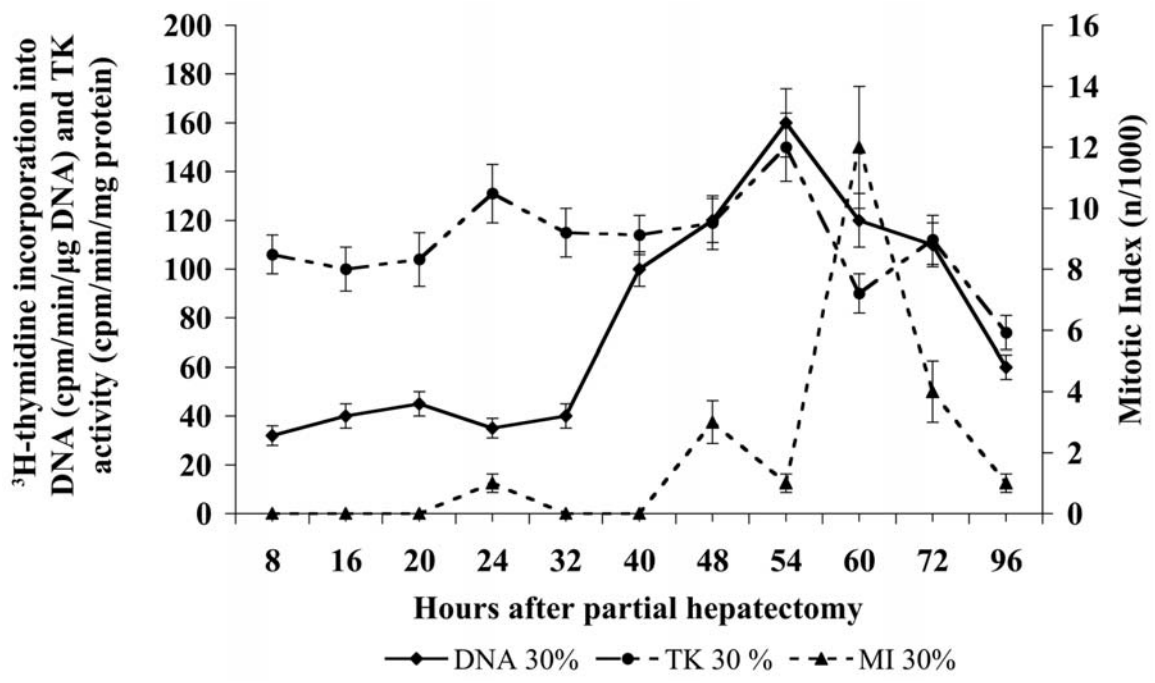


Figure 5. *Time-course of liver regeneration as evaluated by: a) [^{3}H]-thymidine incorporation into DNA, b) hepatic thymidine kinase (TK) enzymatic activity, and c) mitotic index (HE sections) in 30-34% partially hepatectomized rats (Group V). Values are expressed as means±SE.*

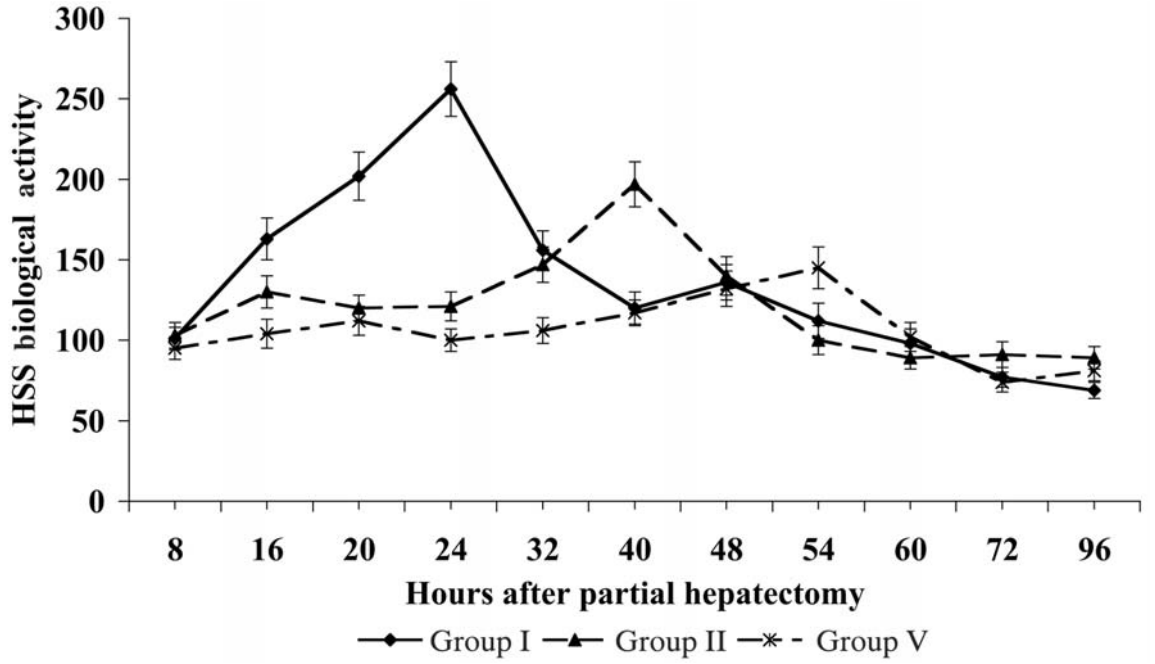


Figure 6. *Time-course of HSS biological activity in 65-70% partially hepatectomized rats having received 24 h prior to partial hepatectomy: a) saline (Group I), b) cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) (Group II), and in rats subjected to 30-34% partial hepatectomy (Group III). HSS was isolated from livers of all subgroups of Groups I, II and III and administered to 30-34% partially hepatectomized rats. HSS biological activity was evaluated by the rate of liver regeneration in 30-34% partial hepatectomized rats. Liver regeneration was reflected by [^{3}H]-thymidine incorporation into DNA. Values are expressed as means±SE.*

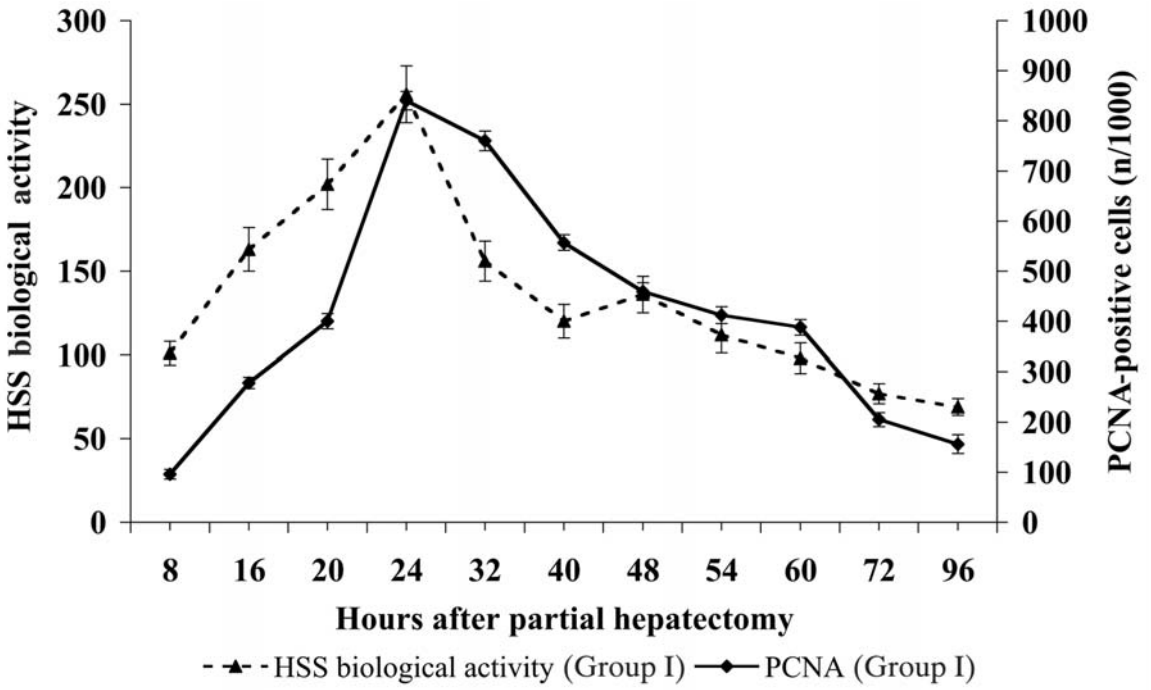


Figure 7. *Time-course of liver regeneration and HSS biological activity in 65-70% partially hepatectomized rats having received saline 24 h prior to partial hepatectomy (Group I). HSS was isolated from livers of all subgroups of Group I and administered to 30-34% partially hepatectomized rats. HSS biological activity was evaluated by the rate of liver regeneration in 30-34% partial hepatectomized rats and reflected by [^{3}H]-thymidine incorporation into DNA. Values are expressed as means±SE.*

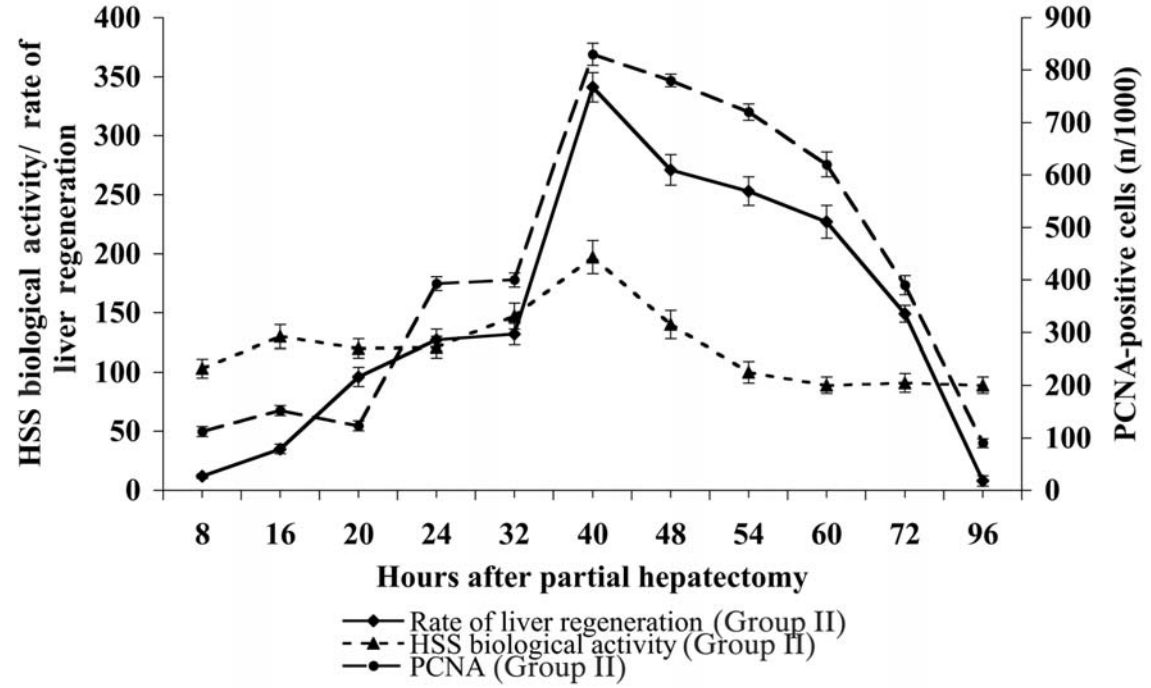


Figure 8. *Time-course of liver regeneration, HSS biological activity and PCNA-positive cells in 65-70% partially hepatectomized rats having received cadmium chloride (2.5 mg $CdCl_2$/kg bodyweight) 24 h prior to partial hepatectomy (Group II). HSS was isolated from livers of all subgroups of Group II and administered to 30-34% partially hepatectomized rats. HSS biological activity was evaluated by the rate of liver regeneration in 30-34% partial hepatectomized rats and was reflected by [^{3}H]-thymidine incorporation into DNA. Values are expressed as means±SE.*

In 30-34% partially hepatectomized rats (Group V), liver TK activity was at low levels for all time-points examined (Figure 5) and the distinct peaks, which were observed in Group I, were absent. [^{3}H]-Thymidine incorporation into hepatic DNA was low for the first 32 h after partial hepatectomy in rats subjected to 30-34 % partial hepatectomy, while relatively high levels were observed between 32 and 96 h after partial hepatectomy. The mitotic index was maximal at 60 h for Group V rats, with relatively high values at 48 and 72 h after partial hepatectomy (Figure 5).

HSS biological activity, as evaluated by the rate of [^{3}H]-thymidine incorporation into hepatic DNA in 30-34% partially hepatectomized rats, started increasing at 16 h after partial hepatectomy in Group I and peaked at 24 h, coinciding with the maxima of other regenerative indices. In addition, the activity remained at relatively high levels between 16 and 60 h and a second lower peak was observed at 48 h (Figure 6). In cadmium-treated rats (Group II), the biological activity was at low levels for the first 24 h after partial hepatectomy, started increasing at 24 h and peaked at 48 h (Figure 6). With the exception of 54 h, no significant increase in HSS biological activity was observed for all time-points examined for 30-34% partially hepatectomized rats (Figure 6).

Discussion

The results of this study clearly indicate that cadmium administration suppressed the liver regenerative process during the first 24 h after partial hepatectomy, since all indices of liver regeneration were significantly lower in cadmium-treated rats. In the cadmium-treated Group, the regenerative process resumed after that time-point and evolved in a more prolonged time-pattern. These findings are in accordance with the findings of others (13-14).

The immunochemical detection of PCNA nuclear antigen showed a small percentage of positive cells during the first 24 h after partial hepatectomy in cadmium-treated rats, while a sharp increase was observed in the normal controls by 20 h. PCNA nuclear protein expression is known to be cell cycle-dependent and a sharp increase at protein and mRNA levels of this nuclear factor is known to take place between 17 and 20 h after partial hepatectomy in the rat liver, marking the G1/S transition point (33). From this point of view, there was synchronized entrance of hepatocytes into the S-phase of the cell cycle in normal hepatectomized rats, but no such transition was observed for cadmium-intoxicated rats, finding it compatible with G1 arrest in the cell cycle in the cadmium-treated Group. Liver regeneration resumed at 32 h in the cadmium-treated Group and abrupt increases in the number of positive cells were observed between 32 and 40 h. The above findings are also compatible with cell cycle arrest in the G1-phase in cadmium-treated rats for the first 24 h after partial hepatectomy and indicate that G1/S transition takes place between 32 and 40 h after partial hepatectomy in cadmium-treated rats.

In 30-34% partially hepatectomized rats, no increases in the number of PCNA-positive cells or in the liver thymidine kinase enzymatic activity were observed for all time-points examined, which is also in accordance with the knowledge about cell cycle progression after that degree of partial hepatectomy. According to current knowledge, 30-34% partial hepatectomy represents a partial mitotic stimulus able to trigger entrance of hepatocytes only into the G1-phase of the cell cycle, but not synchronized entrance into the S-phase of the cell phase (2).

To evaluate the biological activity of HSS, the factor was isolated from the regenerating livers of normal and cadmium-treated rats at different time-points and administered to rats subjected to 30-34% partial hepatectomy. This percentage of liver excision is considered a partial or incomplete mitotic stimulus able to cause only entrance of hepatocytes into the G1-phase of the cell cycle (2). HSS, as an incomplete mitogen, provides the necessary stimulus for these already primed hepatocytes to proceed towards DNA synthesis and mitosis and the biological activity of the factor was evaluated by the determination of the rate of liver regeneration in the 30-34% partially hepatectomized rats.

The HSS biological activity in normal hepatectomized rats abruptly increased at 16 h and this increase was maintained at 20 and 24 h, where it peaked. The initial increase in the factor's biological activity coincided with the G1/S transition point, which implies possible involvement of the factor in the complicated network of growth factors that control that checkpoint (Figure 7). Cadmium administration suppressed the biological activity of the factor for the first 32 h after partial hepatectomy and the increase in the activity coincided with the sudden increase in the number of PCNA-positive cells in cadmium-treated rats (Figure 8). The increase in HSS activity also coincided with the G1/S transition point in cadmium-treated rats, as evaluated by the sudden increase in the number of PCNA-postitive cells. From the above, it seems that the suppression of HSS biological activity by cadmium represents an important aspect of its inhibitory effect on liver regeneration. This speculation is further supported by the fact that HSS administration reversed the inhibitory effect of cadmium on liver regeneration and also partially caused a synchronized entrance of hepatocytes into the cell cycle, as indicated by the progress of liver regeneration in these Groups of rats. This finding is also in agreement with the results of Labrecque *et al.*, where the factor shortened the G1-phase, when administered in already primed livers, by 60% (24).

HSS administration is able to reverse cadmium-induced arrest of liver regeneration and it seems that there is a lag-period of 8 h before the factor is able to exert its biological activity. Another striking finding is the fact that the liver

regeneration resumed in HSS-treated rats in a time-pattern similar to that of normal hepatectomized rats, but with a more prolonged replicative period.

Liver TK enzymatic activity was also partially restored by HSS administration, a finding compatible with the proposed action of the growth factor on the cell cycle. It is known that TK is an inducible enzyme and is cell cycle-specific, since there is abrupt induction of its expression at the mRNA level at the late G1 and at the G1/S transition point (34). The suppression of liver regeneration by cadmium has been attributed to the inhibition of liver TK activity by other researchers (13-14), but it seems that cell cycle arrest is able to explain all the observed effects of cadmium on liver regeneration.

According to our findings, HSS has the ability to restore TK activity. TK activity in cadmium-pretreated and HSS-administered rats showed a more prolonged time-pattern, being in accordance with the other indices of liver regeneration.

The results of this study suggest the possible participation of HSS in the network of growth factors that control the G1/S transition. The finding that HSS administration alone in cadmium-treated rats was capable of normalizing entry into the S-phase hints at a possible central role of the factor at the G1/S checkpoint.

In conclusion, cadmium administration seems to arrest liver regeneration during the first 32 h after partial hepatectomy, possibly through G1-phase arrest. Cadmium administration also suppressed HSS biological activity during the first 32 h after partial hepatectomy. HSS is capable of restoring liver regeneration in cadmium-treated rats and normalizing cell cycle progression. From this point of view, the factor seems to participate in the network of growth factors controlling the G1/S checkpoint.

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