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Inhibition of In Vivo DNA Synthesis In Regenerating Rat Liver Following Thermal Injury

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Hepatic repair and regeneration which is extremely important after thermal injuries can be inhibited by the acute inflammatory reaction. Since thermal injury initiates this acute inflammatory reaction, DNA synthesis was studied in the regenerating liver following this injury. In vivo incorporation of [^{3}H]-thymidine into hepatic DNA, autoradiographic determination of a labeling index, and thymidine kinase activity were determined. Incorporation of [^{3}H]-thymidine into hepatic DNA and labeling indices were markedly diminished at 24 hours if partial hepatectomy and thermal injury were carried out concurrently. After partial hepatectomy, the expected elevations in thymidine kinase activity were inhibited by the thermal injury ($p < 0.01$) and elevation of serum fibrinogen, a marker of the acute phase reaction that normally follows thermal injury, was blunted by the partial hepatectomy ($p < 0.05$). The combination of thermal injury and partial hepatectomy resulted in a greatly diminished DNA replicative response as compared to partial hepatectomy alone and suggests that multiplicative injury is more likely to result in multi-system failure.

Acute inflammation following turpentine administration has been shown to successfully delay and impair DNA synthesis following partial hepatectomy (1). As a result of this, impaired hepatocellular proliferation and tissue replacement may occur under conditions in which optimal organ function is critical to survival. Since thermal injury is known to initiate an acute inflammatory reaction similar to turpentine, our study was undertaken to determine whether thermal injury also inhibits or delays DNA synthesis in a regenerating rat liver model.

In other thermal injury studies, DNA synthesis was shown to be diminished when measured by in vivo incorporation of [^{3}H]-thymidine into the intestinal mucosa and by in vitro measurements of thymidine kinase activity in

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supernatants taken from mucosal cells (2). The understanding of mechanism(s) which may be critical to DNA synthesis for hepatocellular replication and intestinal repair after thermal injury may be important to further improvements in patient survival.

METHODS

Female Lewis rats (weight, 150-175 grams) were obtained from Charles River, Wilmington, MA. The animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Two-thirds partial hepatectomies were carried out as described previously (3). The animals were anesthetized between 8 and 10 A.M. using ether. The abdomen was opened and three lobes of the liver were ligated and removed; the abdomen then closed using wound clips. While still under ether anesthesia, the animals were subjected to either thermal injury or sham injury according to modified methods previously described (2). The animal's back was shaven, the animals were placed in a mold which exposed 25% total body surface area of the back, and then the animals were placed on top of a boiling water bath for 10 sec. All the animals were given an intraperitoneal injection of normal saline (15 ml/kg body weight) whether the animal received thermal injury or sham treatment. The rats were returned to the cages after partial hepatectomy and given water *ad libitum*. Both thermal injury and sham treatment were treated in exactly the same fashion because thermal injury has been demonstrated to produce anorexia in rats and guinea pigs and anorexia alone may influence DNA synthesis in the rat liver.

For the determination of *in vivo* incorporation of thymidine, the animals received an intraperitoneal injection of [^{3}H]-thymidine (50μCi, 40-60 Ci/mM, New England Nuclear) 22.5 hours prior to sacrifice. Twenty-four hours after the laparotomy and injury, the animals were sacrificed by cervical dislocation. The livers were removed and the DNA content was determined as described previously (3).

Labeling indices were determined using autoradiography in animals injected with [^{3}H]-thymidine. Briefly, the animals were injected with [^{3}H]-thymidine and sacrificed as above. The liver was removed, rinsed, and fixed in formalin. Subsequently, the tissue was embedded, sectioned, and processed for autoradiography using Kodak Nuclear NTB Emulsion by standard techniques (4).

For the determination of thymidine kinase activity, the animal was sacrificed by cervical dislocation. The liver was removed, homogenized in 10 volumes of 100 mM Tris buffer (pH 8.0), and spun at 105,000 x g for one hour. The thymidine kinase activity was measured in the supernatant fraction as described previously (2).

Plasma fibrinogen was determined by the chemical chemistry laboratory using standard blot formation technique; protein and DNA content were determined by the methods of Lowry and Burton respectively (5,6).

For statistical analysis, comparisons of thymidine incorporation, mitotic indices, and activities of thymidine kinase were made using one-way analysis of variance (ANOVA) and appropriate multiple range tests (Statistical Analysis Systems, Cary, NC). Comparison of specific activities of total radiolabel in the regenerating rat livers was made by the Student's t-test. Probabilities less than 5% were considered significant in each comparison. Analysis of variance for the fibrinogen levels was made using the Waller-Duncan K-ratio t-test.

RESULTS AND DISCUSSION

In these studies, neither partial hepatectomy nor thermal injury was lethal alone or in a combination injury. Mortality was less than 10% from either partial hepatectomy or thermal injury, or both for animals who were not sacrificed at 24 hours.

Plasma fibrinogen is a marker in rats for the acute inflammatory reaction. In the absence of partial hepatectomy, thermal injury increased the plasma fibrinogen level 2-3 fold ($p < 0.05$) and after partial hepatectomy 2-fold ($p < 0.05$) (Table 1). Thermal injury did not elevate the plasma above the levels resulting from partial hepatectomy. These results were consistent with Bernuau's report (1) using turpentine.

In vitro activity of thymidine kinase in the supernatants of hepatocytes taken from the regenerating hepatic lobes was increased as expected when compared to no hepatectomy (Table I). The thymidine kinase activity was also increased in the combined injury (partial hepatectomy and thermal injury), but this increase in thymidine kinase activity was not as great following partial hepatectomy and thermal injury ($p < 0.01$).

Table 1: Effect of Thermal Injury and/or Partial Hepatectomy on Plasma Fibrinogen and In Vitro Thymidine Kinase Activity in Hepatocyte Supernatants

Thermally Injured	Partial Hepatectomy	Plasma* Fibrinogen	Thymidine Kinase† Activity
0	0	0.21 ± 0.03	25 ± 5
0	+	0.40 ± 0.08	510 ± 55
+	0	0.61 ± 0.09	35 ± 15
+	+	0.44 ± 0.02	280 ± 88

* grams/dl

† (DPM/mg protein/15 min)

Thymidine kinase activity was determined in vitro in the 105,000 x g hepatocyte supernatant hepatectomy and/or thermal injury. (Mean ± SD, n=4)

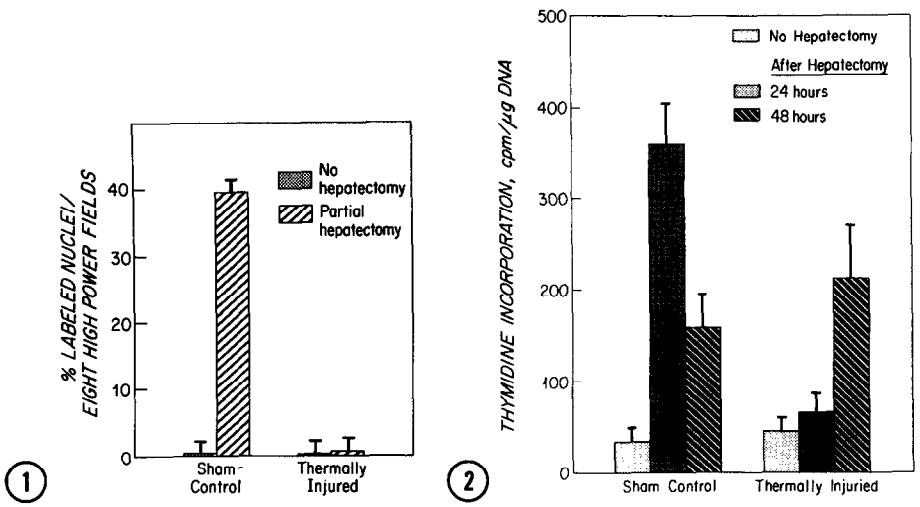

Figure 1. In vivo incorporation of [^{3}H]-thymidine into hepatic DNA after partial hepatectomy and thermal injury. Thymidine incorporation was determined 24 hours after injection of [^{3}H]-thymidine and partial hepatectomy and/or thermal injury. Thermal injury did not influence thymidine incorporation when partial hepatectomy was not performed. A markedly increased thymidine incorporation into hepatic DNA was found after partial hepatectomy in sham-control animals which was not seen if the animal also received a thermal injury simultaneously. (Mean ± SE, n=6)

Figure 2. Mitotic index 24 hours after in vivo labeling of hepatic DNA following partial hepatectomy and thermal injury. The mitotic indices were low in the absence of partial hepatectomy. Following partial hepatectomy, the mitotic index was markedly increased. Partial hepatectomy did not result in a rise in mitotic index if thermal injury immediately followed the partial hepatectomy. (Mean ± SE, n=4)

Partial hepatectomy resulted in a 20-40 fold in vivo enhancement of incorporation of [^{3}H]-thymidine into hepatic DNA 24 hours after partial hepatectomy as compared to animals without hepatectomy (Figure 1); a lower but highly significant elevation in DNA incorporation was still seen at 48 hours (Figure 1). By contrast, only a minor increase in DNA [^{3}H]-thymidine incorporation was found at 24 hours in rats subjected to both partial hepatectomy and thermal injury (Figure 1); by 48 hours, significant elevations in DNA synthesis were evident. Without partial hepatectomy, DNA synthesis was minimal in the livers of either the sham-control or thermally-injured rats. If the thermal injury occurred 18 hours, one month, or two months prior to partial hepatectomy, no inhibition of in vivo DNA synthesis was observed (data not shown).

The differences in incorporation of radiolabeled thymidine into hepatic DNA did not appear to be the result of differences in [^{3}H]-thymidine uptake into the hepatocytes of the regenerating liver. The specific activity (DPM/g liver) of the regenerating liver 24 hours after the partial hepatectomy was not statistically different for the cases of sham-control versus thermally-injured animals (data not shown). Therefore, though the total uptake of [^{3}H]-thymidine into the regenerating liver cells was indifferent to the presence of thermal injury, the incorporation of the [^{3}H]-thymidine into hepatic DNA was significantly decreased by thermal injury.

The fraction of hepatocyte nuclei which were radiolabeled (labeling index) was determined for both sham-treated and thermally-injured animals (Figure 2). After partial hepatectomy many nuclei were radiolabeled at 24 hours in the regenerating liver. In marked contrast, few hepatocellular nuclei were radiolabeled without partial hepatectomy. In confirmation of the previous radiolabeled DNA assay, fewer nuclei were radiolabeled in the regenerating liver when acute thermal injury was carried out immediately following the partial hepatectomy than was seen in the absence of the thermal injury (Figure 2). The labeling index for the sham-treated animals with partial hepatectomy was statistically increased over the other groups ($p < 0.01$).

This study demonstrates for the first time inhibition of <u>in vivo</u> DNA synthesis in regenerating rat hepatocytes following thermal injury. These inhibitory effects were not seen if the thermal injury preceded the partial hepatectomy by 18 hours or more.

Bernuau has suggested a mechanism for this inhibition of DNA synthesis by the induction of the acute phase reaction in which hepatic regeneration and production of acute phase proteins compete at a transcriptional or translational level of protein synthesis (1). Gene expression for acute phase protein expression probably occurs within minutes or hours following exposure of the hepatocyte to inducers of acute phase proteins (7). This rapid sequence of events may compete with oncogenesis which also occurs quite

rapidly after partial hepatectomy in rat liver regeneration systems (8-10). Of course, these systems are quite complicated and competition may occur much earlier at an initial cell receptor-ligand interaction or during second messenger processes. Therefore, one can only conclude that this multiplicative injury more likely results in a multi-system failure.

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