

# Modulation of Reparative Regeneration and CD117 Expression by Liver Cells after Partial Hepatectomy in Mice

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The effects of 3-aminophthalhydrazide and carrageenan on reparative regeneration and expression of CD117 by liver cells after partial hepatectomy were studied in mice. 3-Aminophthalhydrazide stimulated regeneration of the liver and increased the count of CD117<sup>+</sup> hepatocytes. By contrast, carrageenan inhibited liver reparation and CD117 expression.

**Key Words:** *hepatectomy; reparative regeneration; CD117; 3-aminophthalhydrazide; carrageenan*

Fibroblast-derived stem cell factor (SCF) stimulating proliferation and differentiation of hemopoietic cells by the Jak2/STAT signal pathway [4] via interaction with CD117 receptor (c-kit) is now intensively studied. Apart from hemopoietic cell stimulation, SCF plays an important role in gametogenesis and some other physiological processes in mammals [5,7]. CD117<sup>+</sup> cells were found in the pancreas and heart, where they are regarded as the main candidates for regional stem cells. In the liver and kidneys this receptor is present also in mature cells, but the role of CD117 expression in these organs is not yet clear [6].

According to modern concepts, macrophages are involved in the regulation of tissue reparation and remodeling [2,3]. The content of monocyte/macrophages increases during the development of regenerative processes, particularly at their early stages. Changes in functional activity of these cells lead to modification of the reparative potential of the organ [1]. Synergism of some macrophage cytokines and SCF was described [8]. Macrophage inhibitor carrageenan and 3-aminophthalhydrazide, a modulator of the function of these

cells, *a priori* modulate CD117 expression by hepatocytes and the reparative regeneration of the liver.

We studied the effects of carrageenan and 3-aminophthalhydrazide on reparative regeneration and CD117 expression by liver cells in mice after partial hepatectomy.

## MATERIALS AND METHODS

Experiments were carried out on 3-month-old outbred albino mice kept on standard ration at vivarium of Institute of Immunology and Physiology. The animals were divided into 5 groups, 10 per group. Group 1 consisted of intact animals. Group 2 mice were subjected to upper median laparotomy. Group 3 were subjected to partial hepatectomy as described previously [10]. Group 4 mice were intramuscularly injected with 2 mg/kg 3-aminophthalhydrazide 1 h before partial hepatectomy. Group 5 animals were intraperitoneally injected with 10 mg/kg carrageenan 1 h before surgery. The operations were carried out under ether narcosis. The intensity of reparative processes was evaluated 24 h after hepatectomy; this period corresponded to the peak of regenerative processes in the liver [9].

CD117<sup>+</sup> hepatocytes were identified on liver sections by standard indirect immunohistochemical staining with monoclonal anti-mouse CD117 antibodies (clone ACK2, Millipore). The measurements were car-

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ried out in 10 visual fields with semiquantitative interpretation of the results (from 0 to +++).

The intensity of reparative regeneration was evaluated by regenerate weight and content of dry substance and fluid. The count of hepatocytes and the size of these cells and their nuclei, and the nuclear/cytoplasmic index were evaluated on histological preparations. The total counts of binuclear hepatocytes and the counts of these cells expressing Ki-67 proliferation marker were determined. Ki-67<sup>+</sup> cells were detected by indirect immunohistochemical staining with monoclonal anti-human Ki-67 clone B56 antibodies (BD).

The data were statistically analyzed using Statistica 6.0 software. The hypothesis on homogeneity of two independent samples was verified using nonparametric Mann–Whitney test. The data are presented as the mean±standard error of the mean. The differences were considered significant at  $p<0.05$ .

## RESULTS

The weight of the remaining liver tissue increased by 181% of the initial postoperative value 24 h after resection of the organ. 3-Aminophthalhydrazide promoted an additional increment of the regenerating fragment (liver weight increased by 212%), presumably due to macrophage stimulation. By contrast, intraperitoneal injection of carrageenan decelerated the increase in liver weight. Analysis of the percentage of the tissue dry weight in the remaining fragment of the liver showed that the weight of dry substance increased in all experimental groups, which attested to the development of regenerative processes in the organ.

The count of binuclear hepatocytes and the nuclear/cytoplasmic index increased in mice after partial hepatectomy, this indicating the development of cellular and intracellular regeneration processes. The mice injected with 3-aminophthalhydrazide 1 h before surgery developed more pronounced increase in the

counts of binuclear hepatocytes. The counts of Ki-67<sup>+</sup> binuclear cells in the livers of these animals also increased. This fact indicates more intense hepatocyte cytotoxicity and reparative regeneration of the liver in general. Carrageenan inhibited reparative processes in the liver, which manifested in lower counts of binuclear cells, including the Ki-67<sup>+</sup> ones (Table 1).

Immunohistochemical study of CD117<sup>+</sup> cells in mouse livers showed finely granular DAB-positive staining of hepatocyte cytoplasm. About 20% hepatocytes located mainly periportal and around the central veins expressed CD117 in intact and laparotomized mice. The degree of CD117 expression, evaluated by staining intensity, was mainly weak in intact animals (Fig. 1).

The counts of CD117<sup>+</sup> hepatocytes increased in the livers of hepatectomized mice, including cells with medium expression of the receptor. A small portion of cells with high expression of CD117 was detected. Similarly as in intact mice, CD117<sup>+</sup> hepatocytes were located in the periportal and pericentral compartments of lobules.

Preventive injection of 3-aminophthalhydrazide led to an increase in the count of CD117<sup>+</sup> cells, reaching 60% after 24 h. The expression of SCF receptor varied from slight to pronounced, while the number of cells with medium and pronounced expression of CD117 was higher than in mice subjected to hepatectomy alone. The CD117<sup>+</sup> hepatocytes were located mainly in the perivascular zones.

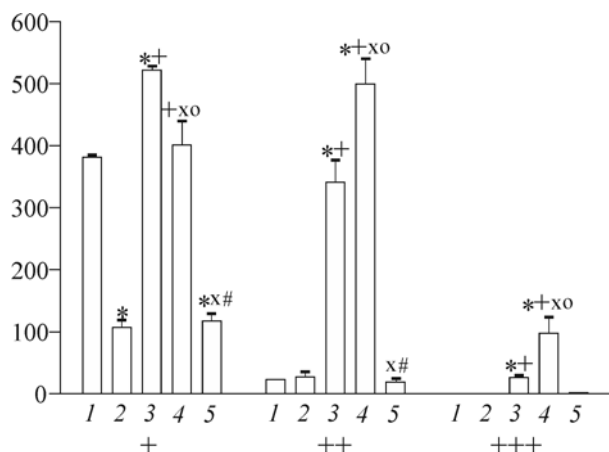
By contrast, injection of carrageenan 1 h before hepatectomy drastically reduced the count of CD117<sup>+</sup> hepatocytes, the greater part of them just slightly expressing this receptor (Fig. 1).

Hence, 3-aminophthalhydrazide stimulated the expression of CD117 and potentiated reparative processes in damaged liver, while carrageenan significantly inhibited organ regeneration. The opposite effects of these substances can be explained (at least partially) by their opposite effects on functional ac-

**TABLE 1.** Effects of 3-Aminophthalhydrazide and Carrageenan on Liver Regeneration Parameters in Mice after Partial Hepatectomy ( $M\pm m$ )

Parameter	Intact	Laparotomy	Hepatectomy	Hepatectomy+ 3-aminophthalhydrazide	Hepatectomy+ carrageenan
Hepatocyte count per mm <sup>2</sup>	1392±52	1232±23*	1101±33**	1245±44** <sup>o</sup>	990±23** <sup>x#</sup>
Total count of binuclear hepatocytes per mm <sup>2</sup>	243±33	148±18*	343±14**	414±12** <sup>xo</sup>	116±15** <sup>x#</sup>
Count of Ki-67 <sup>+</sup> hepatocytes per mm <sup>2</sup>	15.5±1.0	7.4±2.2*	9.3±2.8	19.8±1.1** <sup>xo</sup>	3.1±1.2** <sup>x#</sup>
Nuclear/cytoplasmic index	0.270±0.006	0.305±0.005*	0.349±0.008**	0.324±0.006** <sup>x</sup>	0.335±0.008**

**Note.**  $p<0.05$  compared to: \*intact group, <sup>\*</sup>laparotomy, <sup>\*</sup>hepatectomy, <sup>#</sup>hepatectomy+3-aminophthalhydrazide, and <sup>o</sup>hepatectomy+carrageenan.



**Fig. 1.** Changes in the counts of hepatocytes with different intensity of CD117 expression in mice after partial hepatectomy under the effect of 3-aminophthalhydrazide and carrageenan. 1) intact mice; 2) laparotomy; 3) hepatectomy; 4) hepatectomy+3-aminophthalhydrazide; 5) hepatectomy+carrageenan. "+" slight expression, "++" medium expression, "+++" strong expression.  $p < 0.05$  compared to: \*1, +2, x3, #4, °5.

tivity of macrophages. Our data suggest investigating the details of mechanisms of the detected biological effects of 3-aminophthalhydrazide and search for ef-

fective stimulants of reparative regeneration of the liver among other stimulants/modulators of macrophage function.

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