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AN INDUCTION OF HEME OXYGENASE AND ITS POSSIBLE RELATION TO THE DECREASE OF CYTOCHROME P-450 CONTENT DURING LIVER REGENERATION*

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The alterations of various enzymes responsible for drug metabolism and heme metabolism were examined in regenerating livers of male rats. Microsomal cytochrome P-450 content and aminopyrine demethylase activity were significantly decreased during liver regeneration. In contrast, microsomal heme oxygenase activity was markedly increased under the identical conditions. The increased heme oxygenase activity which appeared within 4 h and reached maximum at 1 d after partial hepatectomy was sustained for 5 d. In sham-operated rats, the changed patterns of these parameters were similar, but to a lesser extent as compared to partially hepatectomized rats. The increase of heme oxygenase activity following partial hepatectomy was blocked by the administration of cycloheximide or actinomycin D. Other enzymes involved in heme synthesis did not change appreciably during liver regeneration.

The inverse relationship between the decrease of cytochrome P-450 content and the increase of heme oxygenase activity was also observed in female rats and male mice. These findings suggest that the increase of heme oxygenase activity in regenerating rodent liver would be correlated to the decrease of cytochrome P-450 content.

Adrenalectomy enhanced the increase of heme oxygenase activity following partial hepatectomy, though the decrease of cytochrome P-450 content was less extensive under the experimental conditions. The results also suggest that the inverse relationship between the increase of heme oxygenase activity and the decrease of cytochrome P-450 content would be a biochemical phenomenon seen in regenerating liver and that the phenomenon would not be simply due to the surgical stress to the animals.

Keywords —— heme oxygenase; cytochrome P-450; liver regeneration; partial hepatectomy; adrenalectomy

INTRODUCTION

The reduction of microsomal drug-metabolizing enzyme activities including cytochrome P-450 content has been well-defined in regenerating rat liver following partial hepatectomy.[1-6] It has also shown that there is a delayed response, during the early stages of liver regeneration, to the inducers of drug-metabolizing enzymes such as phenobarbital.[3-6] But, the response to the inducers has restored during the later stages of liver regeneration at those times when the liver had been essentially completely regenerated. Based on these facts, it is suggested that the reduced levels of microsomal drug-metabolizing enzymes including cytochrome P-450 during liver regeneration resulted from a competence with the process of rapid cell proliferation.[3-6] However, Presta *et al.*[7] have recently proposed that the decrease of drug-metabolizing enzyme activities in regenerating

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rat liver is due to the mere surgical stress to the animals, because the extent of the decrease of the enzymes and the response to phenobarbital were similar in both hepatectomized and sham-operated rats. Thus, an exact mechanism by which cytochrome P-450 dependent drug-metabolizing enzyme activities are decreased during liver regeneration still remains to be elucidated.

Recent studies have shown that there is an inverse relationship between the increase of microsomal heme oxygenase activity, a rate limiting enzyme in heme breakdown,[8,9] and the decrease of cytochrome P-450 content.[10-17] Concomitant with the increase of heme oxygenase activity following the administrations of hemin,[10,11] heavy metals,[12-15] and endotoxin,[16,17] there was a remarkable decrease of cytochrome P-450 content as well as drug-metabolizing enzyme activities. The administrations of insulin, glucagon and epinephrine, which are thought to be important factors for hepatocellular proliferation and are increased in blood levels following partial hepatectomy,[18] also resulted in the increase of heme oxygenase activity of rat liver.[19]

Since data on the possible changes of heme metabolizing enzyme activities during liver regeneration are very scanty, the present investigation has been designed to examine whether there are possible alterations of microsomal heme oxygenase activity and other enzymes involved in heme synthesis in regenerating liver after partial hepatectomy. Additionally, to ascertain a possible role of adrenal gland for the surgical stress, the adrenalectomized rats were also subjected to partial hepatectomy for examining the changes of cytochrome P-450 content as well as heme oxygenase activity.

MATERIALS AND METHODS

Chemicals — Hemin, protoporphyrin IX, NADP, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, cycloheximide and actinomycin D were obtained from Sigma Chemical Co. All other chemicals were of the highest grade available commercially.

Animals and Treatment — Male and female Wistar rats, weighing 130 – 150 g, and male ddY mice, weighing 23 – 25 g, were used in these experiments. The animals were subjected to either partial hepatectomy (about 70%) under ether anesthesia as described by Higgins and Anderson,[20] or a sham-operation, in which a similar abdominal incision was made and the liver was manipulated. The animals were allowed free access to food and water. In some experiments, the animals were subjected to bilateral adrenalectomy under ether anesthesia. After the operation, they were allowed free access to food and 0.9% NaCl solution. Four days after the adrenalectomy, the animals were again subjected to partial hepatectomy as described above. Cycloheximide or actinomycin D, dissolved in 0.9% NaCl solution, was injected intraperitoneally to rats just after the partial hepatectomy. The animals were sacrificed at the times indicated in Figs. and Tables. The liver was perfused *in situ* with cold 0.9% NaCl solution, excised, and homogenized with 4 vol. of 1.15% KCl solution. The homogenate was centrifuged at 9000 × ***g*** for 20 min. The resulting supernatant fraction was centrifuged again at 105000 × ***g*** for 60 min. The pellets were washed once and resuspended in 0.1 M Phosphate buffer (pH 7.4). The cytosol, prepared from normal rat liver homogenate, was saved and used as a source of biliverdin reductase for the determination of heme oxygenase activity. When necessary, mitochondrial fraction was also prepared for the determination of ferrochelatase activity. Total homogenate was used when δ-aminolevulinic acid synthetase activity was assayed.

Enzyme Assays — Microsomal cytochrome P-450 content was determined by the method of Omura and Sato.[21] Aminopyrine demethylase activity was measured by the procedure of Cochin and Axelrod.[22] Heme oxygenase activity was assayed according to the method of Tenhunen *et al.*,[23] as described previously.[15] Mitochondrial ferrochelatase activity was determined by the method of Porra *et al.* [24] δ-Amino-

levulinic acid synthetase activity was assayed according to the method of Marver *et al.* [25]

Protein Concentration — Protein concentration was determined according to the method of Lowry *et al.* [26] with bovine serum albumin as the standard.

Statistical Analysis — The results were subjected to statistical analysis according to the Student's t-test.

RESULTS

Changes of Microsomal Cytochrome P-450 Content and Aminopyrine Demethylase Activity During Liver Regeneration

Fig. 1 shows the time course of the changes of hepatic microsomal cytochrome P-450 content and aminopyrine demethylase activity in both sham-operated and partially hepatectomized rats. Both cytochrome P-450 content and aminopyrine demethylase activity were significantly decreased during liver regeneration following partial hepatectomy. The reduced levels of both parameters which reached minimum at 3 d following partial hepatectomy were sustained for 7 d, and then completely returned to control levels by 14 d. In sham-operated rat liver, both parameters were similarly reduced, but to a lesser extent as compared to those of partially hepatectomized rats. The changed pattern of both cytochrome P-450 content and aminopyrine demethylase activity during liver regeneration following partial hepatectomy are well compatible to those of the published reports.[1-7] The results indicate that the decrease of drug-metabolizing enzyme activities, such as aminopyrine demethylase, would be mainly due to the decrease of cytochrome P-450 content as shown by other investigators.[3,4,6]

Changes of Microsomal Heme Oxygenase Activity and Other Enzyme Activities Involved in Heme Synthesis during Liver Regeneration

To examine a mechanism through which cytochrome P-450 content is decreased during liver regeneration, we investigated the possible alterations of the enzyme activities involved in heme synthesis and degradation. Fig. 2 shows the time course of the changes in microsomal heme oxygenase activity in both sham-operated and partially hepatectomized rat liver. At 4 h after partial hepatectomy, microsomal heme oxygenase activity was increased to about 2 times the control levels. The increased heme oxygenase activity which reached maximum at 1 d following partial hepatectomy was sustained for 5 d, and returned to control levels by 7 d. The changed pattern of heme oxygenase activity in sham-operated rat liver was almost similar to

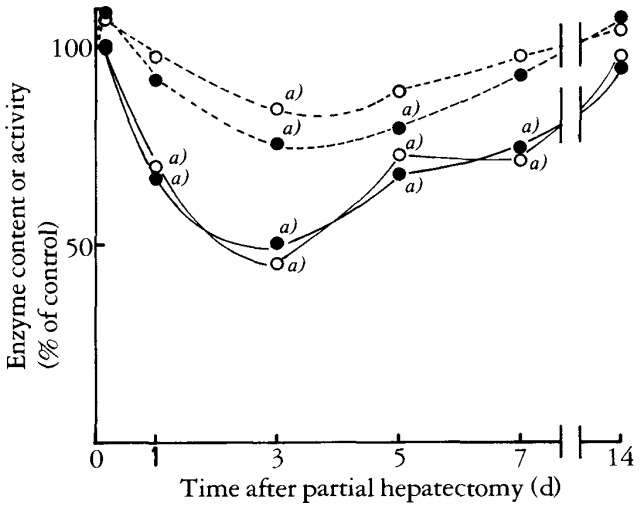

FIG. 1. *Time Course of Changes in Microsomal Cytochrome P-450 Levels and Aminopyrine Demethylase Activity during Liver Regeneration of Male Rats*

Rats were subjected to partial hepatectomy and sacrificed at the times indicated for the determinations of cytochrome P-450 levels and aminopyrine demethylase activity. The assay conditions and other details are described in Materials and Methods. Values, expressed as percentage of the corresponding control values at each time period, are the mean of three or four rats. The control values of cytochrome P-450 levels and aminopyrine demethylase activity ranged from 0.74 to 1.09 nmol/mg protein and from 5.43 to 6.61 nmol/mg protein/min during the time course study, respectively.

○ cytochrome P-450; ● aminopyrine demethylase; ----- sham-operated; —— partially hepatectomized.

a) $p < 0.05$.

that seen in partially hepatectomized rat liver, except that the onset, intensity and duration of the increase of the enzyme activity were different in both groups.

The increase of heme oxygenase activity after partial hepatectomy was blocked by the administration of a well-known inhibitor of protein synthesis (cycloheximide) or m-RNA synthesis (actinomycin D), suggesting that the increased formation of the enzyme protein occurred in regenerating liver, as shown in Fig. 3. The basal heme oxygenase activity was not changed significantly by the administration of cycloheximide or actinomycin D, as reported by other investigators.[11]

In contrast to such a remarkable changes of heme oxygenase involving in heme breakdown, mitochondrial δ-aminolevulinic acid synthetase activity, a rate limiting enzyme in heme synthesis, was not changed significantly during the process of liver regeneration (data not shown). In addition, allylisopropylacetamide, a well-known inducer of δ-aminolevulinic acid synthetase, was able to induce the enzyme when administered to rats either 1 h before or after partial hepatectomy (data not shown). Hepatic ferrochelatase activity, a final step enzyme in heme synthesis, did not change significantly during liver regeneration (data not shown). All of these results suggest that hepatic heme synthesizing machinery is not changed in regenerating rat liver.

Next, to examine as to whether the increase of heme oxygenase during the process of liver regeneration is a generalized phenomenon, we determined the enzyme activity as well as cytochrome P-450 content in partially hepatectomized female rats and male mice. Table I shows the results of these experiments. Microsomal heme oxygenase activity was also increased to 2.4 times and 5.0 times the control levels in sham-operated and partially hepatectomized female rat liver, respectively. Conversely, cyto-

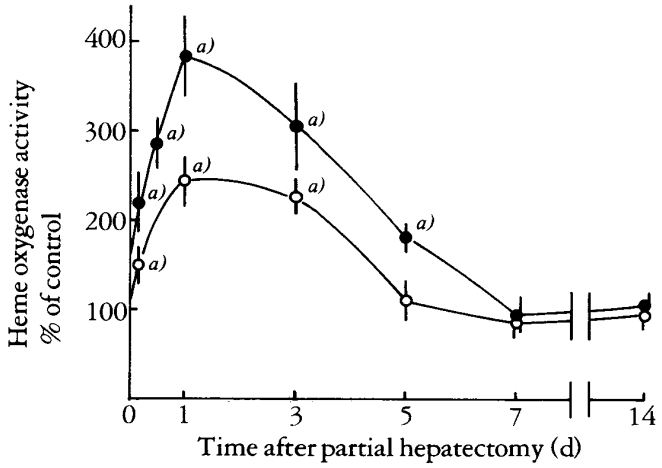

FIG. 2. *Time Course of Changes in Microsomal Heme Oxygenase Activity during Liver Regeneration of Male Rats*

Rats were subjected to partial hepatectomy and sacrificed at the times indicated for the determination of heme oxygenase activity. Values, expressed as percentage of the corresponding control values, are the mean ± S. E. of three or four rats. The control values ranged from 0.57 to 1.25 nmol/mg protein/h during the time course study.

○ —○ *sham-operated;* ● —● *partially hepatectomized.*

a) $p < 0.05$.

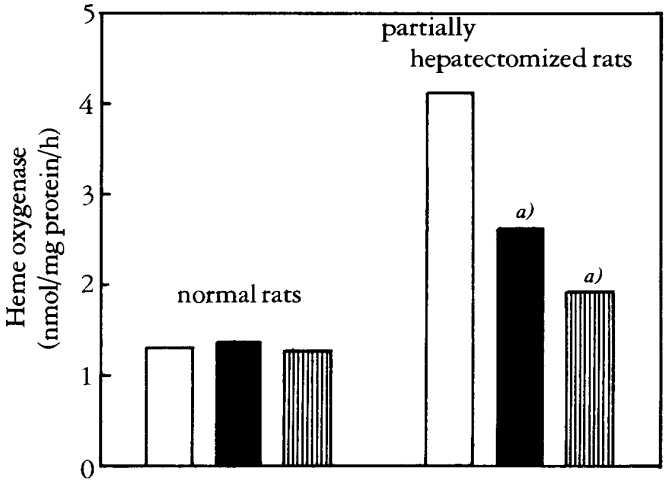

FIG. 3. *Effects of Actinomycin D and Cycloheximide on the Increase of Heme Oxygenase Activity in Regenerating Rat Liver*

Male rats were injected with actinomycin D (1 mg/kg, i.p.) or cycloheximide (1 mg/kg, i.p.) just after partial hepatectomy and sacrificed 24 h after the injection. Each value is the mean of three or four rats.

□ *control;* ■ *actinomycin D;* ▥ *cycloheximide.*

a) $p < 0.05$.

chrome P-450 content was decreased significantly as compared to the controls. It was also found that there was the inverse relationship between the increase of heme oxygenase activity and the decrease of cytochrome P-450 content in sham-operated and partially hepatectomized mice. Thus, it is reasonable to point out that the increased activity of heme oxygenase would be a generalized phenomenon in regenerating rodent liver. Such an increased activity of heme oxygenase could lead to the enhanced breakdown of heme available for the synthesis of cytochrome P-450, thus may leading to the decrease of hemoprotein under the identical experimental conditions.

Effect of Adrenalectomy on the Increase of Heme Oxygenase Activity and the Decrease of Cytochrome P-450 Content during Liver Regeneration

Presta *et al.* [7] have proposed that the decrease of drug-metabolizing enzyme activities during liver regeneration would be resulting from the mere surgical stress to the animals. In this respect, we examined the effect of adrenalectomy on the increase of heme oxygenase activity during liver regeneration, since the adrenal gland is one of the major organs responsible for the stress. The results of these experiments are shown in Table II. Adrenalectomy did not change heme oxygenase activity in this experiments. But, cytochrome P-450 content was decreased to about 70% of the normal rats. When adrenalectomized rats were subjected to partial hepatectomy, there was an enhanced increase of heme oxygenase activity as compared to the corresponding normal rats who had been partially hepatectomized. In sham-operated rats, heme oxygenase activity was also tended to increase. Inversely, cytochrome P-450 content tended to decrease in adrenalectomized-partially hepatectomized rat liver. The results suggest that the decrease of cytochrome P-450 content as well as drug-metabolizing enzyme activities during liver regeneration would not be merely due to the surgical stress to the animals, but rather biochemical changes in heme metabolism, espacially the increase of heme oxygenase activity, could be ascribed to such a decrease of the enzyme systems.

DISCUSSION

This study demonstrates that there is an inverse relationship between the decrease of cytochrome P-450 content and the increase of heme oxygenase activity during the course of liver regeneration. Partial hepatectomy produced a decrease of cytochrome P-450 content and an increase of heme oxygenase activity in male and female rats and male mice (Figs. 1, 2 and Table I). Time course study performed in male rats indicate that the increased activity of heme oxy-

TABLE I. *Changes in Microsomal Cytochrome P-450 Content and Heme Oxygenase Activity in Regenerating Liver of Female Rats or Male Mice*

	Cytochrome P-450 (nmol/mg protein)		Heme oxygenase (nmol/mg protein/h)	
	Female rats	Male mice	Female rats	Male mice
Control	0.63 ±0.03	0.80 ±0.03	0.83 ±0.07	1.26 ±0.14
Sham-operated	0.54 ±0.06	n.d.	1.99 ±0.31 [a)]	n.d.
Partially hepatectomized	0.47 ±0.02 [a)]	0.46 ±0.01 [a)]	4.14 ±0.54 [a, b)]	3.29 ±0.37 [a)]

Female rats and male mice were subjected to partial hepatectomy and sacrificed 24 h after the operation. The assay conditions and other details are described in Materials and Methods section. Values are the mean ± S.E. of three female rats. In the case of mice, the remnant livers from three or four mice were pooled for one determination and values are the mean ± S.E. of three independent determinations. n.d.: not determined. Significantly ($p < 0.05$) different from the control a) or sham-operated b) values.

genase is followed by the decrease of cytochrome P-450 content as well as aminopyrine demethylase activity (Figs. 1 and 2). The increase of heme oxygenase activity during liver regeneration would be due to the increased formation of the enzyme protein (induction), because the administration of either cycloheximide or actinomycin D blocked the increase of the enzyme activity following partial hepatectomy (Fig. 3). Since heme oxygenase is a rate limiting enzyme of heme breakdown,[8,9] the increase of the enzyme may lead to the accelerated breakdown of heme in regenerating liver, thus may leading to a decreased concentration of heme available for the synthesis of cytochrome P-450. This idea could be supported by the facts that hepatic heme synthesizing enzyme activities did not change appreciably during the course of liver regeneration, especially δ-aminolevulinic acid synthetase, a rate limiting enzyme in heme synthesis, remained at control levels under the experimental conditions. Such a mechanism for the decrease of cytochrome P-450 content, occurring as a consequence of the increase of heme oxygenase activity, has been demonstrated by the administrations of hemin,[10,11] heavy metals[12-15] and endotoxin.[16,17] The induction of heme oxygenase following the administrations of these compounds is followed by the decrease of cytochrome P-450 content as well as drug-metabolizing enzyme activities. However, it can not be excluded that apo-cytochrome P-450 synthesizing machinery is exaggerated during the course of liver regeneration, because it has been shown that there is a delayed response, during the early stages, to the inducers of the enzyme systems.[3-6]

Adrenalectomy enhanced the increase of heme oxygenase activity in regenerating rat liver (Table II). Considering the importance of this endocrine responsible for the stress, the results suggest that the decrease of cytochrome P-450 content as well as drug-etabolizing enzyme activities during liver regeneration[1-7 and this investigation] would not be simply due to the surgical stress to the animals as proposed by Presta *et al.*[7] They have proposed the surgical stress theory on the basis of the similarities in the decrease of aminopyrine demethylase and aniline hydroxylase activity between partially hepatectomized and sham-operated rats, and of the response of partial hepatectomy to phenobarbital. However, they did not show the changes of cytochrome P-450 content under the same experimental conditions. In the present investigation, on the other hand, we have confirmed the

TABLE II. *Effect of Adrenalectomy on the Increase of Heme Oxygenase Activity and the Decrease of Cytochrome P-450 Content Following Partial Hepatectomy*

	Cytochrome P-450 (nmol/mg protein)	Heme oxygenase (nmol/mg protein/h)
Normal rats		
Control	1.08 ±0.01	0.64 ±0.06
Sham-operated	0.97 ±0.08	1.55 ±0.14 a)
Partially hepatectomized	0.72 ±0.02 a)	2.56 ±0.25 a)
Adrenalectomized rats		
Control	0.73 ±0.01	0.65 ±0.11
Sham-operated	0.66 ±0.13	1.90 ±0.15 a)
Partially hepatectomized	0.60 ±0.03 a)	6.11 ±0.71 a, b)

Male rats were subjected to bilateral adrenalectomy. Four days after adrenalectomy, the animals were again subjected to partial hepatectomy and sacrificed 24 h after the operation. Values are the mean ± S.E. of three or four rats. Significantly ($p < 0.05$) different from the corresponding control a) or sham-operated b) values.

decrease of cytochrome P-450 content in regenerating liver as shown by other investigators[3,4,6] and added further insight into the increase of heme oxygenase activity in relation to the decrease of the hemoprotein after partial hepatectomy in normal and adrenalectomized rats. Though the present experimental conditions and biochemical variables determined were somewhat different from those of Presta *et al.*,[7] the results of this study would suggest that the decrease of cytochrome P-450 content as well as drug-metabolizing enzyme activities in regenerating liver is not simply due to the surgical stress, but rather associated with the increase of heme oxygenase activity.

Sardana *et al.*[27] have shown that cobalt-mediated induction of heme oxygenase and the decrease of cytochrome P-450 content are enhanced in adrenalectomized rats as compared to normal rats. They have also shown that the treatment of the adrenalectomized rats with hydrocortisone normalizes the metal-mediated induction of heme oxygenase. The findings indicate that the adrenal gland controls heme oxygenase activity. Therefore, the enhancement of the induction of heme oxygenase in adrenalectomized rats following partial hepatectomy, as shown in Table II, would be a reasonable phenomenon.

It remains to be elucidated that what kind of factors is involved in the induction of heme oxygenase during liver regeneration. In this respect, a published report by Bakken *et al.*[19] is very suggestive. They have shown that the administrations of insulin, glucagon and epinephrine resulted in the increase of heme oxygenase activity of rat liver. These hormones which are able to increase heme oxygenase activity are thought to be hepatotrophic factors for liver regeneration and increased in blood levels during early stages of liver regeneration.[18] Thus, such changes of these hormones concentration might be involved in the induction of heme oxygenase in regenerating liver. On the other hand, if there were derangement of heme concentration and/or labilization of heme from cytochrome P-450 in the remnant liver, such endogenous heme might be a candidate for an inducer of heme oxygenase in regenerating liver, as seen in the case of hemin[10,11] or endotoxin administration.[16,17] But, further in-depth studies will be necessary to clarify the mechanism for the induction of heme oxygenase in regenerating rodent liver.

Considering the induction of heme oxygenase during liver regeneration, it is of interest to point out that biliverdin, the enzyme-catalized metabolite of heme, is able to induce mitosis of liver cells and the exogenous administration of the compound also causes the increase of mitotic index of rat liver.[28] Based on these facts, the persistent increase of heme oxygenase activity during the early stages of liver regeneration (Fig. 2), thus may leading to a sustained increase of biliverdin concentration in the liver, seems likely to be an adaptive biochemical change. The findings that heme oxygenase activity is under the increased state in fetal[29] and neonatal[30,31] rat liver might be well compatible with the results seen in the present investigation. Accordingly, it could be possible to note that the increased activity of heme oxygenase will be seen under some conditions of the growing liver.

In conclusion, the present findings indicate that the induction of heme oxygenase would be correlated to the decrease of cytochrome P-450 content as well as drug-metabolizing enzyme activities during the course of liver regeneration. Additionally, the findings that the induction of heme oxygenase seen in regenerating liver would provide information on a biochemincal event of heme metabolism under the experimental conditions.

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