Induction of Serum Haptoglobin by Administration of Ethionine to Cows

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ABSTRACT. In order to assess the association of cow serum haptoglobin with fatty liver, haptoglobin concentrations were evaluated by single radial immunodiffusion in sera from cows after administration of ethionine (an analogue of methionine). Haptoglobin concentrations were found to be increased in the sera of the ethionine-administered cows. This result suggests that the induction of haptoglobin is associated in the pathogenesis of fatty liver.—KEY WORDS: ethionine, fatty liver, haptoglobin.


Haptoglobin (Hp) is one of the acute phase proteins and is believed to be induced by acute inflammation [1, 7]. Evidence for the induction of Hp other than by inflammation has been accumulated recently. Through the pioneering work by Minoccheri [9], it became evident that Hp was induced in cows by transportation exhaustion. We have reported that a (35K + 23K) protein could be detected in cow serum in natural cases of fatty liver [13], and this protein was identified as Hp [17]. Fatty liver develops as a result of several complicated factors, including excess feeding before parturition, stress, starvation and negative energy balance attributable to milk production [3, 10]. Because this condition particularly occurs at the peripartum period, when levels of steroid hormones such as glucocorticoids and estrogens are greatly altered [14], the hormones are postulated to be involved in the development of fatty liver [4, 12, 17, 18].

Ethionine, an analogue of methionine, induces fatty liver in rats by inhibiting protein synthesis in liver [16], particularly that of apolipoprotein [2, 5, 6]. We have recently succeeded in inducing fatty liver in cows by ethionine administration [15]. Hp concentration was expected to increase in the sera of the ethionine-administered cows because of the association of Hp with normal cases of fatty liver. However, Hp could not be detected in the cows by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). We have recently developed a method for evaluating Hp by single radial immunodiffusion using anti-Hp rabbit serum [18]. The present study was therefore undertaken to evaluate Hp concentrations by this immunologic method in the ethionine-administered cows.

Ethionine (Nakarai Chemical Co., Kyoto, Japan) was administered intraperitoneally to four cows [3 normal cows (E1, E2, E3) and 1 cow with fatty liver (E4)], at a dose of 25 mg of ethionine/kg body weight at 1 week intervals [15]. The E4 cow was included, because the coincident decreases of both serum triglyceride (TG) and apoB-100 (a main TG-binding protein) concentrations were clearly demonstrated [15]. Another cow (control) received vehicle (0.9% NaCl) alone. Fatty accumulation to liver was monitored by biopsy, for which the right flank was incised for 1–2 cm, with a 1-week interval. All ethionine-administered cows showed a significant increase of liver TG contents. Blood was collected before feeding and serum was stored at −20°C until use. Single radial immunodiffusion was carried out [18]. Serum cortisol and α1-acid glycoprotein (AGP) concentrations were evaluated using kits from Diagnostic Product Corporation (Los Angeles, CA, U.S.A.) and Saikinagakaku Institute (Sendai, Japan), respectively.

Using single radial immunodiffusion, Hp could be detected in sera of cows E1, E2 and E3 (Table 1). Hp was undetectable in serum of the control cow, indicating that the surgical incision of skin and peritoneum for biopsy had no effect on Hp induction. In cow E4, which had 119 mg of TG/g liver before administration [15], Hp was detected in serum before administration, which was consistent with our previous reports [13, 15, 17]. After the decrease at day 8, Hp concentration was again increased at day 10.

The serum cortisol concentration was evaluated to examine the possibility of participation of glucocorticoids in inducing Hp in the ethionine-administered cows, because the rat Hp gene includes a glucocorticoid-responsive element [8], and Hp is induced by dexamethasone administration to fasted cows [18]. In cows E1-E3, the increase of cortisol concentrations was followed, at least partly, by that of Hp (Table 1). For example, the increase in E1 was observed at day 12 (cortisol) and day 16 (Hp). Similarly, the increased concentrations were noted in E2 at day 12 (cortisol) and day 14 (Hp), and in E3 at day 4 (cortisol) and day 6 (Hp). However, the cortisol concentration also increased in the control in which Hp was not induced. It remains unanswered at present whether glucocorticoids play a causal role in the induction of Hp in ethionine-administered cows. The serum concentration of AGP (a marker for inflammation) was also measured (Table 1). The concentration was not changed in cows E1 and E2. The slight, but significant increases were observed at days 12 and 14 in E3 and at days 8 and 10 in E4. The increase in E3 was preceded by Hp induction at day 6 and fatty accumulation of liver at day 7 [15], suggesting that AGP is induced at late phase of fatty liver development. The relatively higher value at day 0 in E4, compared to those in E1-E3, also supports this possibility.

As we suggested previously [13], the present study provided evidence that cow Hp was associated with the experimentally induced fatty liver, as with the natural

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cases of fatty liver. Because Hp is thought to have immunosuppressive activity [11], the reduced immune competence observed in fatty liver cows [3] may be causally linked to the Hp appearance in serum. The induction of Hp, as well as the decreased concentration of apolipoprotein B-100 [15] further strengthen the hypothesis that ethionine-induced fatty liver is a useful model for the study of the pathogenesis of fatty liver in cows.

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REFERENCES