Pergamon

Biochemical Pharmacology, Vol. 50, No. 8, pp. 1309–1312, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00265-0

Short Communication

EFFECT OF FOOD INTAKE ON THE ACTIVITY OF LIVER ENZYMES IN PARTIALLY HEPATECTOMIZED RATS TREATED WITH TUMOR NECROSIS FACTOR

WALID G. YASMINEH,*† H. STEPHEN BEYER,‡ JANELLE I. CASPERS‡ and ATHANASIOS THEOLOGIDES‡

*Department of Laboratory Medicine and Pathology, University Hospital, and ‡Department of Medicine, Hennepin County Medical Center, University of Minnesota Medical School, Minneapolis MN 55455, U.S.A.

(*Received* 24 *February* 1995; *accepted* 27 *April* 1995)

Abstract—After partial hepatectomy (PHx), there are significant changes in the activity of a number of enzymes in the regenerating rat liver. Administration of low doses of recombinant human tumor necrosis factor-α (rHu-TNF) to normal rats induces similar changes in some of the enzymes but not in others. Because certain observations suggest that TNF may play a dominant role in liver regeneration, we speculated that the discrepancies in enzyme activities may be due to the decrease in food intake caused by PHx. Accordingly, the activities of eleven liver enzymes of 70% PHx rats additionally treated i.p. with rHu-TNF (20–50 μg/kg/day for 3–4 days) were compared with those of (i) PHx controls fed *ad lib.*, and (ii) PHx controls pair-fed the same amount of food. When pair-fed controls were used, the discrepancies in the activities of the enzymes that are affected by fasting tended to disappear, suggesting that the decrease in the food intake was responsible for the differences.

Key words: enzymes; TNF; liver regeneration; food intake

After 70% PHx§ in the rat, there is a marked change in the activity of several hepatic enzymes. Most notably there is a significant increase in G6PD, TA, and AP, and a decrease in ALT and CAT [1–11]. Similar changes have been observed in rat liver following i.p. injection of small doses of rHu-TNF [12–14]. On the other hand, the activities of several other hepatic enzymes are different in PHx and TNF-treated rats. G6P, which decreases after TNF treatment, is unchanged after PHx [9, 10, 15, 16], while PEPCK, LD, AST and 5′-NT, which are unchanged after TNF treatment, are increased [10, 15–19].

Certain observations suggest that TNF may play a prominent role in liver regeneration. After PHx, rat liver regeneration is enhanced significantly by endotoxin [20] and inhibited by antibodies to TNF [21]. In this study, since food intake is reduced drastically after PHx, we attempted to determine whether the differences observed between the enzyme activities of TNF-treated and PHx rats are due to the difference in food intake.

† Corresponding author: Walid Yasmineh, Ph.D., University of Minnesota Hospital and Clinic, Box 609, Mayo Memorial Building, 420 Delaware Street SE, Minneapolis, MN 55455. Tel. (612) 626-5121; FAX (612) 625-6994.

§ Abbreviations: PHx, partial hepatectomy or partially hepatectomized; rHu-TNF, recombinant human tumor necrosis factor-α; ShO, sham-operated; PHx-TNF, partially hepatectomized and TNF-treated; G6P, glucose-6-phosphatase (EC 3.1.3.9); FDP, fructose-1,6-diphosphatase (EC 3.1.3.11); PEPCK, phosphoenolpyruvate carboxykinase (EC 4.1.1.32); TA, transaldolase (EC 2.2.1.2); CAT, catalatic activity of catalase (EC 1.11.1.6); PER, peroxidatic activity of catalase; G6PD, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); LD, lactate dehydrogenase (EC 1.1.1.27); AST, aspartate aminotransferase (EC 2.6.1.1); ALT, alanine aminotransferase (EC 2.6.1.2); AP, alkaline phosphatase (EC 3.1.1.3); and 5′-NT, 5′-nucleotidase (EC 3.1.3.5).

Materials and Methods

Animals. All animal studies were approved by the Animal Use Committee of Hennepin County Medical Center. Male Sprague-Dawley rats (190 ± 14 g) were maintained on a 12-hr light/dark cycle and fed Purina rat chow as indicated below. Two-thirds partial hepatectomy was performed under ether anesthesia as previously described [22]. Sham operation consisted of laparotomy and liver manipulation.

Chemicals. rHu-TNF was donated by the Asahi Chemical Industry of America and contained a negligible amount of endotoxin as contaminant (2.33×10^6 units TNF/mg protein; 0.35 pg endotoxin/10^6 units TNF; equivalent to 0.001 pg endotoxin/μg TNF) [13].

Experimental. Two experiments were performed. The first included ten PHx rats injected i.p. every 4 hr with 7.5 μg/kg rHu-TNF for 4 days and, as controls, eight ShO and six PHx rats injected with the vehicle only (10% glycerol). All animals were fed *ad lib.*, and the daily food intake was recorded. The second experiment included five PHx rats injected i.p. every 4 hr with 3.3 μg/kg rHu-TNF for 3 days, and five control PHx weight-matched rats that were injected with the vehicle only and pair-fed daily the same amount of food consumed by their TNF-treated partners on the previous day. Food intake was measured by giving each animal a weighed amount of food each morning and subtracting the weight left over the following morning. In a slight modification of this experiment, the experimental animals were injected for 2 days with TNF prior to PHx, and for 3 more days after PHx, while the control rats were injected for 5 days with the vehicle only.

On the day following the end of the injection schedule, all PHx, PHx-TNF and ShO rats were anesthetized with ether and killed by guillotine decapitation. Pair-fed PHx control rats were killed 1 day later. The livers were excised and homogenized in 0.2 M phosphate buffer, pH 7.4, as previously described [13]. The homogenates were centrifuged at 30,000 *g* for 30 min, and the supernatant fluids were kept at −20° until assayed.

Biochemical analyses. Protein concentration and enzyme ac-

tivities of the liver extracts were determined as previously described [12–14, 23].

Statistical analysis. All data were statistically analyzed by the unpaired, two-tailed Student's *t*-test.

Results and Discussion

When fed *ad lib.*, the mean food intake of ShO rats was slightly lower (20%; $P < 0.05$) than that of normal rats on day 1 after the operation, but was back to normal on the following day. In contrast, the mean food intakes of both PHx rats and PHx-TNF rats were significantly lower than those of ShO rats. PHx rats consumed 91% less food ($P = 0.0001$) on day 1 after the operation, 60% less on day 2 ($P = 0.0001$), 40% less on day 3 ($P = 0.0003$), and the same amount of food on day 4. In the PHx-TNF rats, the decrease in food intake was comparable to that of the PHx rats on days 1 and 2 after partial hepatectomy (or 85 and 71% lower than that of ShO rats, respectively), but was more severe on day 3 (61% lower; $P < 0.01$), and was still significantly low (54% lower; $P = 0.001$) on day 4. Thus, food intake was reduced appreciably in the PHx rats, and more so in the PHx plus TNF-treated rats. In contrast, the treatment of rats with low levels of rHu-TNF alone for several days has been shown previously to cause only a small decrease (about 20%) in food intake on day 1 of treatment [13], as was obtained here after sham operation. Since TNF has a short half-life in the circulation (about 20 min), these results suggest that partial hepatectomy may cause a continuous endogenous release of TNF and/or other cytokines which, together with the additional exogenous TNF administration, causes a prolonged decrease in food consumption.

Table 1 shows the hepatic enzyme activities (means ± SD, units per g protein) of ShO rats, PHx rats and PHx-TNF rats, fed *ad lib.* Comparison of the activities of PHx-TNF rats with those of PHx rats as controls indicated that TNF treatment caused significant changes in eight enzymes ($P \leq 0.005$). These included PEPCK and TA, which increased by 119 and 38% in the TNF-treated rats, and G6P, FDP, CAT, PER, LD and ALT, which decreased by 53, 35, 38, 46, 19, and 47%, respectively. Since, however, the control animals were fed *ad lib.*, some of these changes probably resulted from the decrease in the food intake of the TNF-PHx rats.

When a similar comparison was made between PHx-TNF rats and ShO rats as controls, the same changes occurred in six of the eight enzymes, but the changes were more pronounced because of the more pronounced decrease in food intake (see above). Thus, the activities of PEPCK and TA increased by 148 and 89%, while those of G6P, FDP CAT and PER decreased by 62, 53, 68, and 60%, respectively ($P < 0.0001$; not shown in table). The remaining two enzymes showed slightly different results; ALT activity again decreased, but the decrease was smaller (29%; $P < 0.01$), while LD showed a small increase in activity (12%; $P < 0.005$) instead of a small decrease.

Four enzymes (G6PD, AST, AP and 5′-NT) did not show any significant difference between PHx-TNF and PHX rats. However, they showed significant differences when the activities of PHx-TNF rats were compared with those of ShO rats. These included large increases in the activities of G6PD and AP (115 and 280%; $P < 0.0001$), and moderate increases in AST and 5′-NT activity (23 and 38%; $P < 0.05$). Close examination of the activities of PHx-TNF versus PHx rats indicated that three of these four enzymes, G6PD, AST and AP, also showed increases of 38, 9 and 19%, respectively, which, however, did not attain statistical significance.

When the enzymatic activity changes of PHx-TNF versus PHx rats fed *ad lib.* (Table 1) were compared with those previously obtained after the treatment of rats with rHu-TNF alone [12, 14], seven of the twelve enzyme activities showed similar patterns, including TA, G6P, CAT, PER, AST, ALT and 5′-NT. The remaining five, FDP, PEPCK, G6PD, LD and AP, showed appreciable discrepancies that had to be explained. Since the treatment of rats with low doses of rHu-TNF alone has only a minor effect on food intake [13], and the food intake of PHx-TNF rats is reduced much more drastically than that of PHx rats (see above), these observations suggested that the discrepancies were due mainly to the difference in food intake.

Table 2 shows the enzyme activities (means ± SD) of PHx rats treated with rHu-TNF (3.3 μg/kg every 4 hr for 3 days) compared with those of PHx control rats whose daily food intake was equally reduced by pair-feeding. Comparison of the mean enzyme activities of the PHx-TNF with those of the PHx-PF control partners showed that seven of the twelve activities were significantly different. The differences included a significant increase in TA (28%), G6PD (114%) and AP (220%), and

Table 1. Hepatic enzyme activities of sham-operated rats (ShO), partially hepatectomized rats (PHx), and partially hepatectomized rats treated with TNF (PHx-TNF) fed *ad lib.*

Enzyme	Enzyme activity (units/g protein) ShO (N = 8)	PHx (N = 6)	PHx-TNF* (N = 10)	TNF/PHx†
G6P	14.8 ± 1.1	11.8 ± 2.6	5.6 ± 2.1‡	0.47
FDP	51 ± 4.4	37 ± 0.9	24 ± 3.1‡	0.65
PEPCK	23 ± 2.3	26 ± 9.6	57 ± 15‡	2.19
TA	46 ± 3.3	63 ± 15.4	87 ± 11.0‡	1.38
CAT§	434 ± 28	226 ± 11	139 ± 4.4‡	0.62
PER	1786 ± 106	1321 ± 97	714 ± 45‡	0.54
G6PD	11.3 ± 1.3	17.6 ± 6.5	24.3 ± 9.0	1.38
LD	5270 ± 177	7273 ± 534	5902 ± 560‡	0.81
AST	1184 ± 93	1338 ± 137	1456 ± 230	1.09
ALT	168 ± 44	223 ± 33	119 ± 19‡	0.53
AP	3.2 ± 0.7	10.3 ± 5.5	12.3 ± 6.7	1.19
5′-NT	39 ± 4.0	61 ± 8.8	54 ± 20	0.88

Values are means ± SD.

* The PHx-TNF rats received, after partial hepatectomy, an i.p. dose of 7.5 μg/kg TNF every 4 hr for 4 days.

† TNF/PHx = PHx-TNF (column 4) over PHx (column 3) ratio.

‡ Enzyme activities of PHx-TNF rats significantly different from those of PHx rats at $P < 0.005$.

§ CAT activity is in kilounits.

Table 2. Hepatic enzyme activities of partially hepatectomized rats treated with TNF (PHx-TNF) and their corresponding PHx pair-fed controls (PHx-PF)

Enzyme	Enzyme activity (units/g protein) PHx-PF (N = 5)	PHx-TNF* (N = 5)	TNF/PF†	P value
G6P	8.9 ± 1.0	0.8 ± 0.6	0.09	<0.0001
FDP	32.2 ± 2.9	30.8 ± 2.1	0.96	NS‡
PEPCK	15.4 ± 3.8	15.9 ± 2.5	1.03	NS
TA	58 ± 5.3	74 ± 12	1.28	0.02
CAT§	225 ± 33	103 ± 21	0.46	<0.0001
PER	906 ± 109	349 ± 39	0.39	<0.0001
G6PD	10.9 ± 1.2	23.3 ± 8.4	2.14	<0.0001
LD	5149 ± 461	5232 ± 360	1.02	NS
AST	1329 ± 119	1199 ± 322	0.90	NS
ALT	184 ± 53	108 ± 7.3	0.59	0.01
AP	5.9 ± 1.6	18.9 ± 3.0	3.20	<0.0001
5′-NT	24.6 ± 3.9	24.8 ± 7.2	0.99	NS

Values are means ± SD.

* The PHx-TNF rats received after partial hepatectomy an i.p. dose of 3.3 μg/kg TNF every 4 hr for 3 days.

† TNF/PF = PHx-TNF over PHx-PF ratio.

‡ NS = not significant.

§ CAT activity is in kilounits.

a significant decrease in G6P (91%), CAT (54%), PER (61%) and ALT (41%). The remaining five enzymes, FDP, PEPCK, LD, AST and 5′-NT, showed little or no change. When these changes were compared with those previously obtained for rats injected with TNF alone ([12–14]; Table 3), a good correlation was obtained; namely, in both cases there was a significant increase in TA, G6PD and AP, a decrease in G6P, CAT, PER and ALT, and little or no change in FDP, PEPCK, LD, AST and 5′-NT. These results indicate that the discrepancies in the activities of the five enzymes (FDP, PEPCK, G6PD, LD and AP) observed in Table 1, using PHx rats fed *ad lib.* as controls, were resolved upon equalization of the food intake. The most important of these corrections were those involving the activities of the gluconeogenic enzymes PEPCK and FDP, whose activities have been shown previously to increase by 300% and decrease by 60%, respectively, after a 24-hr fast [14]. The third gluconeogenic enzyme, G6P, which also has been shown to increase appreciably in activity (300%) after a 24-hr fast [14], was not among the discrepant enzymes, but upon equalization of food intake showed a more pronounced decrease in activity than previously obtained in rats treated with TNF alone (91 vs 44%; Table 3).

When PHx-TNF rats were additionally pretreated for 2 days with TNF prior to hepatectomy and compared with those of pair-fed controls, the mean enzyme activities were essentially similar to those shown in Table 2, suggesting that TNF pretreatment prior to hepatectomy does not affect the magnitude of the changes significantly.

Thus, the results indicate that when PHx pair-fed rats are used as controls, the changes in the regenerating liver of PHx-TNF rats are remarkably similar to those previously observed in our laboratory after TNF treatment alone [12–14]. The hepatic enzymes involved in these changes are varied and include key enzymes in gluconeogenesis (G6P, ALT), the pentose phosphate pathway (G6PD, TA), and the scavenging of hydrogen peroxide (CAT/PER). The biochemical implications of these changes have already been discussed [12–14, 24]. Briefly, they suggest that after low-dose TNF treatment, the hepatocyte is concerned primarily with the elimination of hydrogen peroxide (and, consequently, of oxygen-derived free radicals) by releasing CAT into the circulation and increasing the activity of G6PD [13, 24]. On the other hand, in contrast to the increase in gluconeogenesis which occurs upon fasting, gluconeogenesis was inhibited mainly by a significant decrease in G6P activity (Table 3), which, incidentally, also increased the concentration of glucose phosphate to be metabolized via the pentose phosphate shunt. The significant increase in AP activity has been shown histologically to be associated with the canalicular regions of the hepatocytes [12], but its significance is still unknown. The increase in TA is probably a reflection of the increase in mitotic activity and nucleic acid synthesis [12].

Table 3. Mean relative enzyme activities of PHx-TNF rats (from Table 2) and TNF-treated rats (from previous reports) listed as fractions of their respective controls

Enzyme	TNF/PF ratio*	TNF†	Reference‡
G6P	0.09	0.56	14
FDP	0.96	0.87	14
PEPCK	1.03	0.91	14
TA	1.28	1.14	14
CAT	0.46	0.60	13
PER	0.39	0.58	13
G6PD	2.14	1.66	13
LD	1.02	0.98	13
AST	0.90	0.85	12
ALT	0.59	0.40	12
AP	3.20	3.09	12
5′-NT	0.99	0.98	12

* This is the PHx-TNF over PHx-PF ratio from Table 2.

† Previous data from rats treated with TNF alone (100 μg/kg/day, for 5 days, i.p.). This low-dose TNF treatment has little or no effect on food intake [13].

‡ References from which TNF data were obtained.

Acknowledgements—We wish to acknowledge the technical assistance of Kathy McAllen. This work was supported by a grant from Hennepin Faculty Associates of the Minneapolis Medical Research Foundation.

REFERENCES

1. Taketa K, Tanaka A, Watanabe A, Takesue A, Aoe H and Kosaka K, Undifferentiated patterns of key carbohydrate metabolizing enzymes in injured livers. *Enzyme* **21:** 158–173, 1976.
2. Schlaeger R, Haux P and Kattermann R, Studies on the mechanism of the increase in serum alkaline phosphatase activity in cholestasis: Significance of the hepatic bile acid concentration for the leakage of alkaline phosphatase from rat liver. *Enzyme* **28:** 3–13, 1982.
3. Wright GH, Changes in plasma membrane enzyme activities during liver regeneration in the rat. *Biochim Biophys Acta* **470:** 368–381, 1977.
4. Van Noorden CJF, Vogels IMC and Houtkooper JM, Cytophotometric analysis of alkaline phosphatase and 5′-nucleotidase activity in regenerating rat liver after partial hepatectomy. *Cell Biochem Funct* **6:** 53–60, 1988.
5. Gawlik Z, Najberg G, Aleksandrowicz R and Wisniewska IE, The activity of alkaline phosphatase in the process of regeneration of rat liver. *Folia Histochem Cytochem* **14:** 91–97, 1976.
6. Goldenberg H, Hüttinger M, Böck P and Kramar R, Influence of subtotal hepatectomy on peroxisomes and peroxisomal enzymes of rat liver and isolated liver cell fractions. *Histochemistry* **44:** 47–56, 1975.
7. Liotti FS, Menghini AR, Guerrieri P, Mariucci G, Locci P and Bruschelli G, Variations in catalase, glutathione peroxidase and superoxide dismutase in regenerating rat liver. *Cell Mol Biol* **33:** 611–617, 1987.
8. Bengmark S, Ekholm R and Olsson R, Transaminase activity in subcellular fractions of the normal and regenerating rat liver. *Acta Hepato-splenol* **6:** 80–88, 1976.
9. Camargo ACM and Migliorini RH, Gluconeogenesis in liver slices from partially hepatectomized rats. *Proc Soc Exp Biol Med* **136:** 962–966, 1970.
10. Brinkmann A, Katz N, Sasse D and Jungermann K, Increase of the gluconeogenic and decrease of the glycolytic capacity of rat liver with a change of the metabolic zonation after partial hepatectomy. *Hoppe Seyler's Z Physiol Chem* **359:** 1561–1571, 1978.
11. Heinrich PC, Morris HP and Weber G, Behavior of transaldolase (EC 2.2.1.2) and transketolase (EC 2.2.1.1) activities in normal, neoplastic, differentiating, and regenerating liver. *Cancer Res* **36:** 3189–3197, 1976.
12. Yasmineh WG, Manivel JC, Nakleh RE, Caspers JI, Parkin JL and Theologides A, Tumor necrosis factor causes enzymatic changes in liver comparable to those in extrahepatic cancer. *Proc Soc Exp Biol Med* **203:** 193–199, 1993.
13. Yasmineh WG, Parkin JL, Caspers JI and Theologides A, Tumor necrosis factor/cachectin decreases catalase activity in rat liver. *Cancer Res* **51:** 3990–3995, 1991.
14. Yasmineh WG and Theologides A, Effect of tumor necrosis factor on enzymes of gluconeogenesis in the rat. *Proc Soc Exp Biol Med* **199:** 97–103, 1992.
15. Katz N, Correlation between rates and enzyme levels of increased gluconeogenesis in rat liver and kidney after partial hepatectomy. *Eur J Biochem* **98:** 535–542, 1979.
16. Andersen B, Zierz S and Jungermann K, Alteration in zonation of succinate dehydrogenase, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in regenerating rat liver. *Histochemistry* **80:** 97–101, 1984.
17. Horio Y, Sakakibara R, Tanaka T, Taketoshi M, Obaru K, Shimada K, Morino Y and Wada H, Molecular cloning of rat mitochondrial glutamic oxaloacetic transaminase mRNA and regulation of its expression in regenerating liver. *Biochem Biophys Res Commun* **134:** 803–811, 1986.
18. Schofield PS, Kerbey AL and Sugden MC, Hepatic pyruvate metabolism during liver regeneration after partial hepatectomy in the rat. *Int J Biochem* **18:** 453–458, 1986.
19. Masuda Y, Nishimura T, Nojiri T and Murano T, Changes in some marker enzyme activities of liver plasma membranes during regeneration after partial hepatectomy in rats. *Biochim Biophys Acta* **426:** 335–338, 1976.
20. Cornell RP, Acute phase responses after acute liver injury by partial hepatectomy in rats as indicators of cytokine release. *Hepatology* **11:** 923–931, 1990.
21. Akerman P, Cote P, Yang SQ, McLain C, Nelson S, Bagby GJ and Diehl AN, Antibodies to tumor necrosis factor-α inhibit liver regeneration after partial hepatectomy. *Am J Physiol* **26:** G579–G585, 1992.
22. Beyer HS, Stanley M and Theologides A, Tumor necrosis factor-α increases hepatic DNA and RNA and hepatocyte mitosis. *Biochem Int* **22:** 405–410, 1990.
23. Yasmineh WG, Chung M and Caspers JI, Determination of serum catalase activity on a centrifugal analyzer by an NADP/NADPH coupled enzyme reaction system. *Clin Biochem* **25:** 21–27, 1992.
24. Yasmineh WG and Theologides A, Catalase as a roving scavenger of hydrogen peroxide: A hypothesis. *J Lab Clin Med* **122:** 110–114, 1993.