TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2009; 39 (6): 875-880
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0804-20

Does L-carnitine increase serum TNF -α and IGF-1 during liver regeneration in the rat?

Mevlüt PEHLİVAN[1]
Abdurrahman COŞKUN[2]
Ahmet ZENGİN[1]
Arif ASLANER[1]
Tevfik YAVUZ[3]

[1] Department of General Surgery, Faculty of Medicine, Düzce University, Konuralp, Düzce - TURKEY
[2] Clinical Biochemistry, Faculty of Medicine, Düzce University, Konuralp, Düzce - TURKEY
[3] Microbiology, Faculty of Medicine, Düzce University, Konuralp, Düzce - TURKEY

Aim: We sought to evaluate the effect of L-carnitine administration on the levels of IGF-1, TNF-α, and lipid peroxidation during hepatic regeneration for up to 72 h in an experimental partial hepatic resection rat model.

Materials and methods: Sixty rats were divided into 3 groups: control and low-dose (100 mg/kg) and high-dose (200 mg/kg) L-carnitine. Each group was divided into 2 sub groups (24 and 72 h after partial hepatectomy). Partial (70%) hepatectomy was performed after 7 days of intra-peritoneal administration of L-carnitine. After partial hepatectomy, L-carnitine was also administered to rats until sacrifice. Serum IGF-1, TNF-α, and tissue malondialdehyde levels were determined in all groups before and at 24 and 72 h after surgery.

Results: Serum TNF-α increased significantly in the control group during the regeneration period. The low dose of L-carnitine (100 mg/kg) decreased the elevation of TNF-α whereas the high dose (200 mg/kg) increased it. In the control group, the IGF-1 level decreased in the first 24 h after surgery and then increased. The IGF-1 level behaved similarly in the L-carnitine groups. The malondialdehyde level in the control group increased during the first 24 h and then decreased. Similarly in the L carnitine groups, the malondialdehyde level increased during the first 24 h and then decreased significantly in a dose-dependent manner.

Conclusion: Three types of biochemical pathways are essential in liver regeneration: cytokine, growth factor, and metabolic pathways. We conclude that, in addition to its effects on energy metabolism, high-dose L-carnitine may promote liver regeneration by increasing IGF-1, TNF-α, and decreasing malondialdehyde.

Key words: Liver regeneration, partial hepatectomy, L-carnitine, TNF-α, IGF-1

L-karnitin, karaciğer rejenerasyonu sırasında serum TNF-α ve IGF-1 düzeylerini artırıyor mu?

Amaç: Rat deneysel parsiyel hepatektomi modelinde L-karnitin uygulamasının 72. saate kadar IGF-1, TNF α ve lipid peroksidasyonuna etkisini araştırmayı amaçladık.

Yöntem ve gereç: Atmış rat kontrol, düşük (100 mg/kg) ve yüksek doz (200 mg/kg) L-karnitin olmak üzere 3 gruba ayrıldı. Her bir grup, parsiyel hepatektomiden sonra 24 ve 72. saat olmak üzere 2 alt gruba ayrıldı. Yedi gün intra-peritoneal L-karnitin uygulamasından sonra parsiyel (% 70) hepatektomi yapıldı. L-karnitin, parsiyel hepatektomiden sonra da ratlara verilmeye devam edildi. Tüm gruplarda cerrahi operasyondan önce, 24 ve 72 saat sonra serum IGF-1, TNF-α ve doku malondialdehid düzeyleri ölçüldü.

Bulgular: Kontrol grubunda, serum TNF-α düzeyleri, rejenerasyon boyunca anlamlı derecede arttı. Düşük doz (100 mg/kg) L-karnitin, TNF-α düzeylerini azaltırken yüksek doz (200 mg/kg) artırdı. IGF-1 düzeyi, kontrol grubunda cerrahi operasyondan sonra ilk 24 saate azalırken daha sonra artmaya başladı. L-karnitin gruplarında IGF-1 düzeylerinde benzer değişimler gözlendi. Malondialdehid düzeyleri kontrol grubunda ilk 24 saatte artarken daha sonra azalmaya başladı. Benzer şekilde L-karnitin gruplarında malondialdehid düzeyleri ilk 24 saatte artarken daha sonra doza bağımlı olarak azaldı.

Sonuç: Karaciğer rejenerasyonunda sitokinler, büyüme faktörleri ve metabolik yollar olmak üzere üç temel unsur bulunmaktadır. Enerji metabolizmasındaki etkilerine ilaveten, yüksek doz L-karnitinin, IGF-1, TNF-α düzeyini artırarak ve malondialdehid düzeyini de azaltarak karaciğer rejenerasyonunu hızlandırabileceğini düşünmekteyiz.

Anahtar sözcükler: Karaciğer rejenerasyonu, parsiyel hepatektomi, L-karnitin, TNF-α, IGF-1

Received: April 08, 2008
Accepted: August 17, 2009

Correspondence
Abdurrahman COŞKUN
Department of Biochemistry, Faculty of Medicine, Düzce University, Konuralp, Düzce - TURKEY

coskun2002@gmail.com

Introduction

Liver regeneration is a process in which liver mass is restored by a compensatory hyperplastic response following liver tissue loss or injury. With advances in anatomical knowledge and surgical techniques, liver resections are performed with reasonable mortality and morbidity. In clinical practice, main types of liver resection are primary malignant tumors and metastases; benign tumors, such as hemangiomas, adenomas, and hydatid cysts; trauma; and donor hepatectomy-related liver transplantations. Recent advances have resulted in extended resections in advanced liver lesions. For this reason, regenerative responses and factors related to liver regeneration are major concerns in liver surgery.

Various nutritional and pharmacological agents have been used to promote liver regeneration in animal models (1,2). In the early phase of liver regeneration, the energy substrate changes from glucose to fatty acids (3). L-carnitine is an important carrier of long-chain free fatty acids from the cytosol to the mitochondria for β-oxidation and has been suggested to play a key role in liver regeneration (4,5). L-carnitine is present in the diet and is synthesized de novo. The last step of L-carnitine biosynthesis is the hydroxylation of butyrobetaine to L-carnitine, which in rats occurs primarily in the liver (6). Blaha and co-workers found that the administration of L-carnitine stimulated liver regeneration in a dose-dependent manner (4). Liver regeneration involves a large number of genes and requires 3 types of pathways: cytokine, growth factor, and metabolic pathways (7). In the present study, we investigated the effect of L-carnitine on the levels of tumor necrosis factor (TNF)-α, insulin-like growth factor (IGF)-1, and lipid peroxidation during liver regeneration for up to 72 h using a rat model.

Materials and methods

The study was approved by the Animal Care and Ethics Committee of Düzce School of Medicine. Experimental procedures were performed in the animal research laboratory at the same institute. Male Wistar albino rats (n = 60) weighing 230-290 g (mean, 240 g) were randomly assigned to 6 groups (10 per group), and partial (70%) hepatectomy (PH) was performed after 7 day intra-peritoneal administration of L-carnitine (CARNITINE®, Sigma Tau, Industrie Farmaceutiche Riunite S.p.A., Pomezia, Italy) as described in Table 1. After partial hepatectomy, L-carnitine was also administered until sacrifice. All rats were fed a commercial pellet diet and were allowed water ad libitum.

Anesthesia and surgery

Surgical Technique: All animals were fed in standard fashion, and no bowel preparation was applied. After 12 h of fasting, all animals were anesthetized by intramuscular ketamine 50 mg/kg (Ketalar, Parke-Davis, Eczacıbaşı) and chlorpromazine 0.1 mg/kg (Largactil, 25-mg ampoule; Rhone-Poulenc, Eczacıbaşı). The skin was cleansed with povidone-iodine after shaving. The liver was exposed through a midline laparotomy; the left lateral and median lobes were exposed, and a 70% hepatectomy was carried out as described by Higgins and Anderson (8). The incision was closed with 4/0 silk, in a continuous fashion. Rats were re-operated according to the time plan for each group (Table 1). Residual liver tissue was resected, and a 2-mL blood sample was obtained from the inferior vena cava. Tissue samples were used for the determination of wet tissue malondialdehyde (MDA), a product of liver peroxidation.

Biochemical measurements

Serum TNF-α levels were measured by a solid-phase sandwich enzyme linked immunosorbent assay (ELISA; Biosource, Nivelles, Belgium). The minimum detection limit and within assay CV were 4 pg/mL and 2.7%, respectively. Serum IGF-1 levels were measured by enzyme immunoassay (EIA; DSL, Webster, Texas, USA). The minimum detection limit and within assay CV were 30 ng/mL and 5.3%, respectively. Tissue MDA levels were measured using the method described by Mihara et al. (9).

Statistical analysis

Data are expressed as means ± SDs. Differences between groups were analyzed using analysis of variance. For significant F values, differences between means were analyzed with a post-hoc Tukey test. Values of $P < 0.05$ were deemed statistically significant.

Table 1. Study design and characteristics of control and treatment groups.

Groups (n = 10)	*Medication*	*Surgical procedure*	*Second procedure*
Group 1	Standard feeding (SF)	70% hepatectomy	Residual liver resection and sacrifice after 24 h
Group 2	SF	70% hepatectomy	Residual liver resection and sacrifice after 72 h
Group 3	SF + L-carnitine (100 mg/kg)	70% hepatectomy	Residual liver resection and sacrifice after 24 h
Group 4	SF + L-carnitine (100 mg/kg)	70% hepatectomy	Residual liver resection and sacrifice after 72 h
Group 5	SF + L-carnitine (200 mg/kg)	70% hepatectomy	Residual liver resection and sacrifice after 24 h
Group 6	SF + L-carnitine (200 mg/kg)	70% hepatectomy	Residual liver resection and sacrifice after 72 h

Results

TNF-α

The TNF-α levels in the groups are presented in Table 2. In the control group, the serum TNF-α level increased significantly during regeneration (161.3 ± 24.5 pg/mL at 72 h, $P < 0.001$). In comparison, the elevation of TNF-α decreased in the low-dose L-carnitine group (56.9 ± 26.9 pg/mL at 72 h, $P < 0.001$) and increased in the high-dose L-carnitine group (193.0 ± 38.6 pg/mL at 72 h, $P < 0.001$).

IGF-1

The serum IGF-1 levels in the groups are summarized in Table 3. In the control group, the IGF-1 level at 24 h was decreased compared with that at 0 h (762 ± 381 ng/mL vs. 2011 ± 295 ng/mL, respectively); the IGF-1 level was increased significantly by 72 h (1513 ± 436 ng/mL) but was still below that at 0 h. At 0 h, the IGF-1 level in the high-dose L-carnitine group was below the levels in the control and low-dose L-carnitine groups. In comparison with the changes in IGF-l levels in the control and low-dose L-carnitine groups, the IGF-1 level in the high-dose L-carnitine group decreased at a lower rate during the first 24 h and then increased at a higher rate by 72 h ($P < 0.01$).

MDA

The liver MDA levels in the groups are shown in Table 4. In the control group, the MDA level increased significantly during the first 24 h ($P < 0.001$) and then slightly decreased, although this decrease was not statistically significant ($P > 0.05$). In the low-dose L-carnitine group, the MDA level increased during the first 24 h and then decreased significantly ($P < 0.01$). In the high-dose L-carnitine group, the elevation of the MDA level during the first 24 h was not statistically significant; however, after 24 h, the MDA level decreased significantly ($P < 0.01$) in comparison with the levels at 0 and 24 h.

Discussion

In mammals, including humans, liver regeneration is one of the most rapid forms of tissue growth. In the

Table 2. Serum TNF-α levels in control and treatment groups administered L-carnitine.

Groups	*TNF-α at 0 h*	*TNF-α at 24 h*	*TNF-α at 72 h*
Control	25.9 ± 6.1 pg/mL^{b,c}	61.9 ± 23.0 pg/mL^{a,c}	161.3 ± 24.5 pg/mL^{a,b}
L-Carnitine (100 mg/kg)	28.8 ± 11.8 pg/mL^{c}	36.9 ± 12.7 pg/mL	56.9 ± 26.9 pg/mL^{a}
L-Carnitine (200 mg/kg)	13.5 ± 5.0 pg/mL^{c}	37.0 ± 12.6 pg/mL^{c}	193.0 ± 38.6 pg/mL^{a,b}

Values are given as means ± SD. a: significantly different vs. 0 h; b: significantly different vs. 24 h; c: significantly different vs. 72 h.

Table 3. Serum IGF-1 levels in control and treatment groups administered L-carnitine.

Groups	*IGF-1 at 0 h*	*IGF-1 at 24 h*	*IGF-1 at 72 h*
Control	2011 ± 295 ng/mL[b,c]	762 ± 381ng/mL[a,c]	1513 ± 436ng/mL[a,b]
L-Carnitine (100 mg/kg)	1935 ± 205ng/mL[b,c]	892 ± 187ng/mL[a,c]	1411 ± 425ng/mL[a,b]
L-Carnitine (200 mg/kg)	1370 ± 288ng/mL[b]	838 ± 152ng/mL[a,c]	1407 ± 280ng/mL[b]

Values are given as means ± SD. a: significantly different vs. 0 h; b: significantly different vs. 24 h; c: significantly different vs. 72 h.

Table 4. Liver MDA levels (nmol/mg wet tissue) in control and treatment groups administered L-carnitine.

Groups	*MDA at 0 h*	*MDA at 24 h*	*MDA at 72 h*
Control	29.3 ± 9.8[b,c]	55.1 ± 16.4[a]	47.8 ± 11.1[a]
L-Carnitine (100 mg/kg)	37.7 ± 12.8[b]	77.0 ± 18.4[a,c]	54.0 ± 15.6[b]
L-Carnitine (200 mg/kg)	52.6 ± 22.0[c]	71.3 ± 18.9[c]	29.9 ± 10.1[a,b]

Values are given as means ± SD. a: significantly different vs. 0 h; b: significantly different vs. 24 h; c: significantly different vs. 72 h.

present study, we investigated whether L-carnitine, in addition to its role in lipid metabolism, promotes liver regeneration by modulating key molecules, such as TNF-α and IGF-1, which are critical in the initiation and process of liver regeneration in a rat model.

L-carnitine is an amino acid synthesized from lysine and methionine by an iron- and ascorbate-dependent pathway in the liver, kidney, and brain. Its main function is the transport of long-chain fatty acids from the cytosol into the mitochondria for β-oxidation and ATP production. In addition, L-carnitine stabilizes membrane fluidity, which is important in maintaining cellular homeostasis through the regulation of several membrane-linked systems (10,11). In adult humans, one-third of the daily L-carnitine requirement is met by endogenous synthesis, and two-third is from the diet (12). L-carnitine deficiency has been observed in cirrhosis, renal failure, long-term total parenteral or enteral nutritional therapy, and premature infants (12). It has been reported that after PH, the L-carnitine concentration in the remnant liver and serum were sufficient for liver regeneration (5). Lai et al. have suggested that kidney and skeletal muscle release their stored L-carnitine into the serum after PH, and consequently the flow of L-carnitine to the liver increases (5).

In the priming phase, which occurs during the first few hours after PH, a wide variety of genes, many of which are related to cytokine networks, are differentially expressed, and liver mRNA and serum levels of TNF-α and interleukin-6 are increased (13-15). The cytokine cascade is initiated by the binding of TNF-α to its receptor, TNFR1, resulting in the activation of nuclear factor-kappa B (NF-κB) in non-parenchymal cells, the production of interleukin-6, and the activation of signal transducer and activator of transcription 3 (STAT-3) in hepatocytes (7). After PH, the cytokine network is activated within 30 min (7). In our study, serum TNF-α increased significantly in the control group; however, low-dose L-carnitine (100 mg/kg) decreased the elevation in TNF-α, and high-dose L-carnitine increased it compared with the control and low-dose groups. We thought that high-dose L-carnitine administration might promote liver regeneration by increasing the TNF-α level during the regeneration period after PH. After PH, the activity of several matrix metalloproteinases (MMPs) increases, and cytokines, such as TNF-α, activate MMPs, which may be a link between cytokines and growth factors (7,16). During liver regeneration, the activation of cytokines and growth factor receptors may initiate several intracellular pathways, which are important for cell survival and proliferation (7). For example, Argast and co-workers found that, in

cultured hepatocytes, TNF-α activated TGFα converting enzyme, resulting in the release of TGFα, the activation of epidermal growth factor receptor, and cell proliferation (17).

Cytokines, growth factors, and metabolic pathways interact during liver regeneration (7). In the priming phase, cytokine network activation causes quiescent hepatocytes to enter the cell cycle (G0 to G1), and then growth factors, such as hepatocyte growth factor (HGF) and the epidermal growth factor receptor ligand family, drive cell cycle progression (18,19). Borowiak and co-workers demonstrated that HGF/c-met signaling was essential for cell cycle entry after PH (20). Furthermore, Huh et al. concluded that HGF/c-met signaling was important in the healing of hepatocytes after CCl_4 administration (21). The activation of cytokine receptors, particularly TNF-α, is important for the initiation of liver regeneration, whereas growth factors usually act later on hepatocytes (22,23). IGF-1 is synthesized primarily in the liver; however, it has no significant effect on hepatocytes because hepatocytes do not express IGF-1 receptors (24). In contrast, IGF-1 has paracrine effects within the liver, because non-parenchymal cells, such as Kuppfer cells, do express IGF-1 receptors and thus IGF-1 can stimulate these cells (25). Patricia and co-workers studied growth hormone receptor (GHR) and IGF-1 gene expression before and after PH (26). They found a significant decrease in GHR and IGF-1 mRNA levels during the first 24 h after PH compared with the basal levels before surgery (26). They also found that GHR levels returned to basal levels within 4 days after surgery, whereas IGF-1 levels returned to basal values within 7 days after surgery (26). In our study, we found that L-carnitine administered before surgery decreased IGF-1 levels compared with control levels. In all of the groups, serum IGF-1 levels decreased within the first 24 h after PH and then increased significantly by 72 h. L-Carnitine administration slowed the decrease in IGF-1, and IGF-1 increased at a higher rate and in a dose-dependent manner after 24 h. High-dose L-carnitine administration also returned IGF-1 to 0 h levels within 3 days after surgery.

In our study, the administration of L-carnitine before surgery increased MDA levels, which decreased after surgery. The anti-oxidative effects of L-carnitine have been examined in various studies (27,28). Derin et al. have shown that L-carnitine pretreatment increases catalase activity after ischemia reperfusion in rats (27). Reznick et al. suggested that L-carnitine scavenges hydroxyl radicals and inhibits hydroxyl radical formation by the Fenton reaction (28).

In summary, our data show that the administration of L-carnitine before PH decreased the TNF-α and IGF-1 levels and increased the MDA level in a dose-dependent manner. In contrast, the administration of L-carnitine after PH had positive effects on the TNF-α and IGF-1 levels and also had anti-oxidative effects. Based on these and previously reported findings, we conclude that L-carnitine should be administered after rather than before PH to promote liver regeneration in rats.

Acknowledgments

This study was supported by Abant İzzet Baysal University Department of Scientific and Research Projects (2005-07-01-236), Bolu – Turkey.

References

1. Rigotti P, Peters JC, Tranberg KG, Fischer JE. Effects of amino acid infusions on liver regeneration after partial hepatectomy in the rat. J Parent Ent Nutr 1986;10:17-20.
2. Ellis LM, Bryant MS, Copeland EM 3rd, Bland KI, Baumgartner TG, Kao KJ et al. Postoperative enteral versus total parenteral nutrition immediately after partial hepatectomy: effects on hepatic regeneration and function. Curr Surg 1987;44:470-472.
3. Lai HS, Chen WJ, Chen KM. Energy substrate for liver regeneration after partial hepatectomy in rats: effects of glucose vs fat. J Parent Ent Nutr 1992;16:152-156.
4. Blaha V, Simek J, Zivny P, Sobotka L, Zadak Z. Effect of parenteral administration of carnitine on liver regeneration in partially hepatectomized rats. Physiol Bohemoslov 1990;39:233-242.
5. Lai HS, Chen Y, Chen WJ. Carnitine contents in remnant liver, kidney, and skeletal muscle after partial hepatectomy in rats: randomized trial. World J Surg 1998;22:42–47.
6. Bremer J. Carnitine--metabolism and functions. Physiol Rev 1983;63:1420-1480.
7. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology 2006;43:S45-S53.

8. Higgins GM, Anderson RM. Experimental pathology of the liver. Restoration oh the liver of the white rat following surgical removal. Arch Pathol 1931;12:186-206.

9. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271–278.

10. Spector AA, Yorek MA. Membrane lipid composition and cellular function. J Lipid Res 1985;26:1015-1035.

11. Arduini A, Holme S, Sweeney JD, Dottori S, Sciarroni AF, Calvani M. Addition of L-carnitine to additive solution-suspended red cells stored at 4oC reduces in vitro hemolysis and improves in vivo viability. Transfusion 1997;37:166-174.

12. Veldee MS. Nutritional assessment, therapy, and monitoring. In: Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry, 3rd edn. Philadelphia: W.B. Saunders Company, 1999:1359-94.

13. Akerman P, Cote P, Yang SQ, McClain C, Nelson S, Bagby GJ et al. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. Am J Physiol 1992;263:G579–585.

14. Trautwein C, Rakemann T, Niehof M, Rose-John S, Manns MP. Acute phase response factor, increased binding, and target gene transcription during liver regeneration. Gastroenterology 1996;110:1854-1862.

15. Iwai M, Cui TX, Kitamura H, Saito M, Shimazu T. Increased secretion of tumour necrosis factor and interleukin 6 from isolated, perfused liver of rats after partial hepatectomy. Cytokine 2001;13: 60-64.

16. Kim TH, Mars WM, Stolz DB, Michalopoulos GK. Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. Hepatology 2000;31:75-82.

17. Argast GM, Campbell JS, Brooling JT, Fausto N. Epidermal growth factor receptor transactivation mediates tumor necrosis factor-induced hepatocyte replication. J Biol Chem 2004;279:34530-34536.

18. Matsumoto K, Nakamura T. Hepatocyte growth factor: molecular structure, roles in liver regeneration, and other biological functions. Crit Rev Oncogene 1992;3:27-54.

19. Michalopoulos GK, Khan Z. Liver regeneration, growth factors, and amphiregulin. Gastroenterology 2005;128:503-506.

20. Borowiak M, Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. Proc Natl Acad Sci USA 2004;101:10608-10613.

21. Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. Proc Natl Acad Sci USA 2004;101:4477-4482.

22. Fausto N, Laird AD, Webber EM. Liver regeneration. II. Role of growth factors and cytokines in hepatic regeneration. FASEB J 1995;9:1527–1536.

23. Yamada Y, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci USA 1997;94:1441–1446.

24. McElduff A, Poronnik P, Baxter RC, Williams P. A comparison of the insulin and insulin-like growth factor I receptors from rat brain and liver. Endocrinology 1988;122:1933–1939.

25. Zindy F, Lamas E, Schmidt S, Kirn A, Brechot C. Expression of insulinlike growth factor II (IGF-II) and IGF-II, IGF-I and insulin receptors mRNAs in isolated non-parenchymal rat liver cells. J Hepatol 1992;14:30–34.

26. Pennisi PA, Kopchick JJ, Thorgeirsson S, LeRoith D, Yakar S. Role of growth hormone (GH) in liver regeneration. Endocrinology 2004;145:4748-4755.

27. Derin N, Izgut-Uysal VN, Agac A, Aliciguzel Y, demir N. L-Carnitine protects gastric mucosa by decreasing ischemia-reperfusion induced lipid peroxidation. J Physiol Pharmacol 2004;55:595-606.

28. Reznick AZ, Kagan VE, Ramsey R, Tsuchiya M, Khwaja S, Serbinova EA, Packer L. Antiradical effects in L-propionyl carnitine protection of the heart against ischemia-reperfusion injury: the possible role of iron chelation. Arch Biochem Biophys 1992;296:394-401.