EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 28, No 1, 17-23, March 1996

γ-Glutamyltransferase in adult rat hepatocyte during proliferation

Yun Gyu Park,[1,2] Meyoung-kon Kim,[1] Jeongwon Sohn,[1] Gil Hong Park[1] and Chong Kun Ryu[1]

1 Department of Biochemistry, Korea University Medical School, Seoul, 136-701, Korea

2 To whom correspondence should be addressed.

Accepted 29 January 1996

Abbreviations: EGF, epidermal growth factor; GGT, γ-glutamyltransferase; PBS, phosphate buffered saline

Abstract

γ-Glutamyltransferase (GGT) activity is known to increase during hepatocarcinogenesis induced by chemical carcinogens. To understand the possible significance of GGT, we have investigated GGT activities in hepatocytes cultured with or without metabolic inhibitors. Primary cultures of adult rat hepatocytes were established and the cell number, [^{3}H]thymidine incorporation and GGT activity were measured at various points of hepatocyte proliferation. When insulin (0.1 μM) and EGF (0.1 μg/ml) were added to the culture, the initially low hepatocyte density (5-6 × 10^4 cells/cm^2) has increased 1.5-fold after 32 h of incubation. The change of GGT activity was correlated with that of [^{3}H]thymidine incorporation: both the GGT activity and the [^{3}H]thymidine incorporation have increased 2.6-fold and 2.5-fold, respectively, after 3 h of incubation, and then decreased rapidly followed by another increase after 24 h. The effect of inhibitors, such as serine-borate complex and acivicin for GGT during hepatocyte proliferation was also investigated. Serine-borate complex (reversible inhibitor) and acivicin (irreversible inhibitor) inhibited proliferation of hepatocyte in a dose-dependant manner: At the concentration of 1 mM serine-borate complex or 0.5 mM acivicin, hepatocyte proliferation was completely abolished, while GGT activity decreased slightly even at the 5 mM serine-borate complex. When hepatocytes were initially cultured in the presence of the growth factor with 5 mM serine-borate complex for 24 h, and then continued to culture further in the absence of the inhibitor, the cells restored proliferating capability completely, indicating the reversibility of the inhibitor. In conclusion, we have demonstrated that during hepatocyte proliferation increase in GGT activity is temporarily correlated with DNA synthesis, suggesting that GGT may play a role in hepatocyte growth.

Keywords: acivicin, γ-glutamyltransferase, hepatocyte proliferation, serine-borate complex

Introduction

γ-Glutamyltransferase (GGT) (EC 2.3.2.2) is a membrane-bound enzyme which catalyzes transfer of γ-glutamyl moiety of glutathione or glutathione derivatives to amino acid acceptor or to water. The enzyme is known to be involved in glutathione metabolism and amino acid transport through plasma membrane (Meister and Tate, 1976; Orlowski and Meister, 1970). GGT activity is high in fetal and neonatal liver but low in adult liver (Cameron *et al.*, 1978; Higashi *et al.*, 1978; Tsuchida *et al.*, 1979). In contrast to the very low GGT activity in normal adult hepatocyte, its activities are strikingly elevated in hepatocellular carcinoma induced by chemical carcinogen (Fiala *et al.*, 1972; Fiala and Fiala, 1973; Cameron *et al.*, 1978; Jalanko and Ruoslahti, 1979; Capouya and Lindahl, 1983) and in various tumorigenic cell lines derived from mammalian liver (Cheng *et al.*, 1978; Laishes *et al.*, 1978; Huberman *et al.*, 1979). Furthermore, its activities were shown to increase markedly at earlier stages of hepatocarcinogenesis (Kalengayi *et al.*, 1975; Cameron *et al.*, 1978; Laishes *et al.*, 1978; Pitot *et al.*, 1978; Pugh and Goldfarb, 1978).

The use of GGT activity as a positive marker for preneoplastic and neoplastic hepatocyte has been reported (Solt *et al.*, 1977; Cayoma *et al.*, 1978; Pitot and Sirica, 1980; Williams, 1980), although its biological significance and mechanism of its induction remain to be uncertain. On the other hand, evidences from several laboratories indicated that those factors which are not primarily hepatocarcinogenic, such as hormones (Edwards, 1982; Barouki *et al.*, 1983; Edwards and Lucas, 1985), nutritional factors (Adjarov *et al.*, 1982; Edwards, 1982) and growth stimulating factors (Manson and Neal, 1984; Bone *et al.*, 1985; Sulakhe, 1986) also play some role in the GGT gene expression in hepatocytes.

S-oxalylglutathione, one of oxalylthiolesters, have been shown to be an excellent *in vitro* substrate for

GGT (Hamilton, 1988). Since oxalylthiolesters have been observed to be decreased in proliferating cells, either transformed or stimulated, the esters were implicated as normal metabolic inhibitors in controlling cell proliferation (Hamilton, 1985; Skorczynski *et al.*, 1987). Consequently, these authors inferred an importance of GGT in cell proliferation.

In the present study, we have investigated the role of GGT during hepatocyte proliferation. The cell number, [^{3}H]thymidine incorporation, and GGT activity were measured to quantify hepatocyte proliferation using primary cultures of adult rat hepatocytes. The effect of inhibitors, such as serine-borate complex and acivicin for GGT activity during hepatocyte proliferation were also investigated.

Materials and Methods

Materials

Calf serum, collagenase, epidermal growth factor (EGF), insulin, and Williams' medium E. were purchased from Gibco (Grand Island, U.S.A.). Acivicin (L-(αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazole acetic acid), dexamethasone, L-γ-glutamyl-*p*-nitroanilide, glycylglycine, hydroxyurea, L-serine, and sodium borate were from Sigma (St. Louis, U.S.A.). [*Methyl*-^{3}H]thymidine was from Amersham (Buckinghamshire, UK). Glass fiber filter was from Skatron (Lier, Norway). Sprague-Dawley rats were from the Laboratory for Maintenance of Experimental Animal, Korea Institute for Safety Research, Seoul.

Isolation and culture of hepatocyte from adult rat liver

Parenchymal hepatocytes were isolated from adult male Sprague-Dawley rats (weighing about 200 g) by two-step collagenase perfusion method as described (Seglen, 1976). The isolated hepatocytes were cultured in Williams' medium E., pH 7.4, supplemented with 5% calf serum and 1 μM dexamethasone under 5% CO_2 and 30% O_2 in air at 37°C in culture plate (6-well or 96-well) or in 60-mm culture dishes. The cells were attached on the bottom of culture vessels after 4 h of incubation. After removal of nonadherent cells, the attached hepatocytes were further cultured in the same medium. The medium was replaced with fresh Williams' medium E., pH 7.4, with or without 0.1 M insulin and 0.1 g/ml EGF after 16 h of incubation (Nakamura *et al.*, 1983). Initial cell density was 5-6 X 10^4 cells/cm^2. The preparation of rat hepatocytes contained >99% parenchymal hepatocytes by morphological criteria discerned by microscopic examination, and its viability was 75-85%. When estimated by trypan blue dye exclusion test. The cells were counted using hemocytometer. To examine the effect of inhibitors, such as serine-borate complex and acivicin for GGT during hepatocyte proliferation, the medium was supplemented with the various concentrations of inhibitors at pH 7.4.

Measurement of DNA synthesis

To measure DNA synthesis, the cells cultured in 96-well culture plate were pulsed with 1 Ci/well [*methyl*-^{3}H]thymidine (88.2 Ci/mmol) in the presence or absence of 10 mM hydroxyurea for 1 h. The cells from two wells were combined and harvested together onto a glass fiber filter with a cell harvestor (Skatron, Norway). The incorporated radioactivity was measured with a liquid scintillation counter (Packard, Australia). The net radioactivity (cpm/h/cell) was calculated by substracting the value in the presence of hydroxyurea. Data were expressed as the mean ± standard error of quadraplicate experiments.

Assay of GGT activity

For assay of GGT activity, cells cultured in 60-mm culture dishes were washed twice with phosphate buffered saline (PBS) prewarmed at 37°C, and harvested by scrapping with a rubber policeman and centrifuged at 5,000 *g* for 5 min. The harvested cells were resuspended in 1 ml of 0.1 M Tris-HCl, pH 9.0, and kept frozen at -70°C until use. Before assay, samples were thawed and homogenized using a polytron homogenizer (Dremel, U.S.A.) at full speed for 10 s at 4°C. GGT activity was measured by a modification of the method of Strömmer *et al.* (1978). Total enzyme reaction mixture, 0.5 ml, containing 5 mM L-γ-glutamyl-*p*-nitroanilide, 50 mM glycylglycine, 10 mM $MgCl_2$, 100 mM Tris-HCl buffer, pH 9.0, and appropriate amount of cell homogenate was incubated at 37°C for 60 min. The reaction was stopped by addition of 0.5 ml of 1.5 M acetic acid, and the precipitated proteins and cell debris were removed by centrifugation at 5,000 *g* for 5 min. The absorbance of the supernatant at 410 nm was measured against blank in which acetic acid was added prior to the incubation. One unit of enzyme activity is defined as the amount of GGT catalyzing the formation of 1 mol of *p*-nitroaniline (ε_{410} = 8,800 $M^{-1}cm^{-1}$) per min at 37°C.

Result

Growth profile of primary adult rat hepatocytes

Isolated adult rat hepatocytes were split into 2 groups, and were cultured in Williams' medium E. supplemented with (wGF) or without growth factors (w/oGF). 0.1 μM insulin and 0.1 μg/ml EGF were included as growth factors. In contrast to the unchanged cell density in w/oGF (Figure 1A), the density increased

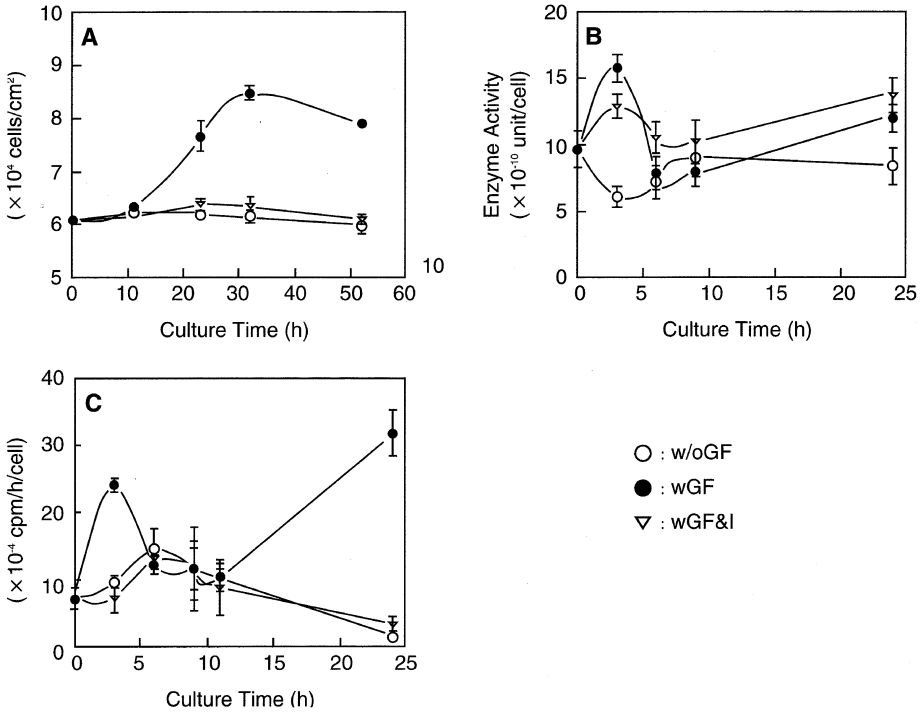

Figure 1. Hepatocyte proliferation **(A)**, γ-GT activity **(B)**, and [^{3}H]thymidine incorporation **(C)** under various culture conditions. Hepatocytes were isolated from adult rat and seeded at an initial density of 6 X 10^4 cells/cm^2 and cultured in Williams' M.E. supplemented with 1 M dexamethasone for 20 h. Then, the medium was replaced by Williams' M.E. supplemented with (wGF) or without (w/oGF); being 0.1 M insulin and 0.1 g/ml EGF as growth factors. Serine-borate complex as GGT inhibitor at the concentration of 5 mM was added to culture media (wGF&I). Cells were analyzed at various time points after the supplement. Values are means ±S.E. from triplicate cultures. Data represent one of several separate experiments that gave almost identical results.

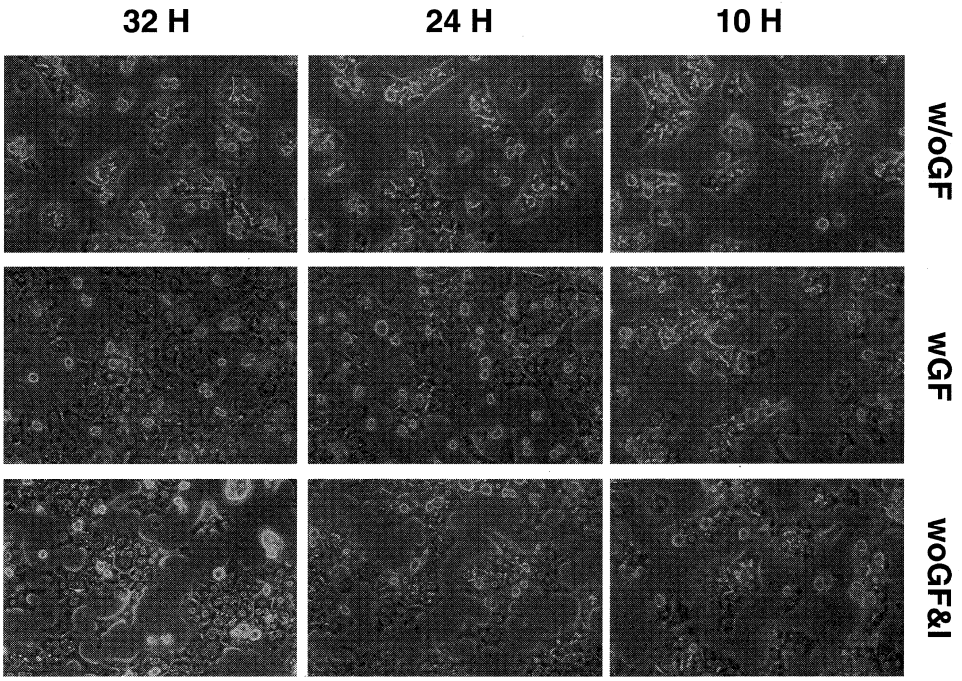

Figure 2. Phase-contrast photomicrographs of adult rat hepatocytes on various culture conditions.
Culture conditions were the same as those for Figure 1. Hepatocytes were cultured in Williams' M.E. supplemented with (wGF) or without (w/oGF) growth factors, and with growth factors and GGT inhibitor (wGF&I). Hepatocytes were photographed at 10, 24 and 32 h after the addition of supplements.

significantly (P<0.005) after 24 h of incubation in wGF (Figure 1A). The low initial hepatocyte density (5-6 × 10^4 cells/cm^2) in wGF increased about 1.5-fold (8-8.5 × 10^4 cells/cm^2) reaching a plateau after 32 h of incubation. During the culture, lag phase (upto 10 h of incubation), exponential phase (from 10 to 32 h), and stationary phase (after 32 h) were observed. Under the present culture condition, adult rat hepatocytes showed a typical normal growth profile. The morphology of the hepatocyte did not change thoroughout the culture under w/oGF (Figure 2, left lane), but showed a typical polygonal shape of normal mature hepatocytes, under wGF (Figure 2, center lane).

Change of GGT activity and DNA synthesis by serine-borate complex

The effect of GGT inhibitor on GGT activity and DNA synthesis during early stage of hepatocyte proliferation was studied. Adult rat hepatocytes were cultured under the same condition as w/oGF, wGF, and wGF&I with growth factors and 5 mM serine-borate complex. The study of inhibitory concentration of serine-borate complex or acivicin will be presented in the following section. During the initial 11 h of cultures, cell numbers did not change irrespective of the different culture conditions (Figure 1A and Figure 2, upper lane). In w/oGF, GGT activities and [^{3}H]thymidine incorporations did not change significantly during the incubation period. In the case of wGF, GGT activity showed a 2.6-fold increase over w/oGF, and [^{3}H]thymidine incorporation increased 2.5-fold after 3 h of incubation, but decreased thereafter to the base line level of w/oGF. In wGF&I, GGT activity showed a 2.1-fold increase but [^{3}H]thymidine incorporation did not change significantly, after 3 h of culture.

The effect of inhibitors for GGT

The effective concentration of GGT inhibitors during hepatocyte proliferation were investigated using serine-borate complex (reversible inhibitor) or acivicin (irreversible inhibitor). In the absence (wGF) or presence (wGF&I) of a GGT inhibitor, adult rat

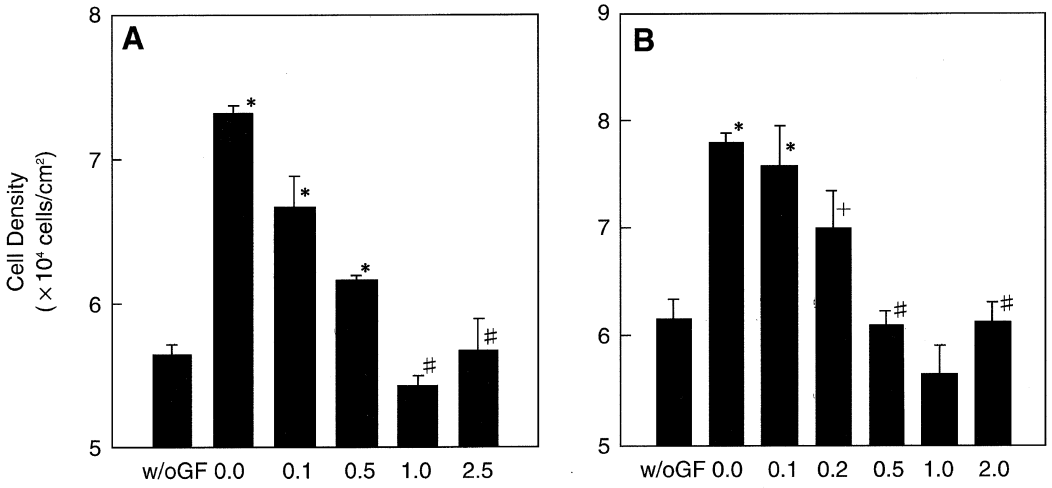

Figure 3. Inhibitory effect of various concentrations of serine-borate complex (**A**) and acivicin (**B**) during hepatocyte proliferation. Hepatocytes were cultured in Williams' M.E. supplemented with growth factors and the indicated amount of the inhibitor, except that the cells in a group named as "w/oGF" were cultured in Williams' M.E. without supplement of growth factors or inhibitor. The cell numbers were measured at 32 h after the supplement. Values are means ±S.E. from triplicate cultures and statistical analy was performed by t-test. Data represent one of three separate experiments that gave almost identical results.*, +, and # indicates $P<0.005$, $P<0.025$, and not significant, respectively.

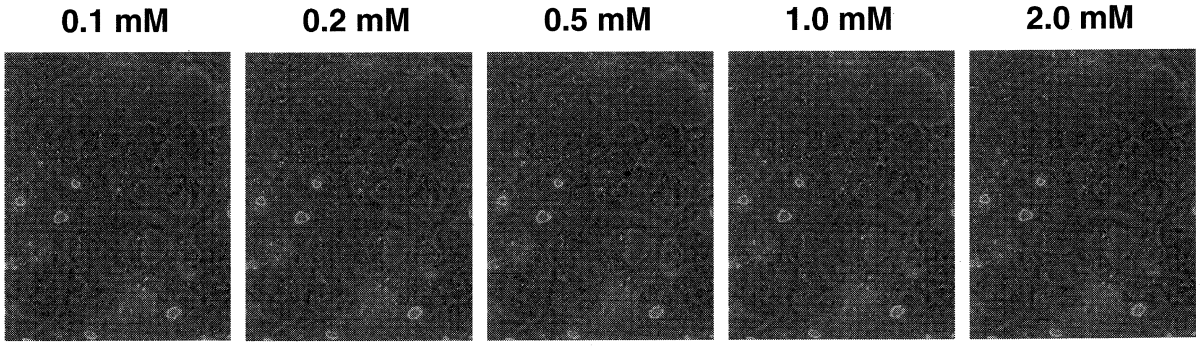

Figure 4. Phase-contrast photomicrographs of adult rat hepatocytes cultured in Williams' M.E. supplemented with acivicin at various concentrations. Culture conditions were the same as those in Figure 3. Hepatocytes were photographed 50 h after the addition of acivicin as indicated concentrations to culture medium.

hepatocytes were cultured in Williams' medium E. supplemented with the growth factors for 32 h. Both serine-borate complex (Figure 3A) and acivicin (Figure 3B and 4) started to inhibit proliferation of hepatocyte at the concentration of 0.1 mM and continued to inhibit in a dose-dependant manner as measured by cell numbers. Serine-borate complex and acivicin abolished hepatocyte proliferation completely at the concentration of 1 mM and 0.5 mM, respectively. The initial hepatocyte density was maintained in the presence of 1-20 mM serine-borate complex (data not shown) or in the presence of 0.5-2 mM acivicin. In wGF&I, the growth factors induced the change of cell shape, in spite of the fact that they did not stimulate hepatocyte proliferation (Figure 2, right lane).

Reversibility of serine-borate complex

To investigate the reversibility of serine-borate complex and to rule out the direct cytotoxicity of the inhibitor, we examined the capacity to restore proliferating activity of hepatocytes which were pretreated with the inhibitor. Adult rat hepatocytes were initially cultured in Williams' medium E. supplemented with the growth factors and 5 mM serine-borate complex for 24 h. Then, the hepatocytes were split into 2 groups and were cultured in the presence or absence of the inhibitor (Figure 5 and Figure 6). In contrast to the decreased cell density in control group (5.2×10^4 cells/cm^2), the density in the absence of inhibitor (7.8×10^4 cells/cm^2) increased significantly ($P<0.005$), about 1.5-fold after 48 h of incubation.

Discussion

The role of GGT during hepatocyte proliferation has been investigated in several laboratories because its activity was shown to increase markedly even at earlier stages of hepatocarcinogenesis (Cameron *et al.*, 1978; Laishes *et al.*, 1978; Pitot *et al.*, 1978). Hamilton *et al.* (1988) proposed an importance of GGT in cell proliferation. However, Sirica *et al.* (1979) and Edwards *et al.* (1987) proposed that an elevated level of the GGT activity might reflect simply a phenotypic expression of dedifferentiated liver cells rather than its key role in hepatocyte proliferation. Most of these laboratories investigated the role of GGT using primary cultures of fetal, neonatal, or adult hepatocytes. The fetal and neonatal hepatocytes are in a rapidly proliferating state, and in contrast to the increased DNA synthesis, the cell number of adult hepatocytes did not change under most of these culture conditions.

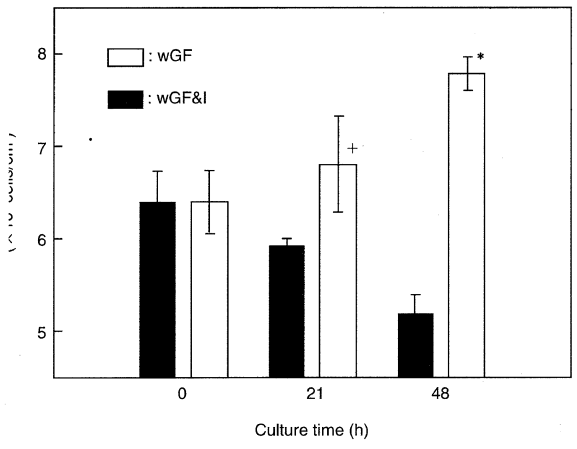

Figure 5. Restoration of proliferating capability of adult rat hepatocyte pretreated with 5 mM serine-borate complex. Adult rat hepatocytes were initially cultured in Williams' M.E. supplemented with growth factors and 5 mM serine-borate complex for 24 h. Then, the medium was replaced with fresh Williams' M.E. supplemented with growth factors. The cell number was measured at various time points after the replacement. Values are means ±S.E. from triplicate cultures and statistical analysis was performed by *t*-test. Data represent one of two separate experiments that gave almost identical results.* and + indicates $P<0.005$, $P<0.025$, respectively.

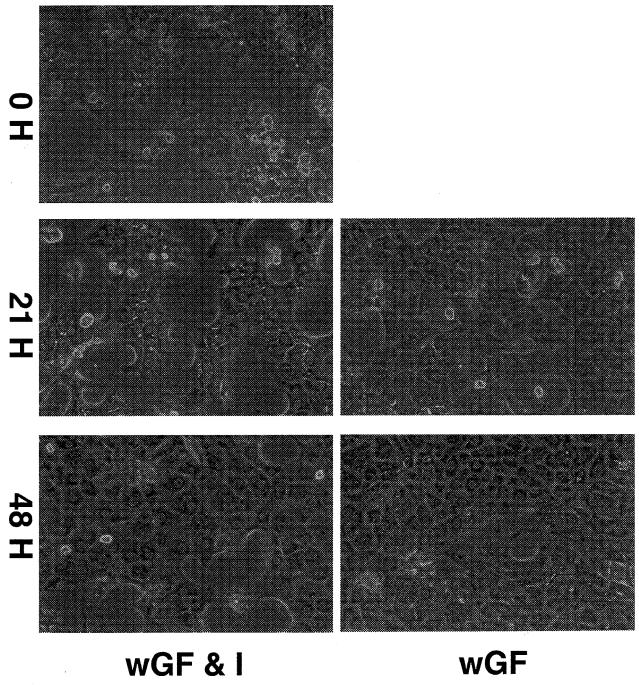

Figure 6. Phase-contrast photomicrographs of adult rat hepatocytes restoring the proliferating capability, which were pretreated with 5 mM serine-borate complex for 24 h. Culture conditions were the same as those in Figure 5. Hepatocytes were photographed at 21 and 48 h after the replacement of culture medium.

Therefore, these conditions may not provide an appropriate system to study the role of GGT during hepatocyte proliferation. In the present study, we have used primary culture of adult rat hepatocytes as described by Nakamura *et al.* (1983).

Among various inhibitors for GGT, serine-borate complex and acivicin have been used to examine various physiological functions of GGT in glutathione metabolism (Ewa *et al.*, 1992; Avishay-Abraham *et al.*, 1993; Gloria *et al.*, 1993), amino acid transport (Ian and Ina, 1994), xenobiotics metabolism (de-Ceaurriz *et al.*, 1994), leukotriene C4 metaboslim (Lois *et al.*, 1991; Keith *et al.*, 1994) and the activation of an anti-tumor prodrug (Joseph *et al.*, 1994). Especially, the serine-borate complex (1:1 mixture) is a transition state inhibitor which mimics a γ-glutamyl-enzyme intermediate or transition state expected to be formed in the normal enzyme reaction (Tate and Meister, 1978). In contrast to substrate analogues which bind nonspecifically to other proteins or nucleic acids, transition state inhibitors are biospecific enzyme inhibitors and also had been reported as therapeutic agents (Brodbeck, 1980). In the present study, we used serine-borate complex and acivicin to investigate the effect of inhibitors for GGT during hepatocyte proliferation.

In wGF, there was an increase of GGT activity and [^{3}H]thymidine incorporation after 3 h of incubation, and the cell number increased after 10 h of incubation. In wGF&I, GGT activity increased after 3 h of incubation, but [^{3}H]thymidine incorporation and cell number did not change significantly during the incubation period. It is quite possible that increase of GGT activity induced by insulin and EGF has been inhibited by serine-borate complex. These results suggest that the increased GGT activity by growth factors primes DNA synthesis during hepatocyte proliferation.

In wGF&I, the initial hepatocyte density was maintained in the presence of serine-borate complex or acivicin, indicating that there was no direct cytotoxic effect of the inhibitors on adult rat hepatocytes. This argument is also supported by the fact that the initial hepatocyte density remained unchanged in the presence of 5 mM serine-borate complex without any growth factors (data not shown). Restoration of proliferating activity in hepatocytes which were pretreated with 5 mM serine-borate complex, provides another line of evidence that the inhibitors for GGT have no direct cytotoxic effect on cells.

To understand the biological significance of GGT, adult rat hepatocytes were isolated and cultured. In the presence of growth factors, the cell number increased 1.5-fold after 32 h of incubation when seeded at a low density. The change of GGT activity was correlated with that of [^{3}H]thymidine incorporation. Serine-borate complex and acivicin inhibited proliferation of hepatocyte in a dose-dependant manner. The hepatocytes, which were pretreated with 5 mM serine-borate complex, restored proliferating capability completely. These results suggest that during hepatocyte proliferation, the increase of GGT activity is correlated with DNA synthesis and that GGT may play a role in hepatocyte growth.

Acknowledgement

This study was supported by a grant from Bio-science Research Institute, Korea University Medical Center, 1995.

References

Adjarov, D., Ivanov, E., Kerimova, M. and Lozanova, L. (1982) Influence of fasting and of high protein diet on the activity of rat liver -glutamyltransferase. *Experientia* 39: 498-499

Avishay-Abraham, S., Errol, Z. and Dennis, A. P. (1993) Glutathione metabolism by γ-glutamyltranspeptidase leads to lipid peroxidation: characterization of the system and relevance to hepatocarcinogenesis. *Carcinogenesis* 14: 183-189

Barouki, R., Chobert, M. N., Finidori, J., Billon, M. C. and Hanoune, J. (1983) The hormonal induction of gamma-glutamyl transferase in rat liver and in a hepatoma cell line. *Mol. Cell. Biochem.* 53: 77-88

Bone, S. N., Michalopoulos, G. and Jirtle, R. L. (1985) Ability of partial hepatectomy to induce γ-glutamyltranspeptidase in regenerated and transplanted hepatocytes of Fischer 344 and Wistar-Furth rats. *Cancer Res.* 45: 1222-1228

Brodbeck, U. (1980) On the design of transition state analog enzyme inhibitors and their future in medical and agricultural chemistry. In *Enzyme Inhibitor*, pp. 3-23, Verlag Chemie

Cameron, R., Kellen, J., Kolin, A., Malkin, A. and Farber, E. (1978) -glutamyltransferase in putative premalignant liver cell populations during hepatocarcinogenesis. *Cancer Res.* 38: 823-829

Capouya, E. R. and Lindahl, R. (1983) Changes in γ-glutamyl transferase activity during N-2-fluorenylacetamide-induced rat hepatocarcinogenesis. *J. Natl. Cancer Inst.* 70: 359-365

de-Ceaurriz, J., Payan, J. P., Morel, G. and Brondeau, M. T. (1994) Role of extracellular glutathione and gamma-glutamyl transpeptidase in the disposition and kidney toxicity of inorganic mercury in rats. *J. Appl. Toxicol.* 14: 201-206

Cheng, S., Nassar, K. and Levy, D. (1978) γ-Glutamyl transpeptidase activity in normal regenerating and malignant hepatocytes. *FEBS Lett.* 85: 310-312

Edwards, A. M. (1982) Regulation of γ-glutamyltranspeptidase in rat hepatocyte monolayer cultures. *Cancer Res.* 42: 1107-1115

Edwards, A. M. and Lucas, C. M. (1985) Induction of γ-glutamyl transpeptidase in primary cultures of normal rat hepatocytes by liver tumor promoters and structurally related compounds. *Carcinogenesis* 6: 733-739

Edwards, A. M., Lucas, C. M. and Baddams, H. M. (1987) Modulation of γ-glutamyltranspeptidase in normal rat hepatocytes in culture by cell density, epidermal growth factor and agents which alter cell differentiation. *Carcinogenesis* 8: 1837-1842

Ewa, R. D. M., Ming S., Minyuen, C., Timothy, W. R., John, G, Nora, H. and Henry, J. F. (1992) Transfection with γ-glutamyl transpeptidase enhances recovery from glutathione depletion using extracellular glutathione. *Toxicol. Appl. Pharmacol.* 114: 56-62

Fiala, S., Fiala, A. E. and Dixon, B. (1972) γ-Glutamyl transpeptidase in transplantable, chemically induced rat hepatomas and "spontaneous" mouse hepatomas. *J. Natl. Cancer Inst.* 48: 1393-1401

Fiala, S. and Fiala, E. S. (1973) Activation by chemical carcinogens of γ-glutamyltranspeptidase in rat and mouse liver. *J. Natl. Cancer Inst.* 51: 151-158

Gloria, S., Neil, K., Murad, O. and Shelly, C. L. (1993) Bidirectional membrane transport of intact glutathione in Hep G2 cells. *Am. J. Physiol.* 265(6 pt 1): G1128-1134

Hamilton, G. A. (1985) Peroxisomal oxidases and suggestions for the mechanism of action of insulin and other hormones. *Adv. Enzymol.* 57: 85-106

Hamilton, G. A., Buckthal, D. J., Kantorczyk, N. J. and Skorczynski, S. S. (1988) S-oxalylglutathione is a substrate for γ-glutamyl transferase (GGT). Implications for the role of GGT in cell proliferation. *Biochem. Biophys. Res. Commun.* 150: 828-834

Higashi, T., Fukui, R., Sekiyama, M., Yoshimoto, F., Tateishi, N. and Sakamoto, Y. (1986) Decreased induction of γ-glutamyl transferase activity by 3-methyl-4-dimethylaminoazobenzene in the liver of rats given carcinogen containing diet for several generations. *Jap. J. Cancer. Res. (Gann)* 77: 139-144

Huberman, E., Montesano, R., Drevon, C., Kuroki, T., St. Vincent, L., Pugh, T. D. and Goldfarb, S. (1979) γ-Glutamyltranspeptidase and malignant transformation of cultured liver cells. *Cancer Res.* 39: 269-272

Ian, A. C. and Ina, S.-K. (1994) A role for γ-glutamyl transpeptidase in the transport of cystein into human endothelial cells: relationship to intracellular glutathione. *Biochim. Biophys. Acta* 1222: 375-382

Jalanko, H. and Ruoslahti, E. (1979) Differential expression of α-fetoprotein and γ-glutamyl transpeptidase in chemical and spontaneous hepatocarcinogenesis. *Cancer Res.* 39: 3495-3501

Joseph, A. P., Rebecca, P. H., Nu, W., Kalyani, M. D. and William, D. B. (1994) γ-glutamyltranspeptidase expression regulates the growth-inhibitory activity of the anti-tumor prodrug γ-L-glutaminyl-4-hydroxy-3-iodobenzene. *Int. J. Cancer.* 56: 874-879

Kalengayi, M. M. R., Ronchi, G. and Desmet, V. J. (1975) Histochemistry of gamma-glutamyl transpeptidase in rat liver during aflatoxin B1-induced carcinogenesis. *J. Natl. Cancer Inst.* 55: 579-588

Keith L. B., Takehiko, B. and William M. P. (1994) Enzymatic barrier protects brain capillaries from leukotriene C_4. *J. Neurosurg.* 81: 745-751

Laishes, B. A., Ogawa, K., Roverts, E. and Farber, E. (1978) Gamma-glutamyl transpeptidase: a positive marker for culture rat liver cells derived from putative premalignant and malignant lesions. *J. Natl. Cancer Inst.* 60: 1009-1016

Lois, A. W., Norma, P. G., David, K. H., Nancy, G. B., Richard, S. B., Henry, A. F. and Jeffrey, M. D. (1991) Leukotriene receptor on U-937 cells: discriminatory responses to leutokrienes C_4 and D_4. *Am. J. Physiol.* 261(2 pt 1): L164-171

Manson, M. M. and Neal, G. E. (1984) The influence of partial hepatectomy on the biphasic response of gamma-glutamyl transpeptidase to aflatoxin B. *Cancer Lett.* 25: 81-87

Meister, A. and Tate, S. S. (1976) Glutathione and related γ-glutamyl compounds: biosynthesis and utilization. *Annu. Rev. Biochem.* 45: 599-604

Nakamura, T., Tomita, Y. and Ichihara, A. (1983) Density-dependent growth control of adult rat hepatocytes in primary culture. *J. Biochem.* 94: 1029-1035

Orlowski, M. and Meister, A. (1970) The gamma-glutamyl cycle, a possible transport system for amino acid. *Proc. Natl. Acad. Sci. U.S.A.* 67: 1248-1255

Pitot, H. C., Barsness, L., Goldsworthy, T. and Kitagawa, T. (1978) Biochemical characterisation of stages of hepatocarcino genesis after a single dose of diethylnitrosamine. *Nature* 271: 456-458

Pitot, H. C. and Sirica, A. E. (1980) The stages of initiation and promotion in hepatocarcinogenesis. *Biochim. Biophys. Acta* 605: 191-215

Pugh, T. D. and Goldfarb, S. (1978) Quantitative histochemical and autoradiographic studies of hepatocarcinogenesis in rats fed 2-acetylaminofluorene followed by phenobarbital. *Cancer Res.* 38: 4450-4457

Sirica, A. E., Richards, W., Tsukada, Y., Sattler, C. A. and Pitot, H. C. (1979) Fetal phenotypic expression by adult rat hepatocytes on collagen gel/nylon mesh. *Proc. Natl. Acad. Sci. U.S.A.* 76: 283-287

Skorczynski, S. S., Mastro, A. M. and Hamilton, G. A. (1987) Oxalyl thioester concentrations are decreased in transformed fibroblasts and in lectin stimulated lymphocytes. *Fed. Proc.* 46: 2189

Strömme, J. H. and Theodorsen, L. (1978) Recommended method for the determination of γ-glutamyltransferase in blood. *Scand. J. Clin. Lab. Invest.* 36: 119-124

Sulakhe, S. J. (1986) The activity of γ-glutamyltranspeptidase in regenerating rat liver. *FEBS Lett.* 204: 302-306

Tate, S. S. and Meister, A. (1978) Serine-borate complex as a transition-state inhibitor of γ-glutamyltranspeptidase. *Proc. Natl. Acad. Sci. U.S.A.* 75: 4086-4809

Tsuchida, S., Hoshino, K., Sato, T., Ito, N. and Sato, K. (1979) Purification of γ-glutamyltransferases from rat hepatomas and hyperplastic hepatic nodules and comparison with the enzyme from rat kidney. *Cancer Res.* 39: 4200-4205