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Allyl mercaptan, a major metabolite of garlic compounds, reduces cellular cholesterol synthesis and its secretion in Hep-G2 cells

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The cytotoxicity, cellular cholesterol synthesis, and secretion of allyl mercaptan, a major metabolite of garlic compounds, were studied in Hep-G2 cells. The cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and treated with 5, 25, 50, 100, 125, 250, and 500 μg of allyl mercaptan/mL for 4, 12, and 24 hours. At concentrations up to 125 μg, no significant cytotoxic effect was noted during those incubation periods. However, at a concentration of 250 μg, cell viability decreased approximately 50% compared with the control ($P < 0.05$) in all three incubation times. At a concentration of 500 μg, allyl mercaptan was highly toxic, causing extensive cell death. The treatment of cells with 5, 10, 25, 50, or 100 μg (noncytotoxic concentration) of allyl mercaptan resulted in a marked inhibition of ^{3}H-acetate incorporation into cholesterol. At concentrations between 5 and 100 μg, the cholesterol synthesis was inhibited 20 to 80% in cells and the cholesterol secretion into the medium decreased 20 to 50% compared with the control ($P < 0.05$). The concentration of allyl mercaptan required to suppress cholesterol synthesis by 50% was approximately 25 μg/mL. Allyl mercaptan treatment of cells incubated with 1 mM of oleic acid also resulted in a significant decrease in the cholesterol synthesis compared with the cells incubated with oleic acid alone ($19.5 \pm 1.2 \times 10^3$ dpm/mg protein/4 h vs. $30.0 \pm 2.6 \times 10^3$ dpm/mg protein/4 h; $P < 0.05$). The present study demonstrates that allyl mercaptan is effective in inhibiting cholesterol synthesis at concentrations as low as 5 μg, and its inhibition is concentration dependent. (J. Nutr. Biochem. 10:654–659, 1999)

Keywords: allyl mercaptan; cholesterol synthesis; cholesterol secretion; cytotoxicity; Hep-G2 cell

Introduction

In recent years, garlic and its products have received increased attention because of their possible beneficial effects in reducing the risk of heart disease. Several garlic extracts have been shown to decrease plasma cholesterol and triglyceride levels in humans[1,2] and animals.[3,4] A significant reduction of cellular cholesterol synthesis also has been demonstrated after exposing primary rat hepatocytes and human Hep-G2 cells to garlic extracts, allicin, and ajoene.[5,6] The depression of activities of the cholesterogenic enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase,[7,8] as well as the lipogenic enzymes including glucose-6-phosphate dehydrogenase and malic enzyme, by garlic compounds has been reported.[9]

Garlic and garlic compounds are metabolized in the intestine and liver, but allicin and some of its transformation products, such as ajoene or diallyl sulfides, have never been found in the blood, indicating that they are converted to other compounds.[10] Some studies have suggested that pure garlic-related organosulfur compounds such as allicin, ajoene, the trisulfides, and perhaps the disulfides are metabolized primarily to allyl mercaptan shortly after entering the blood.[11,12] Allicin also has been shown to transform to diallyl disulfide and allyl mercaptan in the isolated perfused

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rat liver, with the former being rapidly metabolized to allyl mercaptan.[13] Recent studies indicated that allyl mercaptan also reduces cholesterol synthesis in rat hepatocytes, although its inhibitory effect appears to be much less than that of allicin.[14] However, the physiologic role of allyl mercaptan is largely unknown, despite the fact that allyl mercaptan is the major metabolite of garlic compounds.[11–13]

Therefore, the present study was undertaken to investigate the effects of allyl mercaptan on cellular cholesterol synthesis and its secretion using Hep-G2 cells in culture. To find out the nontoxic level of allyl mercaptan, the cytotoxicity was also determined at various concentrations of allyl mercaptan. The human hepatoma-derived cell line Hep-G2 was chosen for the present study because these cells retain many normal hepatic metabolic functions including lipoprotein synthesis and cholesterol metabolism.[15]

Material and methods

Materials

The human hepatoma cell line Hep-G2 was obtained from American Type Culture Collection (Rockville, MD USA). [1-^{3}H]-Acetate, sodium salt (135.4 mci/mmol), and [2-^{3}H]-glycerol (200 mci/mmol) were obtained from New England Nuclear (Boston, MA USA). The allyl mercaptan [85% pure, plus 12% diallyl sulfide and 1% diallyl disulfide by high performance liquid chromatography (HPLC) analysis] was provided by Dr. Larry Lawson (Nature's Way Products, Inc., Springville, UT USA). Dulbecco's modified Eagle's medium (DMEM) was the product of GIBCO (Grand Island, NY USA). Fetal bovine serum (FBS), trypsin, ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA), and the enzymatic cholesterol reagent kit were obtained from Sigma Chemical Co. (St. Louis, MO USA). Oleic acid (sodium salt, 99%+ pure) was purchased from Nu Check Prep (Elysian, MN USA). All other chemicals and solvents were of analytical grade.

Cell culture

The cells were grown in DMEM supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin (100 μg/mL). Stock cultures were maintained in T-25 flasks at 37°C under a humidified atmosphere of 95% air and 5% carbon dioxide. The medium was changed every third day. When cells were grown 70 to 80% confluency, fresh cultures were initiated. The growth medium was removed and the cells were washed twice with Ca^{2+}-, Mg^{2+}-free balanced Hank's salt solution (BHSS). The cells then were harvested by trypsinization, added immediately to DMEM containing 10% FBS to inhibit the tryptic activity, and centrifuged at 600 × g to sediment cells. Aliquots of cell suspension in the growth medium (2.2×10^6 cells) were seeded and grown in T-25 flasks.

Allyl mercaptan solution

Allyl mercaptan was dissolved directly in 100% ethanol (50 mg/mL). The allyl mercaptan solution was then added to DMEM dropwise while stirring (1 mg/mL). This stock solution was prepared fresh just prior to the experiment and was further diluted with DMEM to the appropriate concentrations.

Fatty acid/albumin complex solution

A stock solution of a fatty acid/albumin mixture was prepared under aseptic conditions.[16] Briefly, 20 μmoles of sodium oleate was dissolved in 1 mL of sterilized water at room temperature. Five μmoles (30 mg) of BSA was dissolved in 4 mL of DMEM. The fatty acid solution was then added to the BSA solution dropwise while stirring. The fatty acid/albumin solution (4 mM) prepared was optically clear. The molar ratio of fatty acid to albumin was kept at 4:1.

Cytotoxicity of allyl mercaptan

The cells (1×10^4) were seeded into each well of 24-well culture plates and grown in DMEM containing 10% FBS for 48 hours. By that time, a dense monolayer had formed. The culture medium was removed and the monolayer was washed twice with BHSS. The cells were then incubated with 1 mL of the serum-free medium containing different concentrations of allyl mercaptan (0, 5, 25, 50, 125, 250, and 500 μg/mL) for 4, 12, and 24 hours. The cells then were detached using a solution of trypsin-EDTA (0.005%) in BHSS and were immediately resuspended in DMEM supplemented with 10% FBS to inhibit the tryptic activity. Trypan blue solution (0.4%) and cell suspension were mixed in equal quantities (0.1 mL). The cell numbers were counted using a hemocytometer, and nonstained cells were scored as viable. The control medium contained ethanol (0.05%) in place of allyl mercaptan. Prior to experiments, cells were incubated with various concentrations of ethanol (0, 0.05%, 0.5%, and 1.0%) for 4 and 24 hours. No effect on cell viability was noted in those concentrations of ethanol, which was similar to that reported by Dashti et al.[17]

Incorporation of ^{3}H-acetate into cholesterol and fatty acids

The cells were grown in T-25 flasks in DMEM containing 10% FBS as described above. At day 6, the growth medium was removed and cells were washed twice with Hank's buffer. The cells were then incubated with 3 mL of the serum-free DMEM containing 3 μci of ^{3}H-acetate and various concentrations of allyl mercaptan (0, 5, 10, 25, 50, and 100 μg/mL). In another study, the cells were incubated with 3 mL of the serum-free DMEM containing 1 mM of oleic acid/albumin mixture, 75 μg of allyl mercaptan, and 3 μci of ^{3}H-acetate. Controls received ethanol (0.05%) alone. After 4 hours of incubation, the medium was collected and stored at −20°C. The cells were washed twice with BHSS, harvested by trypsinization, and centrifuged at 2,000 rpm for 5 minutes at 4°C. The cells were resuspended in 1 mL of distilled water and sonicated with an ultrasonic cell disrupter (Cell Disrupter, Polygon, Brinkmann Inst., Westbury, NY USA) at grade 3 for 20 seconds. An aliquot (25 μL) of homogenate was taken and the protein content was determined by the method of Lowry et al.[18] The lipids from the cell homogenate and medium were extracted by the method of Folch et al.[19] The total lipid extracts were saponified in

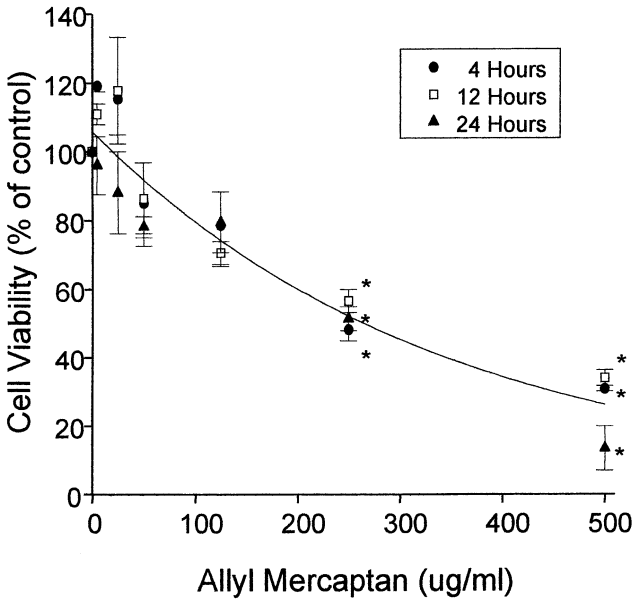

Figure 1 Effects of various concentrations of allyl mercaptan on cell viability. The cells were incubated in a serum-free Dulbecco's modified Eagle's medium containing 5, 25, 50, 125, 250, or 500 μg/mL of allyl mercaptan for 4, 12, and 24 hours. Values are represented as the mean ± SEM (n = 6) of viable cells after treatment, expressed as a percentage of the control.

3 mL of 3.75% methanolic potassium hydroxide at 90°C for 4 hours and acidified with 0.5 mL of 4 N HCl.[20] After saponification, the lipids were extracted again with chloroform/methanol (2:1,v/v) and were separated on silica gel plates using petroleum ether/diethyl ether/acetic acid (85:15:1, v/v/v) as developing solvents. The cholesterol and fatty acid bands were visualized by exposure to iodine vapor. The cholesterol and fatty acid bands were then cut into vials, added to 5 mL of toluene scintillation fluid (0.5% PPO and 0.03% POPOP), and the radioactivity counted in a Beckman Model LS 5800 scintillation counter (Beckman Instruments, Palo Alto, CA USA).

Incorporation of ^{3}H-glycerol into triglyceride and phospholipid

After cells were grown in DMEM for 6 days, the medium was removed and the cells were washed twice with Hank's buffer. The cells were then incubated with 3 mL of the serum-free DMEM containing 1.8 μci ^{3}H-glyerol and 75 μg/mL of allyl mercaptan. After 4 hours of incubation, the medium was collected and stored at −20°C. The cells were homogenated and the total lipids from the cell homogenate and medium were extracted by the method of Folch et al.[19] The lipid extracts were redissolved in chloroform and were separated by thin-layer chromatography as described above. The triglyceride and phospholipid bands were visualized by exposure to iodine vapor. The triglyceride and phospholipid bands were then cut into vials, 5 mL scintillation fluid was added, and the radioactivity was counted as described above.

Statistical analysis

Data are presented as means ± SEM. The data were evaluated statistically using Student's t-test or one-way analysis of variance and Duncan's Multiple Range test to compare the significant difference between the groups at a P-value of less than 0.05.

Results

The effects of various concentrations of allyl mercaptan on viability of Hep-G2 cells during different incubation times are shown in *Figure 1*. At concentrations up to 125 μg/mL, no significant cytotoxic effect was noted during the 4-, 12-, and 24-hour incubation periods. Average viable cell counts ($\times 10^3$) in those concentrations (5–125μg/mL) were 77.7 ± 17.4 (79.5 ± 3.6/control) at 4 hours, 86.4 ± 19.0 (90.0 ± 4.7/control) at 12 hours, and 134.6 ± 11.7 (150.0 ± 9.1/control) at 24 hours. At a concentration of 250 μg/mL, cell viability decreased approximately 50% compared with the control ($P < 0.05$) in all three incubation times. At a concentration of 500 μg/mL, cell viability decreased to 30% during the 4- and 12-hour incubation periods, and it decreased to 10% at 24 hours compared with the control ($P < 0.05$). The viable cells ($\times 10^3$) that remained were 24.0 ± 0.4 at 4 hours, 31.5 ± 1.5 at 12 hours, and 17.0 ± 1.5 at 24 hours. When the monolayer of cells was examined under the light microscope, the cells appeared normal at concentrations up to 100 μg/mL. At 250 and 500 μg/mL, however, morphologic abnormalities including cellular disintegration were very apparent.

Based on cell viability and morphologic appearance,

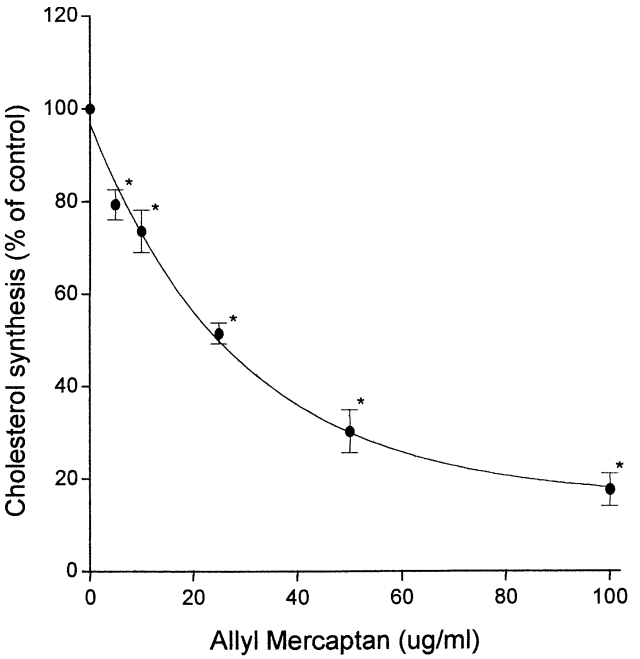

Figure 2 Effects of various concentrations of allyl mercaptan on cholesterol synthesis in Hep-G2 cells. The cells were incubated in a serum-free Dulbecco's modified Eagle's medium containing 5, 10, 25, 50, and 100 μg/mL of allyl mercaptan and ^{3}H-acetate (1 μci/mL) for 4 hours. Values are mean ± SEM (n = 6).

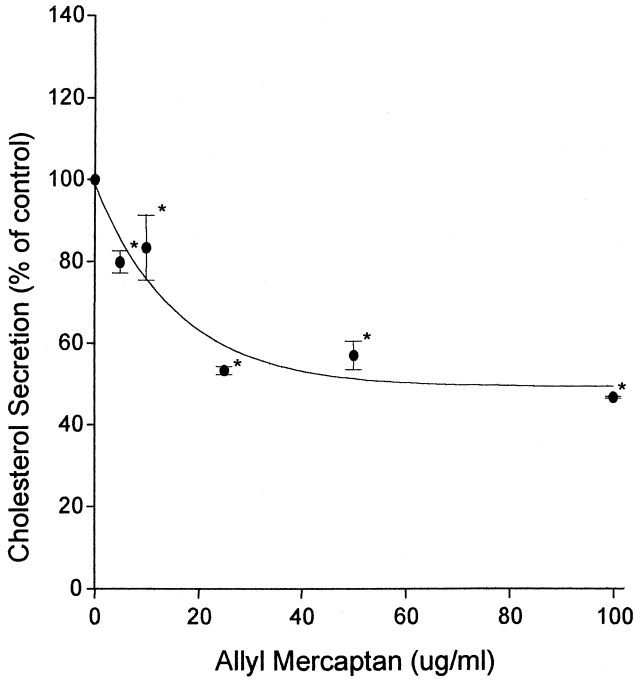

Figure 3 Effects of various concentrations of allyl mercaptan on cholesterol secretion in Hep-G2 cells. The cells were incubated in a serum-free Dulbecco's modified Eagle's medium containing 5, 10, 25, 50, and 100 μg/mL of allyl mercaptan and ^{3}H-acetate (1 μci/mL) for 4 hours. Values are mean ± SEM (n = 6).

concentrations up to 100 μg/mL were chosen as nontoxic levels of allyl mercaptan to carry out subsequent studies. The treatment of cells with 5, 10, 25, 50, or 100 μg/mL (noncytotoxic concentrations) of allyl mercaptan resulted in a marked inhibition of ^{3}H-acetate incorporation into cholesterol in cells (*Figure 2*). Allyl mercaptan exerted a concentration-dependent inhibition of cholesterol synthesis. At a concentration of 5 μg/mL, ^{3}H-acetate incorporation into cholesterol was inhibited approximately 20% compared with the control ($P < 0.05$). The average inhibition of cholesterol synthesis was 30%, 50%, 70%, and 80% with concentrations of 10, 25, 50, and 100 μg/mL, respectively ($P < 0.05$). The concentration of allyl mercaptan required to suppress cholesterol synthesis by 50% (IC_{50}) was approximately 25 μg/mL (0.4 mM).

Allyl mercaptan treatment also resulted in a significant decrease in cholesterol secretion (*Figure 3*). At a concentration of 5 μg/mL, cholesterol secretion into the medium decreased approximately 20% compared with that of the control ($P < 0.05$). A 50% decrease was noted at the concentration of 25 μg/mL, and the decrease leveled off at concentrations above 25 μg/mL (46–53%). However, the treatment of cells with 5, 10, 25, 50, or 100 μg/mL of allyl mercaptan had no apparent effect on the incorporation of ^{3}H-acetate into fatty acids of either cellular or medium lipids (*Table 1*).

The effect of allyl mercaptan on incorporation of ^{3}H-acetate into cholesterol with and without 1 mM of oleic acid treatment is shown in *Table 2*. The treatment of cells with 25 μg/mL of allyl mercaptan significantly decreased ^{3}H-acetate incorporation into cholesterol in cells compared with

Table 1. Effects of allyl mercaptan on ^{3}H-acetate incorporation into fatty acid in cellular and medium lipids*

Concentration (μg/mL)	^{3}H-Acetate incorporation into fatty acid†	
	Cellular lipid	Medium lipid
None	219.6 ± 7.0	2.2 ± 0.2
5	242.7 ± 16.5	2.6 ± 0.3
10	207.0 ± 10.1	2.3 ± 0.1
25	239.9 ± 9.9	2.1 ± 0.4
50	211.1 ± 7.8	2.3 ± 0.2
100	210.1 ± 6.1	2.1 ± 0.1

*Values are mean ± SEM (N = 6).
†dpm × 10^3/mg cell protein/4 h.

that of the control ($10.5 \times 10^3 \pm 1.9$ dpm/mg protein/4 h vs. $22.3 \times 10^3 \pm 1.5$ dpm/mg protein/4 h; $P < 0.05$). The level of radiolabeled cholesterol secretion into the medium also decreased significantly compared with that of the control ($P < 0.05$). The treatment of cells with 1 mM of oleic acid significantly increased cellular cholesterol synthesis compared with the control ($P < 0.05$). Allyl mercaptan treatment of cells incubated with 1 mM of oleic acid resulted in a significant reduction in the cholesterol synthesis in cells ($19.5 \pm 1.2 \times 10^3$ dpm/mg cell protein/4 h vs. $30.0 \pm 2.6 \times 10^3$ dpm/mg cell protein/4 h) and cholesterol secretion into the medium ($0.8 \pm 0.05 \times 10^3$ dpm/mg cell protein/4 h vs. $1.5 \pm 0.1 \times 10^3$ dpm/mg cell protein/4 h) compared with those of oleic acid alone ($P < 0.05$). Although allyl mercaptan significantly inhibited cholesterol synthesis and its secretion, it had no effect on either cellular fatty acid synthesis or saponifiable lipids secreted into the medium. Similarly, ^{3}H-glycerol incorporation into triglyceride and phospholipid in cells and secretion of those lipids into the medium also were not significantly affected by allyl mercaptan (*Table 3*).

Discussion

The present study demonstrates that allyl mercaptan, a metabolite of garlic compounds, caused a low level of cytotoxicity in Hep-G2 cells at concentrations up to 125 μg/mL of incubation medium, but that cell viability decreased to 50% and 30% at concentrations of 250 and 500

Table 2. Effects of allyl mercaptan (AM) on ^{3}H-acetate incorporation into cholesterol and fatty acid in cells and medium*

Treatment†	^{3}H-Acetate incorporation			
	Cellular lipid‡		Medium lipid‡	
	Cholesterol	Fatty acid	Cholesterol	Fatty acid
Control	22.3 ± 2.5^b	113.6 ± 6.2^a	1.7 ± 0.2^a	3.3 ± 0.1
AM	10.5 ± 1.9^c	109.8 ± 5.5^a	0.6 ± 0.0^b	3.7 ± 0.4
Oleic acid	30.0 ± 2.6^a	39.4 ± 0.6^b	1.5 ± 0.1^a	3.3 ± 0.5
Oleic acid + AM	19.5 ± 1.2^b	41.5 ± 3.0^b	0.8 ± 0.0^b	3.2 ± 0.6

*Values are mean ± SEM (N = 6). Values in the same column sharing a different superscript letter are significantly different ($P < 0.05$).
†AM, 25 μg/mL, 0.4 mM; oleic acid, 1 mM.
‡dpm × 10^3/mg cell protein/4 h.

Table 3. Effects of allyl mercaptan on ^{3}H-glycerol incorporation into triglyceride and phospholipid in cells and medium*

Treatment	Triglyceride†		Phospholipid†	
	Cell	Medium	Cell	Medium
Control	30.3 ± 6.0	1.7 ± 0.3	97.2 ± 5.5	4.8 ± 0.3
Allyl mercaptan‡	19.5 ± 2.1	1.3 ± 0.1	83.7 ± 7.9	4.5 ± 0.1

*Values are mean ± SEM ($N = 6$).
†dpm × 10^3/mg cell protein/4 h.
‡25 μg/mL.

μg/mL, respectively. The ingestion of high amounts of garlic and garlic extracts has been reported to cause toxic effects on the liver or the function of liver enzymes.[21] The cytotoxic level of ajoene in human primary fibroblasts also has been reported in the range of 2 to 50 μg/mL.[22] When Hep-G2 cells and rat hepatocytes were exposed to allicin and ajoene, cell death occurred at concentrations above 80 μg/mL (0.5 mM) of the former and 234 μg/mL (1 mM) of the latter.[5] Allyl mercaptan has also been reported to cause cytotoxicity in rat hepatocytes,[14] but its cytotoxicity appeared to start at much higher concentrations than that found in the present study.

Although the mechanism of garlic-induced cytotoxicity is not clearly understood, it has been suggested that ajoene could interfere with cellular sulfur hydryl (SH) homeostasis by affecting the glutathione (GSH) cycle through oxidation of GSH by the sulfoxide group.[23] It has been shown that ajoene inactivates GSH reductase by reacting with the SH group of the enzyme and formation of the mixed disulfide. The depletion of GSH results in protein-SH oxidation, lipid peroxidation, and disturbances of the cellular metabolism, which may lead to cell death.[23,24] However, a recent study has shown that the treatment of human prostate carcinoma cells with 50 μg/mL of S-allylmercaptocysteine and S-allylcysteine increased GSH formation.[25] There also appears to be a concentration-dependent effect of garlic derivatives on GSH: Ajoene stimulated GSH synthesis at low concentrations whereas the GSH level sharply decreased at the high dose of ajoene.[23] Therefore, the overall effect of garlic derivatives on GSH metabolism and their underlying cytotoxic mechanisms needs further clarification.

In the present study, the treatment of cells with allyl mercaptan resulted in a significant inhibition of cholesterol synthesis. The inhibitory effect of cholesterol synthesis by allyl mercaptan was concentration-dependent, with a IC_{50} value of approximately 25 μg/mL (400 μM), and even at a concentration of 5 μg/mL, the cholesterol synthesis decreased approximately 20% compared with the control. The present finding is in agreement with the report that allyl mercaptan exerts an inhibitory effect on cholesterol synthesis, with a IC_{50} value of 450 μM in rat hepatocytes.[14] The inhibition of ^{3}H-acetate incorporation into cholesterol by allyl mercaptan could be due to the decreased activity of HMG-CoA reductase, because water-soluble garlic extracts, allicin, and ajoene have been shown to lower the activity of HMG-CoA reductase in rat hepatocytes.[5,7] The inactivation of HMG-CoA reductase by diallyl disulfide also was reported in rat hepatocytes,[26] and the formation of protein internal disulfides, which are inaccessible for reduction by thiol agents, has been suggested to be the cause of this inactivation.[27]

In the present study, Hep-G2 cells also were incubated with oleic acid to stimulate cholesterol synthesis, because the increased availability of fatty acids has been reported to stimulate HMG-CoA reductase activity in cultured hepatocytes[28] and oleic acid caused the most pronounced rise of HMG-CoA reductase in the perfused rat liver.[29] In agreement with previous studies,[16,29] the incubation of cells with 1 mM of oleic acid resulted in a significant increase in cellular cholesterol synthesis. The possible mechanism that involves oleic acid may be the removal of free cholesterol from its inhibitory site, either by stimulating the esterification of cholesterol to cholesteryl ester or by its utilization of cholesterol for lipoprotein assembly and secretion. It is known that the stimulating effect of excessive free fatty acid on cholesterol synthesis contributes to an increase in the size of the cholesteryl ester storage pool in the liver.[30] In the present study, allyl mercaptan treatment of cells incubated with 1 mM of oleic acid also significantly decreased ^{3}H-acetate incorporation into cholesterol compared with that of cells incubated with oleic acid alone ($19.5 \pm 1.2 \times 10^3$ dpm vs. $30.0 \pm 2.6 \times 10^3$ dpm), neutralizing the stimulatory effect of oleic acid by almost 35%.

The present findings clearly demonstrate that allyl mercaptan is effective in suppressing the synthesis and excretion of cholesterol in Hep-G2 cells. Although it is rather difficult to interpret the relevance of current data to dietary effect of garlic in vivo, allyl mercaptan, which is a major metabolite of garlic compounds circulating in the blood, could be partly responsible for the cholesterol-lowering effect of garlic.

References

1 Jain, R.C. (1977). Effect of garlic on serum lipids, coagulability and fibrinolytic activity. *Am. J. Clin. Nutr.* **30,** 1380–1381
2 Bordia, A. (1981). Effect of garlic on blood lipids in patients with coronary heart disease. *Am. J. Clin. Nutr.* **34,** 2100–2103
3 Augusti, K.T. and Mathew, P.T. (1974). Lipid lowering effect of allicin (diallyl disulphide-oxide) on long term feeding to normal rats. *Experientia* **30,** 468–470
4 Jain, R.C. and Konar, D.B. (1978). Effect of garlic oil in experimental cholesterol atherosclerosis. *Atherosclerosis* **29,** 125–129
5 Gebhardt, R., Halgund, B., and Wagner, K.G. (1994). Inhibition of cholesterol biosynthesis by allicin and ajoene in rat hepatocytes and HepG2 cells. *Biochim. Biophys. Acta* **1213,** 57–62
6 Yeh, Y.Y. and Yeh, S.M. (1994). Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. *Lipids* **29,** 189–193
7 Gebhardt, R. (1993). Multiple inhibitory effects of garlic extracts on cholesterol biosynthesis in hepatocytes. *Lipids* **28,** 613–619
8 Qureshi, A.A., Naji, A., Zafeer, Z.D., Charles, E.E., and Warren, C.B. (1983). Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fractions of garlic. *Lipids* **18,** 343–348
9 Qureshi, A.A., Din, Z.Z., Abuirmeileh, N., Burger, W.C., Ahmad, Y., and Elson, C.E. (1983). Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: Impact on serum lipids. *J. Nutr.* **113,** 1746–1755
10 Lawson, L.D., Ransom, D.K., and Huges, B.G. (1992). Inhibition of whole blood platelet aggregation by compounds in garlic clove extracts and commercial garlic products. *Thromb. Res.* **65,** 141–156
11 Lawson, L.D. and Wang, Z.J. (1993). Pre-hepatic fate of the organosulfur compounds derived from garlic (Allium sativum). *Planta Med.* **59,** A688

12 Lawson, L.D. (1993). Bioactive organosulfur compounds of garlic products: Their role in reducing blood lipids. In *Human Medicinal Agents From Plants* (A.D. Kinghorn and M.F. Balandrin, eds.), pp. 306–330, American Chemical Society, Washington, DC, USA
13 Egon-Schmind, C., Eckardm, R., and Kemper, E.H. (1992). Metabolism of garlic constituents in the isolated perfused rat liver. *Planta Med.* **58,** 301–305
14 Gebhardt, R. and Beck, H. (1996). Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. *Lipids* **31,** 1269–1276
15 Ellsworth, J.L., Erickson, S.K., and Cooper, A.D. (1986). Very low and low density lipoprotein synthesis and secretion by the human hepatoma cell line Hep-G2: Effects of free fatty acid. *J. Lipid Res.* **27,** 858–874
16 Dokko, R.C., Cho, B.H.S., and Chung, B.H. (1998). Cellular uptake of stearic, oleic, linoleic, and linolenic acid and their effects on synthesis and secretion of lipids in Hep-G2 cells. *Int. J. Biochem. & Cell Biol.* **30,** 65–76
17 Dashti, N., Franklin, F.A., and Abrahamson, D.R. (1996). Effect of ethanol on the synthesis and secretion of ApoA-1- and ApoB-containing lipoproteins in HepG2 cells. *J. Lipid Res.* **37,** 810–824
18 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 267–275
19 Folch, T., Lee, M., and Stanlay, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226,** 497–509
20 Yeh, Y.Y. and Leveille, G.A. (1969). Effects of dietary protein on hepatic lipogenesis in the growing chick. *J. Nutr.* **98,** 356–366
21 Agarwal, K.C. (1996). Therapeutic actions of garlic constituents. *Med. Res. Rev.* **16,** 111–124
22 Scharfenberg, K., Wagner, R., and Wagner, K.G. (1990). The cytotoxic effect of ajoene, a natural product from garlic, investigated with different cell lines. *Cancer Lett.* **53,** 103–108
23 Scharfenberg, K., Ryll, T., Wagner, R., and Wagner, K.G. (1994). Injuries to cultivated BJA-b cells by ajoene, a garlic-derived natural compound: Cell viability, glutathione metabolism, and pools of acidic amino acids. *J. Cell Physiol.* **158,** 55–60
24 Miccadei, S., Nakae, D., Kyle, M.E., Gilfor, D., and Farber, J.L. (1988). Oxidative cell injury in the killing of cultured hepatocytes by allyl alcohol. *Arch. Biochem. Biophys.* **265,** 302–310
25 Pinto, J.T., Qiao, C., Xing, J., Rivlin, R.S., Protomastro, M.L., Weissler, M.L., Tao, Y., Thaler, H., and Heston, W.D.W. (1997). Effects of garlic thioallyl derivatives on growth, glutathione concentration, and polyamine formation of human prostate carcinoma cells in culture. *Am. J. Clin. Nutr.* **66,** 398–405
26 Gebhardt, R. (1995). Amplification of palmitate induced inhibition of cholesterol biosynthesis in cultured rat hepatocytes by garlic-derived organosulfur compounds. *Phytomedicine* **2,** 29–34
27 Kumar, R.V.O., Banerji, A., Kurup, C.K.R., and Ramasarma, T. (1991). The nature of inhibition of 3-hydroxy-3-methylglutaryl CoA reductase by garlic-derived diallyl disulfide. *Biochim. Biophys. Acta* **1078,** 219–225
28 Goldfarb, S. and Pitot, H.C. (1972). Stimulatory effect of dietary lipid and cholestyramine on hepatic HMG CoA reductase. *J. Lipid Res.* **13,** 797–801
29 Goh, E.H. and Heimberg, M. (1976). Effect of oleic acid and cholesterol on the activity of hepatic hydroxymethylglutaryl coenzyme A reductase. *FEBS Lett.* **63,** 209–210
30 Goh, E.H. and Heimberg, M. (1973). Stimulation of hepatic cholesterol biosynthesis by oleic acid. *Biochim. Biophys. Res. Co.* **55,** 382–388