[Chem. Pharm. Bull. 17(4) 822—826 (1969)]

UDC 615.28.015 : 547.565-261.09

Biopharmaceutical Studies on Guaiacol Glycerol Ether and Related Compounds. II.[1,2] Hypocholesteremic Action. (1)

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(Received May 17, 1968)

Hypocholesteremic effect of guaiacol glycerol ether[4] (GGE), guaiacol glycerol ether mononicotinate[5] (GGE–MN), and guaiacol glycerol ether dinicotinate[5] (GGE–DN) in rabbits has been reported. Guaiacol glycerol ether provides an apparent hypercholesteremic effect[4a,d] on blood of rabbits, while the chemical shows hypocholesteremic action[4b] on the organs of rabbits fed with lanolin.

Interpretation of these relations was made partly from the following results. Human serum from high-cholesteremia patients and that added with a known amount of cholesterol were each mixed with GGE, incubated at 37° for 24 hours, and these sera were submitted to column filtration over Sephadex.[4c] Chromatography of these sera showed that the amount of cholesterol flowing out with protein decreased while the amount of liberated cholesterol increased markedly. This fact might reveal that GGE liberates cholesterol in the serum and increases the solubility of liberated cholesterol from blood vessels or organs. In the other experiment,[4d] effect of GGE on the choletsterol value in rabbit serum and urine was examined by giving GGE to rabbits with high cholesteremia produced by lanolin-rich diet. It was thereby found that GGE accelerates and increases absorption and excretion of cholesterol. Similar results were obtained when GGE–MN was administered instead of GGE to rabbits.[5b,d] On the other hand, it was observed that nicotinic acid derivatives hydrolysed from GGE–MN in body fluids had the possibility showing hypocholesteremic action to rabbits.[5c]

The purpose of the present work was to determine some mechanisms of hypocholesteremic action of GGE and GGE–MN by intravenous administration of Triton* (oxyethylated tertiary octylphenol formaldehyde polymer), and to show additional results of observations on pathological tissue preparations of an arteria, spleen, and liver after an oral administration of cholesterol and these chemicals to rabbits. Byers, Friedman, and Sugiyama[6] stated that the enhanced rate of hepatic synthesis of cholesterol following Triton injection to rats is sequential to, rather than causal of, the induced hypercholesteremia. The effect of GGE, GGE–MN, assumed metabolites of GGE and GGE–MN, and some of hypocholesteremic agents on Triton hypercholesteremia is shown in Table I. On the other hand, all of the chemicals at the same doses listed in Table I were administered to rats which were not in-

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Table I. Effect of Guaiacol Glycerol Ether (GGE), Guaiacol Glycerol Ether Mononicotinate (GGE–MN), and Related Compounds on Triton–induced Hypercholesteremia in Rats

Group[a]	Dose mg·mole/kg	Serum total cholesterol (mg%)	Inhibition[b] (%)
Normal		84± 2[c]	
Control		404± 7	
GGE–MN	0.33	383±11	7
GGE–MN	0.66	350±21	17
GGE–MN	1.32	331±13	23
GGE–MN	1.98	312±23	29
GGE–MN	2.64	316±19	28
GGE + Nicotinic acid	0.66 + 0.66	412±10	
GGE + Nicotinic acid	1.32 + 1.32	343±19	19
Mesoinositol hexanicotinate	0.25	301±23	32
2,6-Pyridinedimethanol bis(N-methylcarbamate)	0.79	380±39	8
Normal		67± 3	
Control		363±17	
GGE	0.66	372±12	
α-Hydroxy-β-(*o*-methoxyphenoxy)propionic acid	0.66	372±19	
(*o*-Methoxyphenoxy)acetic acid	0.66	339±14	8
Normal		59± 4	
Control		370±18	
Nicotinic acid	0.66	347± 8	7
Nicotinoylglycine	0.66	274±16	31
N^1-Methyl-2-pyridone-5-carboxamide	0.66	291±28	25
Nicotinamide N-oxide	0.66	313±36	18
N^1-Methylnicotinamide	0.66	328±25	14

a) Each group consisted of 5 rats which were bled 18 hours after intravenous injection of 400 mg/kg of Triton, followed immediately by intraperitoneal injection of each chemical except the control group.

b) inhibition (%) $= \frac{\text{control level} - \text{treated level}}{\text{control level} - \text{normal level}} \times 100$

c) standard deviation

jected Triton, and it was ascertained that differences in blood cholesterol level between before and 18 hours after the injection of the chemicals were not significant at 5% level.

No metabolites of GGE were found yet but the metabolites of mephenesin may be helpful to know the supposed metabolites of GGE and GGE–MN. Graves, Elliott, and Bradley[7] reported the isolation of β-(*o*-toluloxy)lactic acid from the urine of man and rabbits which had received mephenesin. Working independently, Riley[8] isolated the same compound as well as a second metabolic acid, β-(2-methyl-4-hydroxyphenoxy)lactic acid from the urine of man. Analogously, Coppi, Sekules, and Pala[9] had separated α-hydroxy-β-(*o*-methoxy-*p*-propionylphenoxy)propionic acid and (*o*-methoxy-*p*-propionylphenoxy)acetic acid from 3-(*p*-propionyl-*o*-methoxyphenoxy)-1,2-propanediol as the metabolites in rats and rabbits. Similary, from GGE, α-hydroxy-β-(*o*-methoxyphenoxy)propionic acid, (*o*-methoxyphenoxy)acetic acid, and β-(2-methoxy-4-hydroxyphenoxy)lactic acid might have formed but, these compounds have not been reported in literature. Therefore, α-hydroxy-β-(*o*-methoxyphenoxy)propionic acid and (*o*-methoxyphenoxy)acetic acid were prepared for the sake of comparison.

7) E.L. Graves, T.J. Elliot, and W. Bradley, *Nature*, **162**, 257 (1948).
8) R.F. Riley, *J. Am. Chem. Soc.*, **72**, 5712 (1950).
9) G. Coppi, G. Sekules, and G. Pala, *Arzneimittel-Forsch.*, **16**, 601 (1966).

Chaykin, Dagani, Johnson, and Samli[10] reported that the metabolites of nicotinamide [7-^{14}C] in mouse urine were separated by paper chromatography and identified by admixture with the authentic samples. A minimum of 98% of the radioactivity in mouse urine after the administration of nicotinamide[7-^{14}C] was present in the following five compounds: N^1-methylnicotinamide, nicotinic acid, nicotinamide N-oxide, N^1-methyl-4-pyridone-3-carboxamide, and nicotinamide. These compounds were also prepared for the sake of comparison of hypocholesteremic effect, since nicotinic acid derivatives should have arisen from the hydrolysed products of GGE–MN *in vivo*.

Kobayashi[11] stated that the blood cholesterol level on hypercholesteremia in a rat caused by administration of Triton WR–1339 reached the maximum 18 hours after the intravenous injection of 200 mg/kg. In the experiment summarized in Table I, 400 mg/kg instead of 200 mg/kg dose of Triton was administered to the rats to examine the variation of cholesterol level in each group. From the results thereby obtained, the following statistical conclusion may be drawn at 95% of fiducial limit.

(a) Difference between the control group and the GGE–MN group given 0.66—1.98 mg·mole/kg is significant but that between the control group and GGE–MN group given 0.33 mg·mole/kg is not.

(b) Significant difference was observed between GGE–MN and equimolar amount of the mixture of GGE and nicotinic acid at 0.66 mg·mole/kg dose, but not at 1.32 mg·mole/kg dose. The reason for this difference may be due to the fact that the hypocholesteremic effect of nicotinic acid is enhanced when the dose is increased. The reason why the decholesterol action of GGE–MN was observed even at a lower dose such as 0.66 mg·mole/kg may be due to the action of nicotinic acid hydrolysed *in vivo*, presumably, in an active state and/or to the action of the molecule of GGE–MN itself.

(c) Difference of cholesterol level between the control and the group given 0.79 mg·mole/kg of 2,6-pyridinedimethanol bis(N-methylcarbamate) was not significant.

(d) Significant difference in cholesterol level between the control and the group given 0.25 mg·mole/kg of mesoinositol hexanicotinate was observed but not between that of mesoinositol hexanicotinate (0.25 mg·mole/kg) and the group given GGE–MN in 0.66—1.32 mg·mole/kg doses.

(e) Differences in cholesterol level between the control group and the groups given GGE (0.66 mg·mole/kg), α-hydroxy-β-(*o*-methoxyphenoxy)propionic acid (0.66 mg·mole/kg), or (*o*-methoxyphenoxy)acetic acid (0.66 mg·mole/kg) were not significant. Accordingly, GGE and the supposed metabolites from GGE have no effect on apparent lowering of blood cholesterol level at doses equimolar to GGE–MN (0.66 mg·mole/kg).

(f) At the dose of 0.66 mg·mole/kg, effect of lowering or blood cholesterol levels was significant in the groups given nicotinoylglycine and N^1-methyl-2-pyridone-5-carboxamide compared to the control group but not significant in the groups given nicotinic acid, nicotinamide N-oxide, or N^1-methylnicotinamide compared to the control group. Leifer, Roth, Hogness, and Corson[12] stated that 56% of N^1-methylnicotinamide, 12% of N^1-methyl-2-pyridone-5-carboxamide, 10% of nicotinuric acid, 6% of nicotinic acid, and 4% of nicotinamide were excreted as the metabolites of nicotinic acid in rats. If nicotinic acid hydrolysed from GGE–MN is metabolized almost completely to nicotinoylglycine and/or N^1-methyl-2-pyridone-5-carboxamide, decholesterol action of GGE–MN may be due to the effect of the metabolites of hydrolysed nicotinic acid. If not, then, apparent lowering of blood cholesterol level should be due to the action of the molecule of GGE–MN itself. The conclusion will be drawn after detailed metabolism of GGE–MN under way now is completed.

10) S. Chaykin, M. Dagani, L. Johnson, and M. Samli, *J. Biol. Chem.*, 240, 932 (1965).
11) T. Kobayashi, *Yakugaku Zasshi*, **81**, 195 (1961).
12) E. Leifer, L.J. Roth, D.S. Hogness, and M.H. Corson, *J. Biol. Chem.*, **190**, 595 (1951).

Pathological examinations were made to ascertain the results mentioned above. Prophylactic effect of equimolar amount of GGE, nicotinic acid, and GGE–MN on hypercholesteremia was examined pathologically by killing the animals in the A, B, C and D groups 3 days after the end of the feeding schedule shown in Table II. Therapeutic effect of the three chemicals

Table II. Feeding Schedule of Rabbits (*ca.* 2.0 kg Body Weight)

Group[a)]	Amount per day, orally, from 1st to 28th day
A	0.6 g of cholesterol+0.99 mg·mole of GGE in capsules, and 200 g of bean–curd refuse
B	0.6 g of cholesterol+0.99 mg·mole of nicotinic acid in capsules, and 200 g of bean–curd refuse
C	0.6 g of cholesterol+0.99 mg·mole of GGE–MN in capsules, and 200 g of bean–curd refuse
D	0.6 g of cholesterol in capsules, and 200 g of bean–curd refuse

Group	Chemical administered orally for 7 days after feeding with 0.6 g of cholesterol and 200 g of bean–curd refuse for 21 days[b)]
E	0.99 mg·mole of GGE
F	0.99 mg·mole of nicotinic acid
G	0.99 mg·mole of GGE–MN
H	no chemical

a) Each group consisted of 3 rabbits.
b) Blood cholesterol levels of all rabbits were above 1000 mg/dl after 21 days.

at the equimolar dose on hypercholesteremia was examined by killing the animals in the E, F, G, and H groups 3 days after the end of the feeding schedule shown in Table II. All the organs were fixed in formaldehyde solution. Although some individual differences were recognized more or less in each group, rough generalization of the observations in each group is shown in Table III.

Table III. Pathological Observations on Cholesterol Deposition in Rabbit Organs

Group	Upper part of artery		Middle part of artery	Spleen	Liver
	Endarterium	Exarterium	Endarterium		
A	++		+	++	++
B	+		+	++	+
C	+		+	++	+
D	+++		+++	+++	+++
E	+[a)]	++		++	+
F	+[a)]	+		++~+++	+~++
G	normal[a)]	normal[a)]		+	+
H	++[a)]	++[a)]		++	++

All specimens stained with Hematoxylin–Eosin.
a) Also, stained with Sudan III.
\+ mild degree ++ moderate degree +++ marked degree

Results of pathological observations are summarized as following:

(a) Groups A and E:

In the prophylactic group A, marked deposition of cholesterol (fatty substances) was recognized due to increased absorption of GGE, but in the therapeutic group E, cholesterol deposited in endarterium was washed out by the solubilizing power of GGE. However, the fact that some deposition of cholesterol in spleen and liver was observed even in the animals of Group E might be due to the fact that metabolism of cholesterol or fatty substances was retained in these organs.

(b) Groups C and G:

Even the prophylactic Group C, deposition of cholesterol was not so marked as in Group B. In the therapeutic group G, deposition of fatty substances was not recognized and better than Group F, even in spleen and liver.

It was found that GGE–MN has good advantages of both GGE and nicotinic acid, while some question remains whether hypocholesteremic effect of GGE–MN is due to that of GGE–MN itself and/or to the metabolites of GGE–MN *in vivo*.

Experimental

Triton Hypercholesteremia——Male rats of Wistar-strain weighing about 120 g were used. All animals were fed commercial laboratory chow before and during the experimental period. Each group consisted of 5 rats injected intravenously with 400 mg/kg of Triton in distilled water. Test sample was administered intraperitonealy immediately after injection of Triton. Each group of animals was killed 18 hours after receiving the Triton injection. At the time of sacrifice, each animal was bled completely, and the blood reserved for analysis.

Assay of Cholesterol——Cholesterol in blood was determined quantitatively by the method of Zak.[13]

***α*-Hydroxy-*β*-(*o*-methoxyphenoxy)propionic Acid**——To a solution of 24.8 g of guaiacol in 100 ml of 12% NaOH, 12.5 g of *β*-chlorolactic acid[14] was added, and the mixture was refluxed for 2 hr. The reaction mixture was adjusted to pH 6.5—7.0 with dilute HCl and steam distilled. The crude acid in the residue was precipitated from the cooled solution by acidification with HCl. The collected product was recrystallized with charcoal from hot acetone to 3.5 g of pure compound, mp 92—94°. *Anal.* Calcd. for $C_{10}H_{12}O_5$: C, 56.60; H, 5.66. Found: C, 56.72; H, 5.61.

(*o*-Methoxyphenoxy)acetic Acid——This compound was prepared by a procedure quite similar to that described above using monochloroacetic acid, and 3.7 g of colorless needles, mp 118°, from 9.9 g of guaiacol was obtained. *Anal.* Calcd. for $C_9H_{10}O_4$: C, 59.34; H, 5.49. Found: C, 59.15; H, 5.62.

N^1-Methylnicotinamide, Nicotinic Acid, Nicotinoylglycine, and Guaiacol Glycerol Ether——These compounds were commercial products (chemical pure grade).

Nicotinamide N-Oxide——Synthesized according to the method of Taylor and Crovetti.[15]

N^1-Methyl-2(1H)-pyridone-5-carboxamide——Synthesized according to the method of Huff.[16]

Acknowledgement The authors are grateful to Dr. K. Nakanoin, Division of Pathology, Public Health Research Institute of Kobe City, for his advices on pathological matters. The authors are much indebted to Dr. K. Okamoto, Kyoto Pharmaceutical Ind. Ltd., for his generous gift of GGE–MN, and to the students, Takashi Akita, Kazuhiko Kitao, and Yasushi Hirata for their technical assistances.

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