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Prevention and reversible solubilization of advanced glycation and products (AGE) by organic germanium compounds as derivatives of amino acids*

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Summary. The amino-carbonyl reaction (The Maillard reaction) of bovine lens crystallin, serum albumin or skin collagen with glucose was investigated to find effective means to prevent the formation of Advanced Glycation End Products (AGE) and induce the reversible solubilization of polymerized glycated proteins. The organic germanium compounds (Ge-132, 373, 385), derivatives of amino acids containing germanium as the linker of framework, were combined by the box titration method to determine the dose that would be most effective, compared with Aminoguanidine-HCl (AMG), α-tocopherol (VE), and pirenoxine (Catalin-K, CK). Although AMG suppressed the formation of AGE, effective concentrations were higher than 20 mM. Ge-385, when administered by itself at a low dose, induced the reversible solubilization of AGE made from crystallin, and albumin. The addition of any two reagents such as AMG, VE, CK and Ge-132 or 385 together to proteins lessened the effective range, and the peaks of smaller molecules in the profiles of HPLC and PAGE were quite remarkable. Examination was made of the effects of Ge-132 on the eyes of SAM mice, which show senescence accelerated cataracts at a relatively young age. The prevention of cataract-genesis and induction of reversible transparency of turbid

* Abbreviations used in this paper: BLC bovine lens crystallin; BSA bovine serum albumin; AsCol acid soluble bovine skin collagen type III; AGE advanced glycation end products; Ge-132 2-Carboxyethylgermanium sesquioxide, Ge-373 2-Carboxy-2-amino-6-phenyl germanium sesquioxide; Ge-385 2-Carboxy-ethyl-2-aminogermanium sesquioxide; AMG or AG aminoguanidine-HCl; V. E. vitamin E or α-tocopherol; CK 1-Hydroxy-5-oxo-5H-pyrido [3, 2-a] phenoxazine-3-carboxylic acid or catalin-K or pirenoxine; PACE polyacrylamide gel electrophoresis; SAM senescence accelerated mouse; HPLC high pressure liquid chromatography; SDS sodium laurylsulfate; FT fructose-p-toluidine.

lenses became evident following the administration of Ge-132 to the eyes 4 times a day. The mode of action of organic germanium compounds was demonstrated quite capable of disconnecting the sugar-parts from AGE by decarbonylation, resulting in the formation of glucosone and amino residues, and further leading subsequently to fewer AGE.

Keywords: Amino acids – The Maillard reaction – Advanced glycation end products (AGE) – Reversible solubilization – Organic germanium compounds – Decarbonylation

Introduction

The Maillard reaction may possibly be the major etiology of cataracts, arteriosclerosis, mutations and even aging. However, research on etiology and therapy for these diseases has just started (Monnier, et al., 1984, and Brownlee, et al., 1988). It is commonly considered that the final glycation products (AGE) cannot be digested by proteinases except Fructosyl-amino acid oxidase from *Corynebacterium* sp. 2-4-1 (Horiuchi, et al., 1989). However, the application of this enzyme for human therapy involves several difficulties such as antigenicity, and drug-delivery and penetration into disease-foci. From this viewpoint it is the most urgent research project to find out the drugs with low antigenicity and molecular size, and which can disconnect AGE into components with smaller sizes.

Although the etiology of all cataracts is not necessarily the Maillard reaction, the amino-carbonyl reaction in the human body would account for this disease. For complete clarification of this point, an in vitro cataract model was first established, and the effectiveness of aminoguanidine-HCl (AMG) (Brownlee, et al., 1986), VE, and CK was subsequently examined and comparison was made with the effects of the organic germanium compounds, Ge-132, 373, and 385, derivatives of amino acids containing germanium as the linker of framework. AMG was found actually prevent the glycation of proteins but only when administered at a very high dose. The Ge-compounds, VE, and CK were effective, even when given alone at various concentrations. The same effectiveness could be attained at a lesser dose of either when both were administered together. They were quite capable of causing reversible solubilization of AGE. The effectiveness of Ge-132 and CK for preventing and solubilizing senile cataracts in SAM mice was confirmed. The effects of Ce-compounds and AMG in the Maillard reaction of BSA and collagen with glucose were also examined, and the results obtained appear to hold potential as a basis for therapy that would prevent the progress of glycation and induce the reversible transparency of polymerized proteins formed by the Maillard reaction in vivo.

Material and methods

Proteins, reagents, and experimental procedures

Bovine lens α-crystallin (BLC), bovine serum albumin (BSA), acid soluble bovine skin collagen type III (AsCol; SIGMA, St. Louis, MO) were dissolved separately in 0.5M

phosphate buffer, pH 7.4, containing 6 mM NaN_3 at a specified concentration. An equal volume of 400 mM glucose in distilled water was mixed with each of these protein solutions. Aminoguanidine-HCl (AMG) (Tokyo Kasei Co., Itabashi, Tokyo), α-tocopherol acetate (vitamin E) (SIGMA), 1-Hydroxy-5-oxo-5H-pyrido [3, 2-a] phenoxazine-3-carboxylic acid (pirenoxine, Catalin-K, CK, Senju Pharmaceutical Co., Osaka) (Ogino, 1955), 2-Carboxyethylgermanium sesquioxide (Ge-132) (Miyao, et al., 1988), 2-Carboxy-2-amino-1-phenylethyl-germanium sesquioxide (Ge-373) and 2-Carboxy-2-aminoethylgermanium sesquioxide (Ge-385) (Fig. 1) were dissolved in 50 mM phosphate buffer, pH7.4 at the 20 times concentrations, and 1/20 volume of the serial diluent was added to each well by the box-titration method. Plates were incubated at 37° C in a moisture saturated plastic box. On the sampling day, 7 μl were taken into a small tube and diluted with 133 μl of 5 mM phosphate buffer, pH6.8 prior to conducting HPLC using a TSK-G3000SW column, UV detector (280 nm, UV-8010), and fluoro-detector FS-8010 (Ext:350 nm, Emt:440 nm) controled by a super system controler SC-8010. The results were automatically calculated and recorded by a PP-8010 recorder (Toso, Co., Akasaka, Tokyo).

(1) 2-Carboxyethylgermanium sesquioxide : Ge-132

$O_3(GeCH_2CH_2COOH)_2$ MW : 339

(2) 2-Carboxy-2-aminoethylgermanium sesquioxide : Ge-385

$O_3(GeCH_2CHCOOH)_2$ MW : 369
NH_2

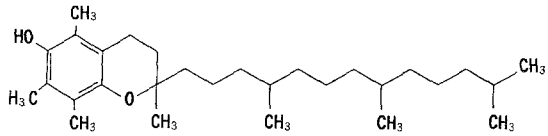
(3) 2-Carboxy-2-amino-1-phenylethylgermanium sesquioxide : Ge-373

$O_3(GeCH\text{-}CHCOOH)_2$ MW : 521
NH_2

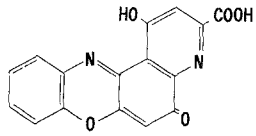
(4) Aminoguanidine·HCl

$H_2N\text{-}C\text{-}NHNH_2 \cdot HCl$ MW : 111
NH

(5) α-Tocopherol, vitamin E MW : 431



(6) 1-Hydroxy-5-oxo-5H-pyrido[3,2a]phenoxazine-3-carboxylic acid :
Pirenoxine, Catalin-K



MW : 308

Fig. 1. Molecular formula of reagents used in this study

Electrophoresis

AGE made from collagen and glucose was analysed by polyacrylamide gel electrophoresis containing 1% sodium laurylsulfate (SDS) (SDS-PAGE) consisting of 4 to 12% continuous

gradient polyacrylamide. The gels were stained by silver stain (Woko Pure Chem. Co., Osaka) (Poehling, et al., 1981) and the profiles analysed by a densitometer (Type DNS-300, Shimadzu, Kyoto).

Senescence accelerated mouse (SAM) as a cataract model

SAM (P-3) mice at various months of age were kindly provided by Dr. M. Namiki, Nagoya University School of Agriculture, Chikusa-ku, Nagoya (Takeda, et al., 1981). One drop of eye solution consisting of 4% Ge-132 in saline and 0.005% pirenoxine (Catalin-K, CK) were applied together or separately to the eye 4 times per day (8, 12, 16, 20 o'clock). The progress of cataract development was followed under an anatomical microscope, with the animals anesthetized by nembuthal and their pupils dilated with a drop of 0.5% tropicamide (Mydrin-P) which had been applied at four to five week interval.

Mode of action of Ge-compounds

Fructose-*p*-toluidine was used as a model for elucidation of the mechanism of reversible solubilization of turbid crystallin (Kawakishi, et al., 1990). Briefly, fructose-*p*-toluidine (FT) 2 mM suspended in 50 mM phosphate buffer, pH7.4, was mixed with Ge-compounds (Ge-132, 373, 385) at various concentrations and incubated at 40°C for 24 hours. As the control reagent, Cu^{2+} 50 μM was used in FT 2 mM. The mixtures were analysed using a Develosil ODS-5 column (4.6 × 150 mm) with a solvent consisting of 3 parts CH_3CN, and one part H_2O, the elution speed was 2.0 ml/min and monitoring was conducted by a UV detector at 240 nm.

Results

Preventive effects of organic Ge-compounds and other reagents on the AGE formation from BLC

When AMG was used alone, the effective range exceeded that at 20 mM. At lesser doses, the effect was small or none. Ge-132 or Ge-385 administered alone was effective up to 51.20 nM or 10.24 nM or less, respectively. Together, their preventive effect was remarkable, as shown in Table 1.

The effects of VE, CK and Ge-132 on AGE formation from BLC were examined and are summarized in Table 2. Administered together, their suppression of AGE formation was markedly more.

The effects of AMG and Ge-compounds on precipitated ready made AGE containing BLC and glucose were examined. AMG 200 mM, Ge-385 at all concentrations and 20 mM Ge-132, when given alone, effectively induced the reversible transparency of turbid BLC. With AMG and Ge-385 together, the transparent spots changed to 64 μM AMG and 32 μM Ge-385. With Ge-132, they shifted to 4.0 mM and 64 μM AMG. In these spots, turbid crystallin became transparent within 13 days following their combined administration (Table 3).

Glycation of BSA and effects of AMG and Ge-compounds

The effects of AMG and Ge-132 on the glycation of BSA are summarized in Fig. 2. AMG or Ge-132 alone was incapable of preventing glycation, though

Table 1. Bovine lens crystallin 25 mg was dissolved in 1 ml 500 mM phosphate buffer containing 6 mM NaN_3, pH7.4, and mixed with 200 mM glucose. Various amounts of aminoguanidine-HCl, Ge-132, and Ge-385 were distributed as indicated in the Table, followed by incubation at 37°C for 30 days and analysis by HPLC equipped with a TSK-G3000SW column. The numerals indicate fluorescence intensity due to crystallin glycation

Aminoguanidine · HCl								
1.28 μM	6.4 μM	32 μM	160 μM	800 μM	4.000 mM	20.000 mM	Incubated for 30 days	
581	559	536	525	643	579	255		Ge-132
435	403	414	481	384	311	237	346	160.000000 μM
343	424	445	447	366	342	252	412	32.000000 μM
321	381	411	478	418	301	256	445	6.400000 μM
280	386	398	421	390	311	224	470	1.280000 μM
293	362	393	420	373	295	196	505	256.00 nM
263	351	380	351	337	278	202	521	51.20 nM
282	336	375	366	308	251	243	626	10.24 nM
								Ge-385
251	326	289	342	303	307	112	273	160.000000 μM
292	274	345	338	298	207	123	258	32.000000 μM
233	250	267	266	243	184	126	225	6.400000 μM
229	276	303	301	251	207	153	262	1.280000 μM
248	257	316	289	260	213	164	288	256.00 nM
278	251	328	268	245	222	169	299	51.20 nM
301	283	334	284	259	236	169	356	10.24 nM
		(Crystal + Gluc): 589 ± 11			(Crystallin): 100 ± 8			

Table 2. Induction of reversible solubilization of AGE made from bovine lens α-crystallin and glucose was measured by a micro ELISA reader (620 nm) inoculating AGE crystallin and various concentrations of Ge-compounds and aminoguanidine-HCl at 37°C for 13 days. The degree of reversible solubilization or transparency of each hole is indicated by the minus symbol, and increase in turbidity by the plus comparing with the hole incubated without any reagent (0)

Aminoguanidine-HCl									
13 μM	64 μM	320 μM	1.600 μM	8.000 mM	40.000 mM	200.000 mM			
−40	−22	− 22	− 21	− 35	− 38	− 59	0		
−72	−21	− 6	− 30	− 69	− 56	− 89	−100	20.000 mM	
−24	+ 9	− 3	− 70	− 65	− 77	− 46	− 96	4.000 mM	
−55	−40	− 56	− 93	−100	− 47	− 31	− 67	800 μM	
−70	−69	−100	− 60	− 64	− 60	− 93	− 19	160 μM	Ge-385
−55	−94	−100	−100	− 81	− 40	− 25	− 50	32 μM	
−79	−60	− 64	− 41	− 41	− 43	− 37	− 21	6.4 μM	
−28	−36	− 32	− 34	− 44	− 21	− 70	− 38	1.3 μM	
−45	−56	− 79	−100	− 86	−100	−100	− 99	20.000 mM	
−22	−77	− 64	− 51	− 37	− 19	− 57	+ 42	4.00 mM	Ge-132
−17	+25	− 4	− 30	− 45	+ 23	− 33	− 5	800 μM	
−31	+19	− 11	+ 1	− 39	− 16	− 48	+ 29	160 μM	

Table 3. Preventive effects of Ge-132, pirenoxine (Catalin-K) and vitamin E on the formation of AGE from α-crystallin and glucose were examined by the box titration method. 96 hole plates were incubated at 37°C for 140 days and aliquots were diluted 20 times by 50 mM phosphate buffer, pH6.8, and applied onto a HPLC using TSK-G3000SW column and the same phosphate buffer as eluting solution, 1 ml/min. Fluorescence intensity (Ext:350 nm, Emt:440 nm) was monitored by FS-8010 detector

Vitamin E								Pirenoxine (Catalin-K)									
0.000640 mM	0.003200 mM	0.016000 mM	0.080000 mM	0.400000 mM	2.000000 mM	10.000000 mM		0.000320 mM	0.001600 mM	0.008000 mM	0.040000 mM	0.200000 mM	1.000000 mM	5.000000 mM			
333	292	264	245	240	282	402		245	204	201	198	296	430	427			
158	193	175	174	165	170	153	179	165	167	176	228	419	439	444	180	20.000000 mM	
172	144	159	176	182	154	174	194	197	184	198	231	302	445	485	196	4.000000 mM	
258	203	131	166	210	168	185	254	215	187	141	208	437	455	588	255	0.800000 mM	
268	175	183	223	180	163	167	331	213	180	208	224	303	459	565	333	0.160000 mM	
231	218	168	163	161	192	189	357	213	192	182	206	300	458	555	359	0.032000 mM	
331	220	134	166	284	261	203	340	189	181	180	218	290	431	563	391	0.006400 mM	Ge-132
291	249	200	187	183	187	229	399	206	187	194	216	295	444	597	410	0.001280 mM	
322	175	191	182	169	184	281	420	231	195	157	219	291	462	511	431	0.000256 mM	
242	333	183	255	238	173	168	434	300	205	291	223	290	422	493	445	0.000051 mM	
544	172	311	339	331	347	299	531	331	364	329	233	309	420	519	535	0.000010 mM	
498 + 25				12 + 1				503 + 29				13 + 1				Controls	
Incubated for 137 days								Incubated for 140 days									

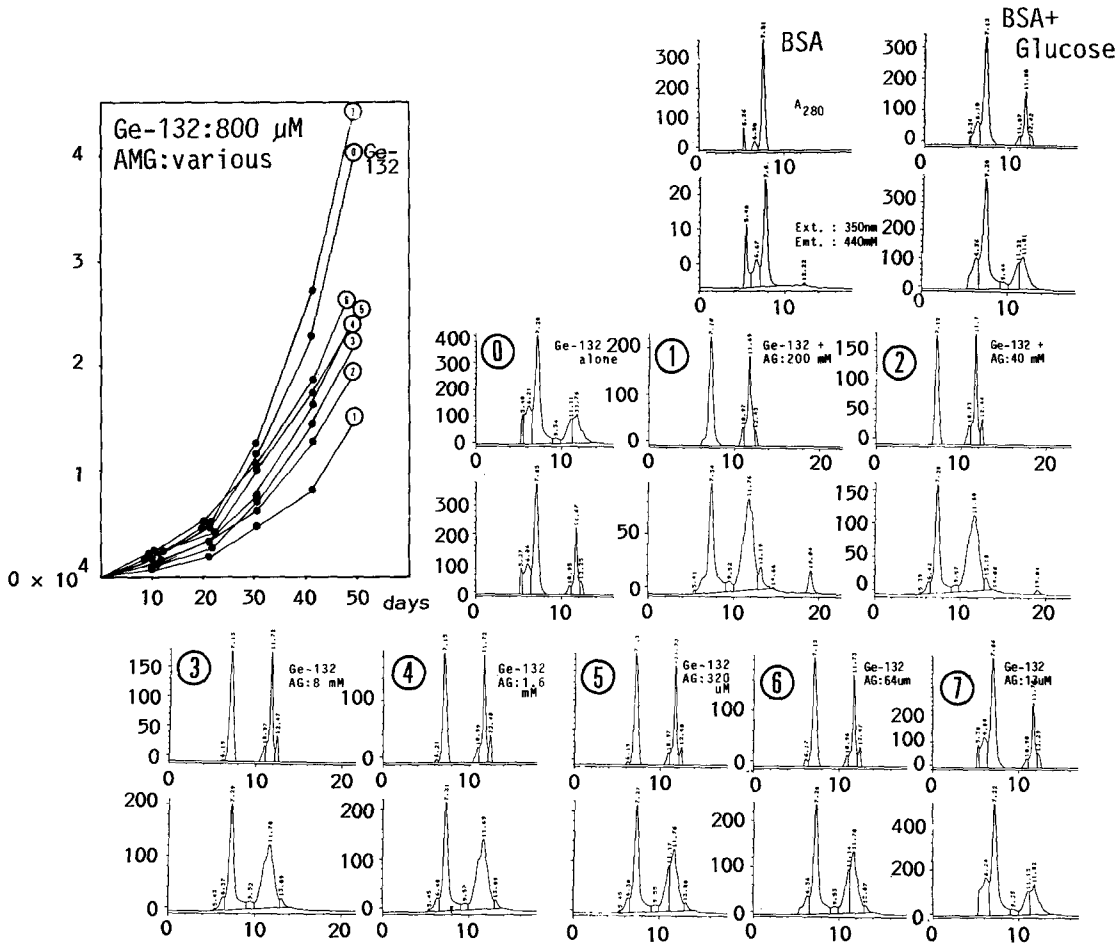


Fig. 2. Effects of aminoguanidine-HCl and Ge-132 on AGE formation from BSA and glucose were examined by mixing 100 mg/ml BSA in 0.5 M phosphate buffer containing 6 mM NaN_3 and an equal volume of 200 mM glucose solution, followed by adding the reagents (AMG or AG, and Ge-132) at various concentrations. On each sampling day, 7 μl were taken and diluted by 5 mM phosphate buffer pH6.8 and analysed by HPLC using a TSK-G3000SW column, and fluorescence intensity was measure (Ext:350 nm, Emt:440 nm)

AMG did so when administered at the highest possible concentration. Their administration together caused decrease in fluorescent intensity with rise in AMG concentration.

The characteristic effects of Ge-385 were comparatively examined with Ge-132 on the glycation of BSA. Even though Ge-385 was present in the mixtures, glycation continued up to day 10 (Fig. 3) Thereafter, depending on the concentration of Ge-385, fluorescence intensity decreased. Ge-132 was incapable of having any such drastic effect.

The collaborative effects of Ge-385 and AMG on ready made AGE consisting of BSA and glucose were examined. With 4 mM Ge-385 alone, glycation accelerated for a while and then decreased up to day 31 (Fig. 4). Of several combinations, 4 mM Ge-385 and 1.6 mM AMG were clearly noted to prevent glycation (Curve No 2, the middle figure in the lower line). Higher concentra-

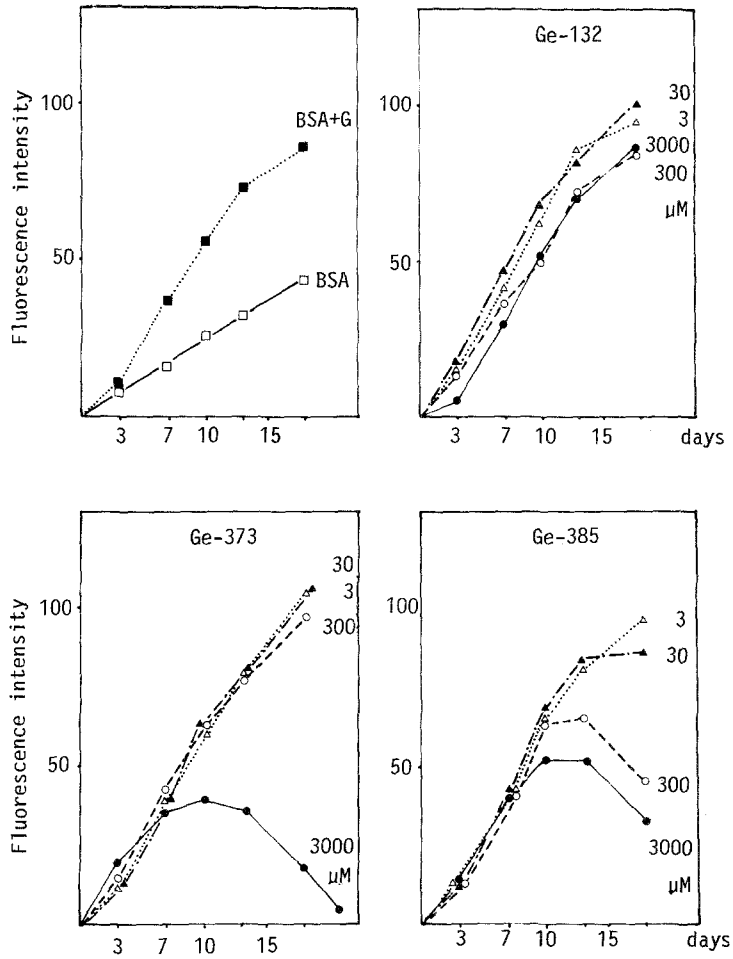


Fig. 3. Effects of Ge-compounds (Ge-132 and Ge-385) on the formation of AGE from BSA and glucose were examined by mixing 100 mg/ml BSA in 0.5 M phosphate buffer and 200 mM glocuse containing 6 mM NaN_3, and Ge-compounds at various concentrations. Fluorescence intensity was measured at an excitation wave length of 350 nm and emission wave length of 440 nm using a fluoro-spectrophotometer type 850 (Hitachi Co., Tokyo)

tions of AMG (AG) generally appeared to suppress further progress of AGE formation.

Glycation of collagen and effects of Ge-compounds and AMG

Acid soluble bovine skin collagen incubated with 200 mM glucose developed AGE, when analysed by PAGE, as shown in Fig. 5. In the profiles of PAGE, each reagent effectively prevented AGE formation at the specified concentration. On using Ge-compounds and AMG together, lesser doses were required for effectiveness, and large molecular AGEs clearly ceased to be evident in the PAGE profile.

The effects of AMG and Ge-compounds on AGE formation as determined by a fluoro-spectrophotometer are summarized in Fig. 6. Although AMG 8.0

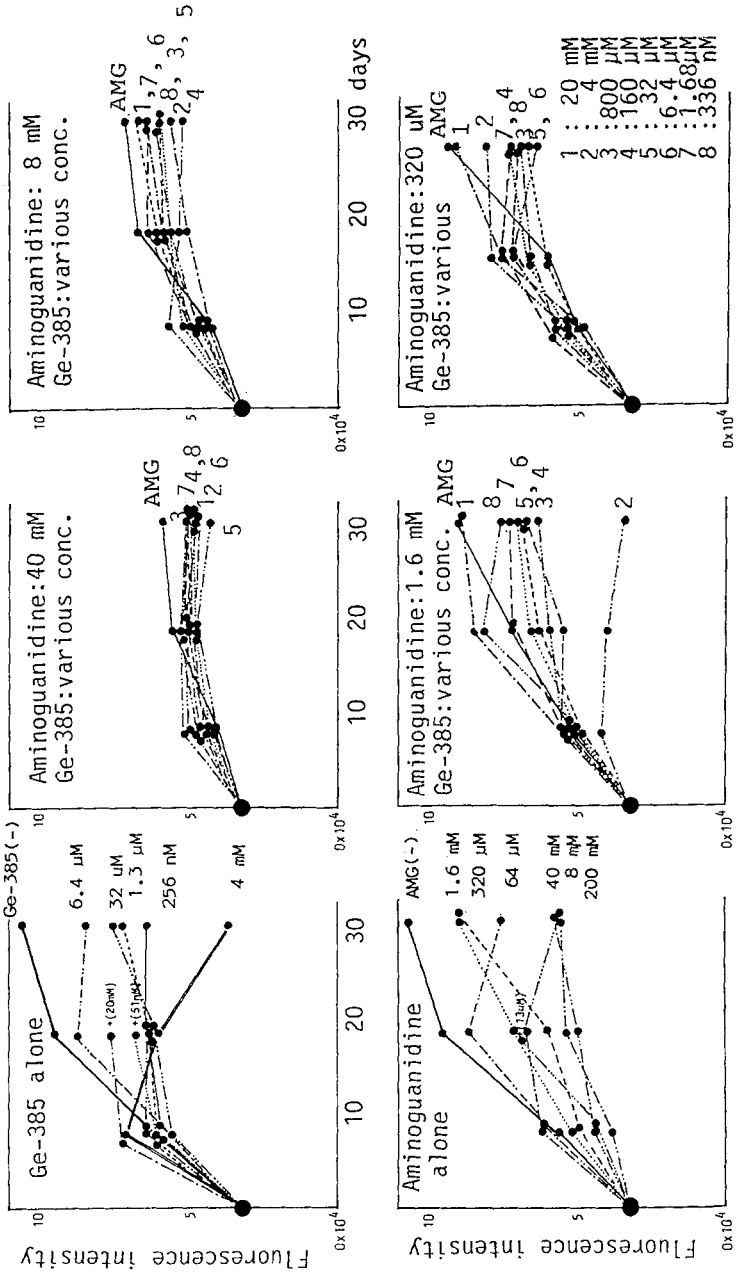


Fig. 4. The collaborative effects of aminoguanidine-HCl (AMG or AG) and Ge-385 on ready' made BSA. AGE was examined following the incubation of 50 mg/ml BSA and 100 mM glucose at 37°C for 70 days. These reagents were added separately or together at the concentrations specified. Fluorescence intensity due to glycation was measured automatically by HPLC using a fluorodetector (Ext:350 nm and Emt:440 nm, a set of type 8010, Toso Co., Akasaka, Tokyo)

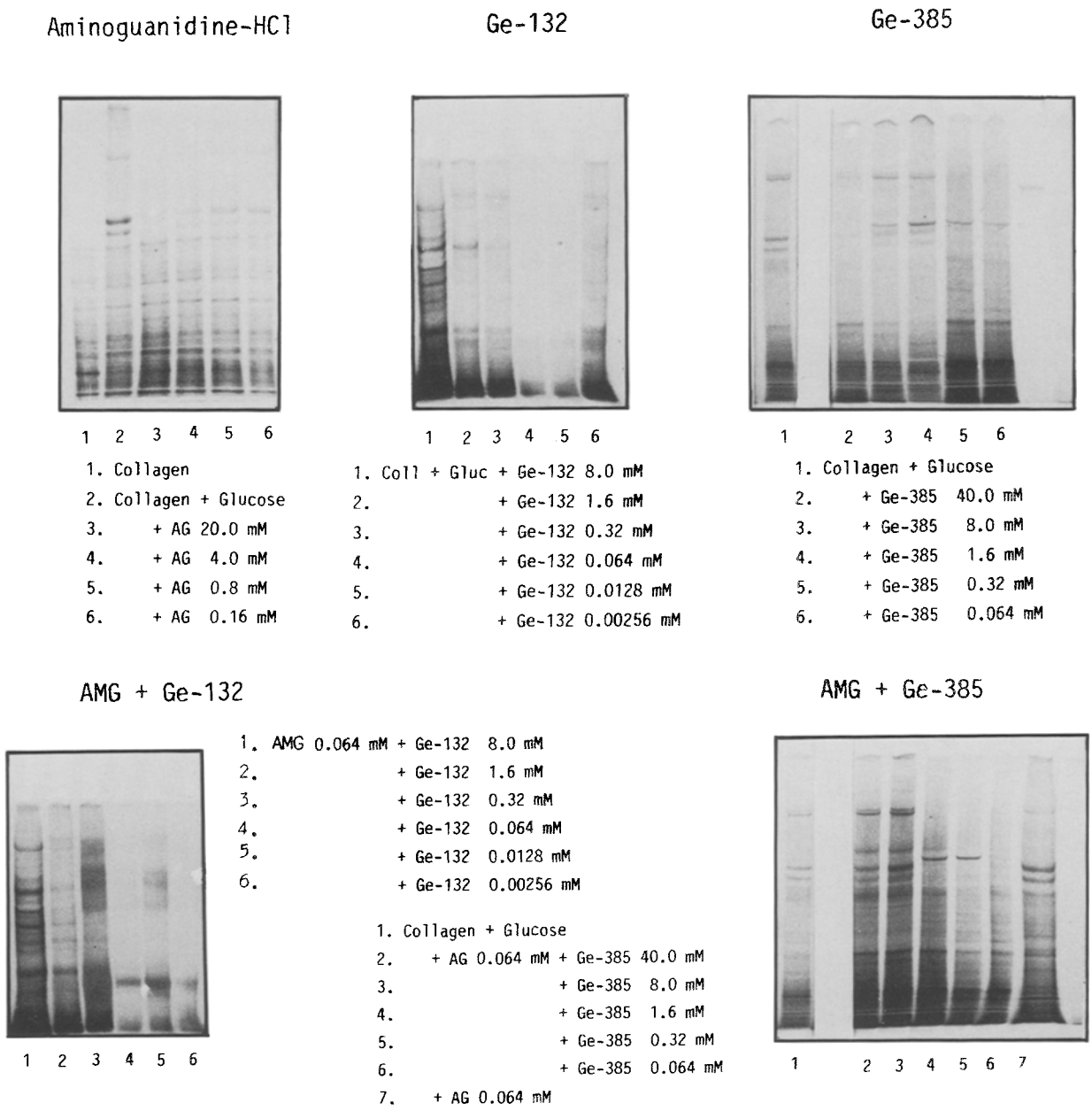


Fig. 5. Profiles by polyacrylamide gel electrophoresis (PAGE) of acid soluble bovine skin collagen. Various concentrations of reagents were added separately or together to the tube containing 10 mg collagen, 200 mM glucose and 3 mM sodium azide in 0.05 M phosphate buffer, pH7.4, and incubated at 37°C. A continuous gradient of 4 to 12% polyacrylamide gel with 1% SDS was used for analysis. The gels were stained by Silver stain

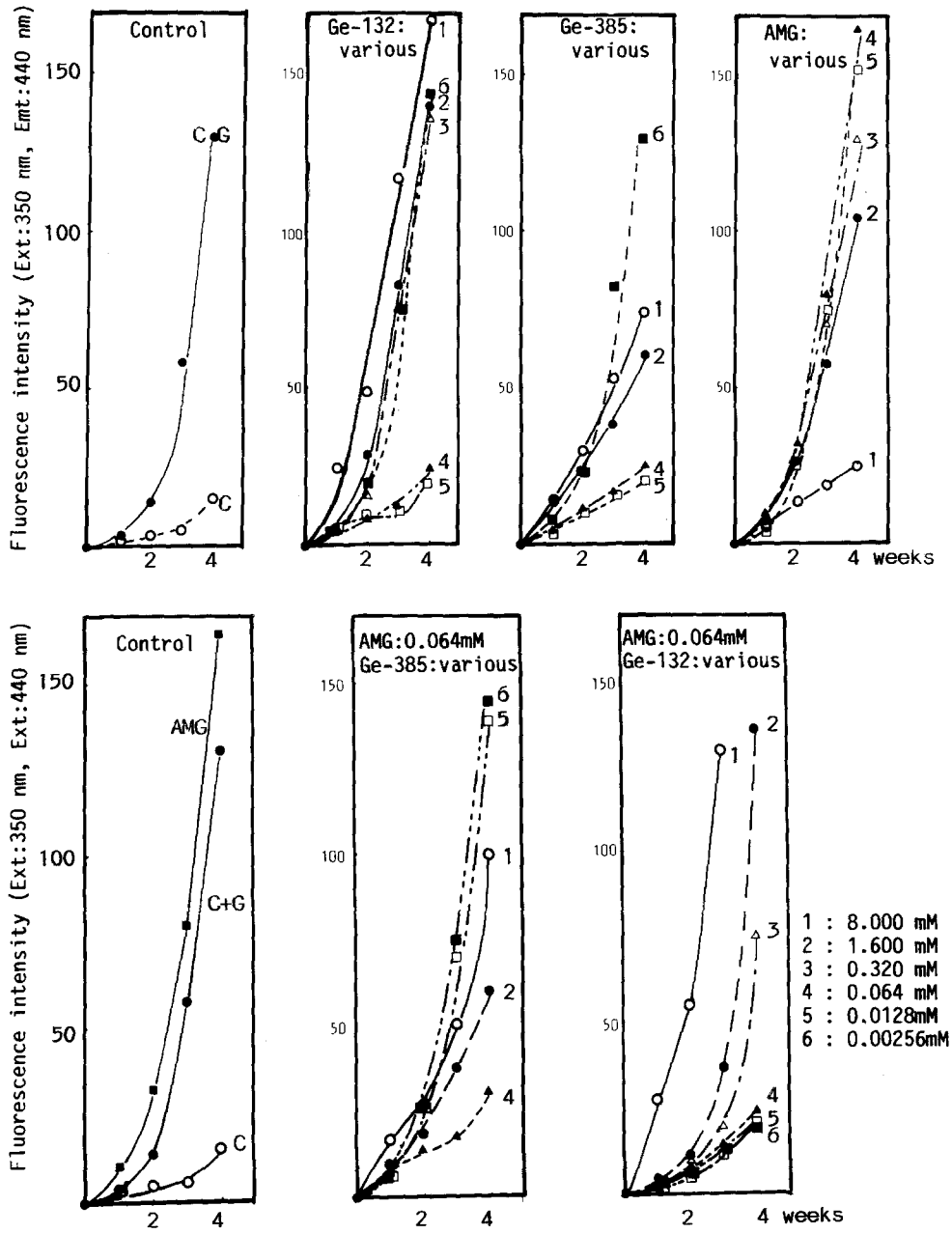


Fig. 6. Preventive effect of aminoguanidine-HCl and germanium compounds on the formation of advanced glycation end products (AGE) from calf skin collagen type III and glucose. In the control, reagent was added to the tube containing 10 mg bovine skin collagen type III and 200 mM glucose. In the experimental groups, various concentrations of reagents, indicated by numerals, were added. Fluorescence intensity was analysed using a fluorescence spectrophotometer (Type 1201, Hitachi-Nisseisangyo Co., Tokyo), at an excitation wave length 350 nm, and emission wave length 440 nm

mM effectively suppressed AGE formation, Ge-compounds could do so at lesser doses, such as 12.8 μM. By application of AMG and Ge-compounds together, Ge-132 was effective at 2.56 μM and AMG at 64 μM.

The addition of Ge-132 3 weeks after incubating collagen and glucose at 37°C accelerated AGE formation, and no definite decrease could be detected in the fluorescence by the effect of AMG or Ge-385. Neither was effective for preventing further progress of AGE formation, once the reaction had started (data not shown).

Effects of Ge-132 on cataracts in senescence accelerated mouse (SAM)

SAM (P-3) mice manifest cataracts at a very high rate (Hosokawa, et al., 1988). The preventive effects of Ge-132 and CK when the mice were 1 month old were examined. One drop of 4% Ge-132 in saline and 0.005% CK in 1% boric acid were administered separately or together to the eyes 4 times per day. The simultaneous administrations of Ge-132 and CK to three SAM mice each one month old completely prevented any change in the lens for as long as 120 days, while all control lens showed macroscopic changes by day 60. The separate administration of each drug caused reduction in the rate of cataract-genesis (Fig. 7).

The effects of Ge-132 at 10 months of age were also studied. As shown in Figure 8, in the group treated by Ge-132, the transparent eyes manifested change from 3 to 4 by 78 days, although transparency had been zero on day 39. In the control group, no such reversibility could be observed by 123 days. SAM mice administered Ge-132 survived longer than the control group (number of eyes in surviving animals is indicated in Fig. 8 with numerals).

Mode of action of Ge-compounds

Ge-compounds were noted to degradate fructose-p-toluidine into all of its separate components, so that glucosone appeared to be free from FT molecules.

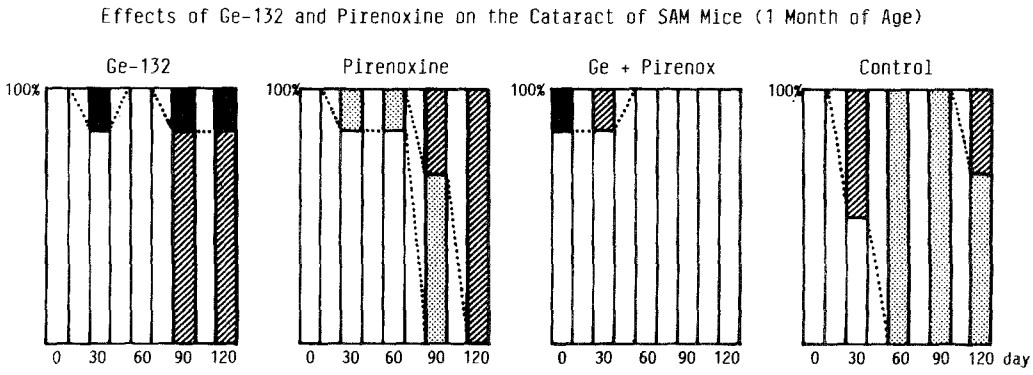


Fig. 7. Preventive effects of Ge-132 and Catalin-K (Pirenoxine) on cataract-genesis in SAM mouse eyes at 1 month of age were examined, the drugs being administered separately or together. Each group consisted of 6 eyes. The numerals indicate days after starting the administration of the drugs 4 times a day. Black column: turbid, diagonally striped column: distortion of lens, meshed column: wedge formation, white column: transparent lens

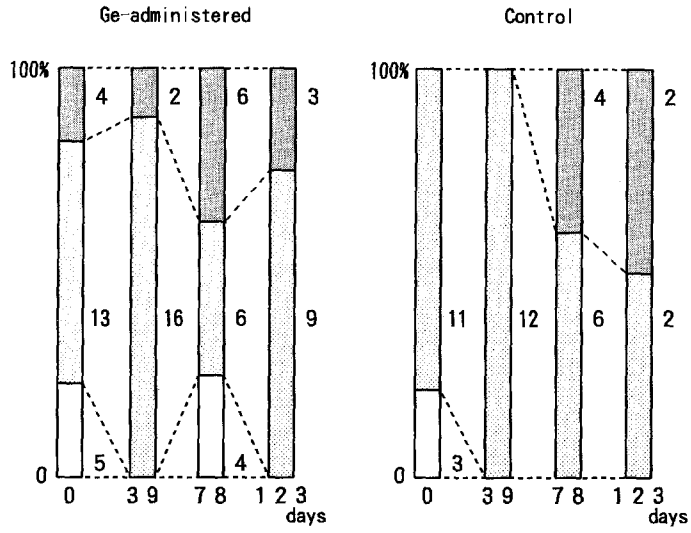


Fig. 8. Induction of reversible transparency in the lens of SAM mice. Numbers on the right margin of each column indicate the number of eyes examined. SAM mice at 10 months of age were used as 0 time, and a drop of Ge-132 was continuously applied 4 times a day. Black column: % of turbid lens, meshed column: nuclear hardening of lens, and white column: transparent lens

Table 4. Mode of action of Ge-compounds was investigated by fructose-*p*-toluidine (FT) 2 mM suspended in 50 mM phosphate buffer, pH7.4, at 40°C for 24 hours. A Develosil ODS-5 column was used to analyse the degradation of FT to glucosone and toluidine, eluting the buffer consisted of CH_3CN:H_2O = 3:1, elution speed:2.0 ml/min, detecting at 240 nm

Oxidative degradation of fructose-p-toluidine with Ge-compounds			
	FT(peak area)	Residual FT(%)	Activity(%)
None	70	100	0
Cu^{2+} 50 μM	7	10	100
Ge-132 3 mM	51	72.9	30.2
0.3 mM	50	71.4	31.7
0.03 mM	52	74.3	28.6
Ge-373 3 mM	36	54.3	50.8
0.3 mM	46	65.7	38.1
0.03 mM	48	68.6	34.9
Ge-385 3 mM	41	58.6	46.0
0.3 mM	49	70.0	33.3
0.03 mM	52	74.3	28.6

The results summarized in Table 4 demonstrate quite clearly that Ge-compounds can cause the breakdown of AGE into glucosone and amino residues. The induction of reversible solubilization of AGE may thus occur through non-enzymatic decarbonylation.

Discussion

The present results show the novel Ge-compounds, Ge-132, 373, and 385, to be effective for preventing the glycation of BLC, BSA, and collagen. In in vitro experiments, AMG was effective at relatively high doses. When AMG was administered along with any of these Ge-compounds, only 1/125 the original dose was effective for bringing about the solubilization of AGE consisting of BLC, and also BSA. The dose of Ge-132 was varied when used with AMG at high dose (20 mM). Ge-132 was generally effective at lower doses.

The present results confirm Ce-132 to be adequate for treating cataracts in SAM mice by application to the eyes. Some turbid lens became transparent on day 78. This compound has long been used in Japan. Basic clinical data on its mode of action have thus accumulated and present authors undertook the present study using the weaker Ge-132, rather than Ge-385, though the later appeared a more effective agent for treatment.

The effect of Ge-385 were much more stronger than 132 for reversing the solubilization of AGE and preventing glycation in BLC, BSA and collagen. This capacity for AGE solubilization revarsal in in vitro systems is the outstanding feature of this drug. However, the authors so far have not used it in in vivo experiments. Ge-385 is a derivative of alanine whose molecular formula is $O_3(GeCH_2CHNH_2COO)_2$, 2-Carboxy-2-aminoethylgermanium sesquioxide. Additional research on its toxicity and effects on protein synthesis has been conducted and neither toxicity nor incorporation into proteins has been detected (data not shown).

In the present study, we did not show the biological activity of Ge-373, a derivative of phenylalanine, because this compound disconnected DNA strands and increased the absorption at 256 nm by the spectrophotometrical analysis (data not shown). Though we found that Ge-373 could also prevent AGE formation from BLC and BSA, such experimental data were not cited to avoid confusion with other Ge-compounds.

The mode of the action of reagents used in this study is the focal point for application to practical clinical treatment. AMG, (molecular conformation, $NH_2C(NH)_2NH_2HCl$), competitively binds to the carbonyl residue in sugar, and prevents further Amadori rearrangement. Ge-compounds supposedly possess activity for decarbonylation from amino-carbonyl complexes. In the model experiment using fructose-p-toluidine, the amino residue and glucosone were isolated as the effect of Ge-compounds. However, this free glucosone is reactive with amino acids through the assistance of trace transitional metals as contaminates. Ge-385, having amino residues in its molecule, may effectively function to bring about decarbonylation and prevent amino residues of AGE separating from the reactive carbonyl residue. Thus, the effects of Ge-compounds particularly significant for reversing the solubilization of AGE produced from various proteins.

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