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EXPERIMENTAL PAPER

Inhibition of glycoxidative modification of proteins by some substances of natural origin

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Summary

Introduction: Advanced glycation end-products (AGE) and advanced oxidation protein products (AOPP) are the main products of glycoxidative modification in diabetes. **Objective:** The aim of this study was to identify the natural substance with the strongest antiglycoxidative properties among dietary supplements or medicines available without prescription in Poland. **Methods**: Bovine serum albumin (BSA), vitamin C (VC), aminoguanidine (A), quercetin (Q) and green tea (GT) were tested *in vitro* in comparison to controls in glycation, oxidation and glycoxidation processes. The decreased AGE and AOPP concentrations were measured as markers of these processes. **Results**: AGE level was reduced by 72% by VC and at least by 43% by all examined substances in the glycation process. AOPP was reduced by 99% by VC and at least by 40% by all examined substances in the oxidation process. Formation of AGE/AOPP was inhibited by 61% by Q and by 97% by A, and at least 49/88% by all examined substances, respectively. This lowering of AGE/AOPP level was statistically significant ($p<0.001$) for all test substances in comparison to the positive control C(+). **Conclusion**: All examined substances are able to inhibit glycative,

oxidative and glycoxidative modification of proteins in different degrees depending on their concentration.

Key words: *protein oxidative modification, protein glycation modification, protein glycoxidation modification, AGE, AOPP, inhibition by natural origin substances, in vitro study*

INTRODUCTION

Hyperglycemia and oxidative stress are the main, almost equally important factors, triggering non-enzymatic modifications (glycation and oxidation, respectively) of many macromolecules, especially proteins, which occur physiologically *in vivo* at a low level. These modifications occur in different ways (directly and indirectly), altering the structure and function of these molecules. Finally, advanced glycation end-products (AGE) and advanced oxidation protein products (AOPP) are created. In human, the glycation and oxidation processes are usually chronic, most often are simultaneous, mutually intensify their adverse effects, and therefore are commonly named glycoxidation. They occur with high intensity, especially in diabetes, leading to glycoxidative modifications of many particles and macromolecules in tissues and organs. Moreover, accumulation of these modified molecules participates in the development of diabetic vascular late complications (DVLC). There is a wealth of information on unfavorable action of chronic hyperglycemia and oxidative stress during the formation of AGE and AOPP in diabetics [e.g. 1, 2, 3]. The effective treatment of diabetes, especially oriented at lowering of hyperglycemia and suppression of oxidative stress, is still an important factor in effective management of this disease. The possibilities of the inhibition or prevention of glycoxidative modifications of proteins, and thereby inhibition of AGE and AOPP formation or lowering their concentration by different agents (conventional medications or substances of natural origin), are still intensively investigated and described, especially in terms of their participation in the delay of the development of DVLC and diabetes-associated diseases [4, 5]. Diabetic patients are usually treated conventionally, however, often take various diet supplements, vitamins, minerals, or herbal medicines. These agents usually contain some substances of well-known antioxidative activity, but less is known about their inhibition of glycation processes, while data about the ability of simultaneous inhibition of AGE and AOPP formation (glycoxidation) are very scarce. Knowledge concerning these properties and possibilities of their application may be helpful both in prevention and treatment of DVLC [6, 7].

The aim of this study was to identify a substance of natural origin, commonly available in Poland in the form of non-prescriptive dietary supplements or medicines, with the strongest antiglycoxidative properties. The most popular natural substances, such as vitamin C, aminoguanidine as well as quercetin and green tea, were chosen for this study. Bovine serum albumin (BSA) was used as a model protein. The potential of these natural substances for inhibition of glycation and oxidation processes as well as simultaneous modification of glycoxidation reflected by the decrease of AGE and AOPP formation were measured.

MATERIAL AND METHODS

Material

Bovine serum albumin (BSA) in the concentration of 40 mg/ml, which corresponds to the physiological concentration of albumin in human blood, was used as a model protein. The BSA solution underwent glycation, oxidation and glycoxidation processes described in detail below. As glycation and oxidation agents glucose solution in a concentration of 500 mg/ml and chloramine T solution at a concentration of 20 mM, respectively, were used. These values were established experimentally in our laboratory and chosen on the basis of our previous research (data not shown). All experiments were conducted in triplicate and the results were presented in 3 figures, where were expressed as the mean of these measurements with ± standard deviations. In each series of experiments a negative [C(-)] and positive [C(+)] control of BSA were made. The C(-) solution of BSA, dissolved in PBS (pH 7.4), was incubated without any additional agents in the same conditions as all examined samples. The C(+) solution of BSA, also dissolved in PBS, was incubated along with glucose or chloramine T, as well as simultaneously with glucose and chloramine T solutions in appropriate concentrations (40 mg/ml, 500 mg/ml, 20 mM, respectively) for the process of glycation, oxidation and glycoxidation of protein, respectively. In the experiments the following substances were used: aminoguanidine in 20, 50 and 80 mM concentrations, labeled as A20, A50 and A80, respectively; vitamin C (in concentrations of 10, 50 and 100 mM) – labeled as VC10, VC50 and VC100, respectively; quercetin (in 10, 25 and 50 mM concentration) – labeled as Q10, Q25 and Q50, respectively. Green tea, in a final concentration of 10 mM of epigallocatechin gallate (EGCG), labeled as GT, was chosen in one concentration resulting both from the applied formulation form (capsules containing the standardized extract of the leaves of GT) as well as an earlier experimentally selected effective concentration of the active substance. An ethanolic solution of quercetin and green tea, and a PBS solution of aminoguanidine and vitamin C, filtered through a 0.22 μm filter (Millipore, USA), were used. All reagents used in this study were purchased from Sigma-Aldrich (USA) except green tea, which was taken from Aboca's (Poland) dietary supplement.

Methods of BSA glycation and AGE measurement

To assess the inhibitory effect of selected natural substances towards protein glycation, reflected by inhibition of AGE formation, a sample labeled as C(+), containing BSA and glucose solutions only, was incubated for 8 weeks at a temperature of 37°C in darkness. In the same conditions samples containing BSA, glucose and appropriate examined natural substances (in a concentration as described above and labeled as mentioned), were incubated. The initial volume of each sample was 3 ml.

The samples were placed on the rotating platform Heidolpf Polymax 1040 (50 rpm). All samples were dialysis in PBS for 48 hours, with one change after 24 hours. They were frozen at a temperature of -80°C until the time of measurements. From each series, in the same conditions, the C(–) sample was also made.

The effectiveness of the glycation process in C(+) as well as the capacity of examined substances for inhibition was assessed by measuring the AGE level in the appropriate samples by the spectrofluorometric method described by Munch *et al.* [8] in our own modification. This method is based on measuring the characteristic fluorescence of created AGE at a specific wavelength of excitation and emission (Ex 370 nm/Em 440 nm) in appropriate diluted samples. The fluorescence was recorded using a Perkin-Elmer LS50B (USA) spectrofluorometer. All samples were measured on one day and calibrated on PBS (pH 7.4) solution. The results were converted into arbitrary fluorescence units (AFU) and expressed as AFU´10^5.

Methods of BSA oxidation and AOPP measurement

In order to assess the inhibitory effect of selected substances on protein oxidation, reflected by inhibition of AOPP formation, a sample labeled as C(+), containing BSA and chloramine T solution at a concentration of 20 mM, was incubated for a total time of 60 minutes at 37°C. The total volume of this sample was 3 ml. In the same conditions, samples containing BSA and chloramine T were incubated with appropriate examined natural origin substances (in concentration as described above and labeled as mentioned). After incubation, samples were dialyzed in PBS with one change of dialyzing solution after 24 hours, and frozen at -80°C until the time of measurements. From each series, in the same conditions, the C(–) sample was also made.

The effectiveness of the oxidation process in C(+), as well as the capacity of examined substances for inhibition, was assessed by measuring the AOPP level in respective samples with a spectrophotometric method described by Witko-Sarsat *et al.* [9]. This method is based on the reaction between AOPP and potassium iodide in the presence of acetic acid, which yields derivatives with maximum absorbance at wavelength λ=340 nm. The intensity of the colored reaction product was measured using the Stat Fax 2100 spectrophotometer. The calibration curve (based on the concentration of chloramine T solution determined in the same way as the samples) was prepared to calculate the AOPP concentration and the results were expressed in μM of chloramine T.

Methods of BSA glycoxidation and AGE/AOPP measurement

To assess the inhibitory effect of selected natural substances on protein glycoxidation, reflected by inhibition both of AGE and AOPP formation, samples were subjected to the glycation process with simultaneous oxidation. The BSA solutions

were incubated with glucose and appropriate natural origin substances (in selected concentrations: VC 10 mM, A 50 mM, Q 50 mM, GT) for 8 weeks at 37ºC. After that, samples were incubated with chloramine T solution at a concentration of 20 mM (for 60 minutes at 37°C). Then, after 48 hours of dialysis in PBS, with one change of dialyzing solution after 24 hours, samples were frozen at -80°C until measurement. In the same conditions samples labeled as C(+), containing BSA, glucose and chloramine T solutions, were incubated. From each series the C(–) sample was also made similarly. The effectiveness of the glycoxidation process in C(+) as well as the ability of examined substances to inhibit glycoxidative modification of protein, by measuring the levels of AGE and AOPP in these samples, was performed as described above.

Statistical analysis

Statistical analysis was performed using Statistica 10.0 PL. The average and standard deviation (SD) were calculated. Next, we performed an analysis of the results for examined samples and a negative control as compared to a positive control using Student's test. A p-value lower than 0.05 was adopted as statistically significant.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

Glycation process

The results of the antiglycative effect of vitamin C, aminoguanidine, quercetin and green tea, expressed as the mean fluorescence of AGE derived from triplicates of each samples, at the end of the glycation process (after 8 weeks of samples' incubation with glucose), with the ± SD (whiskers) and $p<0.001$ values (stars), are presented in the figure 1. Grayscale bars indicate various efficacies of all examined substances in concentrations used in the experiment. The negative and positive controls are also presented in this figure. Fluorescence derived from the positive and negative controls allows one to determine the efficiency of the glycation by comparing these samples. Moreover, fluorescence of C(+) constituted the reference point to estimate the inhibiting properties of the tested substances. Significantly higher values of AGE fluorescence by almost five times (4.95) were measured in C(+) in comparison to C(–) samples, and were statistically significant at $p<0.001$, which confirmed the efficacy of the applied glycation procedure. As shown in figure 1, values of AGE fluorescence and results of statistical analysis clearly show that all examined substances inhibit the glycation process. In the samples incubated with the examined substances the process of BSA glycation was inhibited in different degrees depending on the used concentrations.

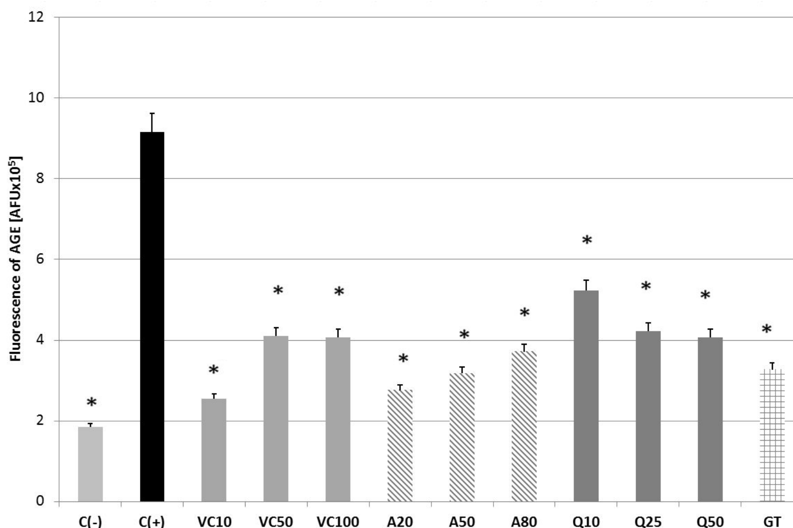

Figure 1.

AGE fluorescence [AFU] after 8 weeks of incubation in control samples (C(–) negative control, C(+) positive control) and samples with aminoguanidine (A), vitamin C (VC), quercetin (Q) and green tea (GT). Numbers after shortcuts present concentration of substances in mM.
*$p<0.001$ compared to C(+).

The changes of AGE fluorescence measured in the samples incubated with aminoguanidine (A), as an antiglycative agent, confirmed its inhibitory effect on AGE formation. A inhibits the glycation process by 60 to 70%, depending on concentration. The lowest fluorescence of AGE, and consequently the highest inhibitory effect, is present in the sample with the lowest concentration of A (20 mM). The value of AGE obtained for A20 is more than 3.3 times lower in comparison with the positive control (with statistical significance, $p<0.001$), and its ability to inhibit these processes was almost 70%. For other concentrations of this compound these values are somewhat lower: 65% for the concentration of 50 mM and nearly 60% for the highest concentration of A (80 mM). All differences between samples and C(+) were statistically significant ($P<0.001$). A similar dependence was observed in samples incubated with vitamin C (VC) – the lowest values of AGE were revealed in samples incubated with its lowest concentration (10 mM), where the ability to inhibit the glycation process was higher than 72% with $p<0.001$, whereas the weakest antiglycative action (about 55%) was revealed in the samples incubated with the medium and highest concentration of VC (50 and 100 mM). All concentrations of VC statistically significantly lowered the AGE level at $p<0.001$ with respect to C(+). In samples incubated with various concentrations of quercetin (Q) the character of this relationship was opposite to the one observed above. The weakest inhibition of the glycation process (almost 43%) reflected by the highest value of AGE fluorescence, has

been demonstrated in samples incubated with the lowest concentration of Q (10 mM). However, in the samples incubated with the highest concentration of this substance (50 mM), the AGE fluorescence was higher than 2.2 times lower in comparison to the C(+) sample, with the statistical significance $p<0.001$, and thus showed the strongest antiglycative effect – the inhibition of glycation was approximately 56%. All differences between samples and C(+) were statistically significant ($p<0.001$). The antiglycative effect of green tea (GT) also indicates its ability to inhibit the glycation process. The value of AGE fluorescence obtained in this sample is about 2.8 times lower in comparison with the positive control, and its ability to inhibit the process was almost 64% and GT was statistically significant at $p<0.001$ with respect to C(+).

Oxidation process

Figure 2 presents the results of the antioxidative effect of aminoguanidine, vitamin C, quercetin and green tea, also expressed as a mean of all three series of experiments. The ±SD (whiskers) and $p<0.001$ values (stars) are also presented. The final AOPP concentration was measured after 60 minutes of incubation with chloramine T in all examined samples. Grayscale bars indicate the efficacy of these substances in concentrations used in the experiment. The negative and positive controls are also presented in this figure. As seen in Figure 2, AOPP concentration in the positive control is significantly higher in comparison with the negative control by about 150 times and was statistically significant at $p<0.001$ with respect to C(+). The AOPP concentration measured in C(–) and C(+) samples enables their mutual comparison and reflects the efficacy of the albumin oxidation process. Moreover, C(+) is a reference point for other tested substances. The concentration of AOPP clearly indicates that in the samples incubated with the examined substances the process of albumin oxidation is inhibited to a different degree depending on the used concentration. An ability to inhibit the oxidation process of albumin is reflected by lowering the AOPP concentration in these samples.

The lowering of AOPP concentrations in the samples incubated with vitamin C at different concentrations confirms its inhibitory effect on oxidation of albumin. The lowest AOPP concentration, and consequently the highest degree of inhibition, were observed in the sample incubated with the highest concentration of VC (100 mM). It exhibited the ability to inhibit the oxidation process by almost 99%, while the AOPP concentration was almost 75 times lower than in the positive control. Conversely, the samples incubated with the lowest VC concentration displayed the weakest antioxidative properties – the AOPP concentrations were only almost 1.7 times lower than in C(+) samples. The medium concentration of VC (50 mM) also strongly inhibited AOPP formation – by about 93%. All differences between samples and C(+) were statistically significant ($p<0.001$). A similar tendency was observed in the samples incubated with various concentrations of aminoguanidine solutions. The lowest

AOPP concentration, and the highest degree of inhibition of oxidation process, were revealed in a sample incubated with the highest concentration of A solution (80 mM). The value of AOPP obtained for A80 is almost 4.2 times lower in comparison with the positive control, and its capacity for inhibition of this process was more than 76%. For A20 the inhibitory action towards AOPP formation is lower – its concentration is more than 2 times smaller than in C(+). All concentrations of A statistically significantly lowered the AOPP level at $p<0.001$ with respect to C(+). In the samples incubated with various concentrations of quercetin, the weakest inhibition of oxidation was displayed in the lowest concentration (10 mM), where the AOPP values were only almost 2.25 times lower than in C(+). The sample incubated with the medium concentration (Q25) showed effective inhibitory action on the inhibition of oxidation (by more than 92%). The AOPP in this sample was more than 13 times lower than in the positive control. However, the results obtained for Q in the highest concentration (50 mM) cannot be taken into account because of the very intense color of the sample (which probably interfered with the color of the reagent mixture in the method). All differences between samples and C(+) were statistically significant ($p<0.001$). As presented in figure 2, green tea, applied in 10 mM, also displays a strong inhibitory effect towards AOPP formation. Samples incubated with this substance demonstrated about 22 times lower AOPP concentration than in C(+), which indicated strong ability of GT to inhibit the oxidation of BSA by almost 95% and was statistically significant at $p<0.001$ with respect to C(+).

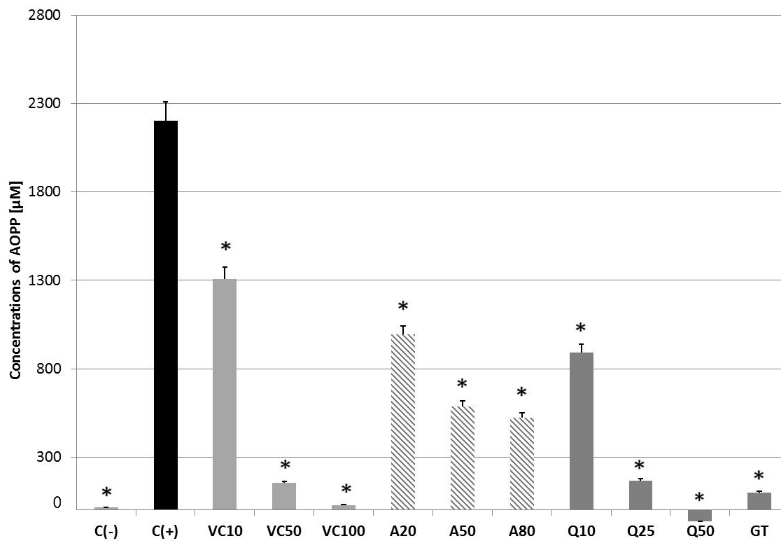

Figure 2.

AOPP concentration [μM] after 60 minutes of incubation in control samples (C(–) negative control, C(+) positive control) and samples with aminoguanidine (A), vitamin C (VC), quercetin (Q) and green tea (GT). Numbers after shortcuts present concentration of substances in mM.
*$p<0.001$ compared to C(+)

Glycoxidation process

The results of the antiglycoxidative effect of vitamin C, aminoguanidine, quercetin and green tea (in concentrations selected by us), expressed as a mean of fluorescence of AGE and concentrations of AOPP measured after the end of the glycoxidation process, the ±SD (whiskers) and $p<0.001$ values (stars) are presented in Figure 3A and 3B. The negative and positive controls are also presented. As shown in figure 3A, the AGE fluorescence values in the positive control are more than 4 times higher than in the negative control, and in the case of AOPP in figure 3B, the concentrations of this value are more than 121 times higher. Both C(–) values were statistically significant at $p<0.001$ with respect to C(+), which confirms the efficiency of the glycoxidative process under the conditions of this experiment.

In samples incubated with VC in a concentration of 10 mM, the AGE formation is more than 2 times lower, and AOPP almost 11 times lower compared to the positive control, which means that VC inhibited glycation of albumin by almost 58% and oxidation by almost 91% in the ongoing glycoxidation process. Fluorescence of AGE was almost 2.5 times lower, and AOPP was more than 31 times lower in comparison to the positive control in samples incubated with A in a concentration of 50 mM. That means that A inhibited glycation by almost 60% and inhibited oxidation by more than 96% in the process of glycoxidation. In samples incubated with Q in 50 mM concentration, an antiglycative effect similar to A was observed – fluorescence of AGE was more than 2.5 times lower in comparison to C(+), and its formation was inhibited by more than 60%. However, the inhibition of AOPP formation was somewhat weaker – concentration of AOPP was more than 8 times lower than in the positive control, so Q inhibited oxidation in the glycoxidative process by more than 87%. GT reduced fluorescence of AGE almost 2-fold and concentration of AOPP more than 13-fold in comparison to the positive control. This means than GT inhibited glycation by almost 50% and oxidation by more than 92% under glycoxidation processes. All differences between samples and C(+) were statistically significant ($p<0.001$). Inhibition of glycation by A was 5% lower and the inhibition of oxidation was 24% higher in glycoxidation, as compared to both processes occurring separately. The VC inhibited AGE formation 14% weaker, while the formation of AOPP was 50% better for glycoxidation, as compared to the processes of glycation and oxidation taking place separately. In the case of Q, these processes have the opposite character – 5% increase in the ability to inhibit glycation, and the inhibition of oxidation decreased by about 4% in glycoxidation compared to the results obtained for these processes carried out separately. Only GT showed a reduction of the efficiency in inhibiting the formation of both AGE (by 3%) and AOPP (by 15%) in glycoxidation compared to separately occurring processes of glycation and oxidation of BSA.

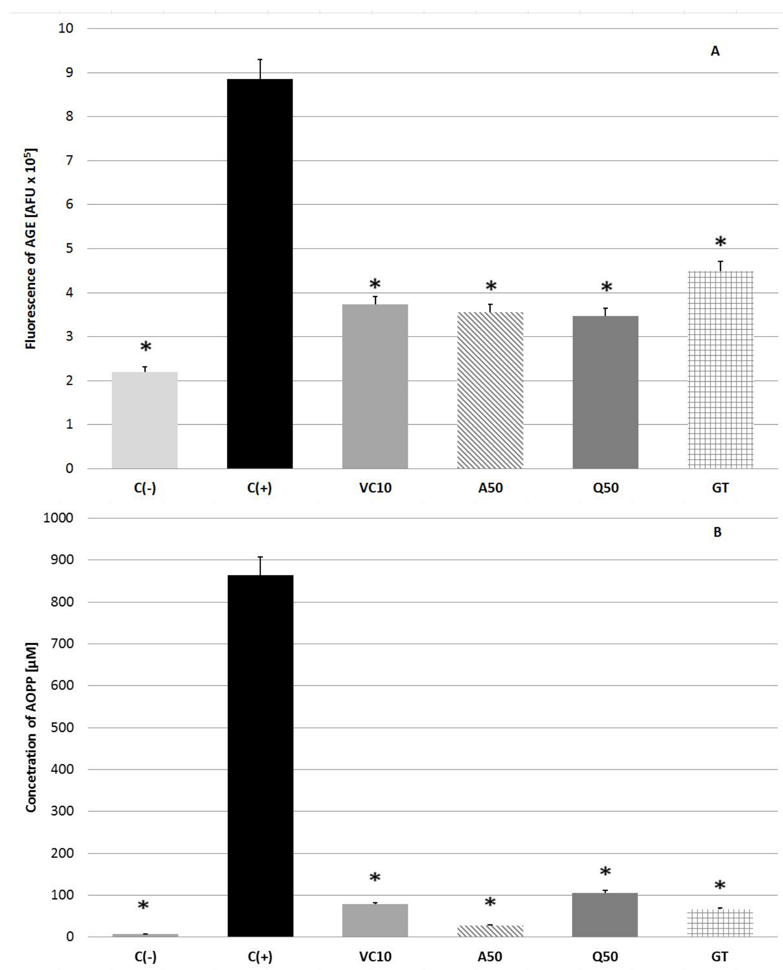

Figure 3.

Results of AGE (3A) and AOPP (3B) measurement obtained after the end of glycoxidation process. C(-) - negative control, C(+) – positive control, (A) – aminoguanidine, (VC) – vitamin C, (Q) – quercetin, (GT) - green tea. Numbers after shortcuts present concentration of substances in mM. $^*p<0.001$ compared to C(+)

DISCUSSION

Hyperglycemia results in increased non-enzymatic glycation, and oxidative stress induces excessive oxidation of many macromolecules, especially proteins, and leads to the formation of advanced glycation end-products and advanced oxidation protein products (AGE and AOPP, respectively). AGE and AOPP have

similar complex structures, they undergo similar transformations (e.g. cross-link reactions, fragmentation, aggregation, accumulation), and both bind to the same RAGE receptor, activating intracellular signal pathways that lead to unfavorable changes in structure and function of tissues (mainly endothelium) and organs (e.g. kidneys, eyes). AGE and AOPP are intensively investigated mainly in the aspects of their participation in the pathogenesis and development of diabetic vascular late complications and diabetes-associated diseases [1, 10]. Inhibition of their formation or deterioration and removal from the human body is still being investigated by many researchers. Many synthetic and natural substances are suspected of exhibiting these properties. Special attention is paid to searching substances of natural origin in the aspect of their dissemination/distribution on the pharmaceutical market, availability and especially current trend to self-medication [6, 11].

Studies using an experimental model of selected macromolecules (e.g. proteins, lipids) are commonly applied in *in vitro* experiments, and the most commonly applied protein is albumin. Albumin constitutes of approximately 65% of all blood proteins, so it is especially vulnerable to damage and modifications as a result of glycation and oxidation as well as glycoxidation. The purified bovine serum albumin is the most frequently used, because of the same properties and structural homology to human albumin and due to its lower cost [12, 13].

In present study, all substances examined, e.g. aminoguanidine, vitamin C, quercetin and green tea, are of natural origin and components of various dietary supplements, readily available without prescription. Their antiglycoxidative properties reflected by abilities of these substances to inhibit AGE and AOPP formation in glycation, oxidation and glycoxidation processes were examined. Available data about the activity of some examined substances concerned examination as inhibitors of glycation or oxidation processes. Those experiments were performed separately without studying these processes occurring simultaneously. In addition, the researchers involved in the aspect of inhibiting both glycation and oxidation carry out tests on each of these processes individually and in long time intervals [9, 14]. In contrast, we attempted to evaluate their antiglycoxidative properties due to the occurrence of glycation and oxidation processes simultaneously. This is important due to the fact that these processes occur in human body at the same time and it is known that each of them increases the action of the other. This strengthen and enhances their adverse effects on the cells and macromolecules. None of the examined substances has been studied yet in context of inhibition of the glycation and oxidation processes occurring at the same time in glycoxidation process and in a single experiment.

Aminoguanidine is widely used in the scientific research as a substance of confirmed antiglycative properties and with the ability to inhibit AGE formation not only in *in vitro* experiments but also in animal and human studies. Thornalley *et al.* [15] investigated the inhibitory action of aminoguanidine on

protein glycation in a concentration from 1 mM to 500 mM, and demonstrated its ability to inhibit AGE formation, but in varying degrees depending on the concentration. It was shown in some *in vitro* studies that aminoguanidine at 10 mM strongly inhibits glycation by 60% [16] to 75% [17, 18] but at a lower concentration (1 mM) only by 52% [19]. The antioxidant properties of aminoguanidine were tested in experiments on rats [20, 21] in which its ability to reduce the production of peroxynitrite and nitric oxide, inhibition of formation of CO groups in proteins and increase in the level of reduced glutathione were studied. There is no research on the capacity for inhibition of formation of AGE and AOPP by aminoguanidine in the process of glycoxidation. Our studies confirmed aminoguanidine's ability to inhibit glycation and oxidation of protein in applied concentrations. It should be noted that the best inhibitory effect of glycation was obtained for the lowest concentration of A (20 mM), whereas the best inhibition of oxidation was observed for the highest concentration of A (80 mM). These results did not allow us to identify the most appropriate concentration of A in both processes, but taking into account all obtained results it was possible to continue the study concerning glycoxidation with of medium concentration, i.e. 50 mM. In the glycoxidation process, the inhibitory activity of aminoguanidine was found to be lower in the case of AGE (60%) than of AOPP (96%), so its effectiveness is higher as antioxidant than as antiglycative agent, as compared to the course of these processes separately.

Vitamin C, in contrast to aminoguanidine, is well known for its antioxidant properties. It shows a very high ability to inhibit the oxidation reflected by inhibiting DPPH radical activity – in different tests at 85% [22] and up to 95% [23] – and by inhibiting ABTS radical activity by up to 95% [22]. In a concentration range from 20 to 100 μg/mL, it has the ability to inhibit the formation of free radicals in the range from 85% to 98% [24]. The VC ability to inhibit protein glycation was tested, however, the results of these studies are diverse. Vinson and Howard [25] demonstrated ability of vitamin C to inhibit the formation of AGE by 73% in a concentration of 20 mM, while Tarwadi and Agte [26] achieved only 20% ability to inhibit glycation. It was also emphasized that even though vitamin C demonstrates the ability to inhibit glycation, this issue should take further examination [27]. In the literature, no experiment concerning the abilities of vitamin C's to inhibit both glycation and oxidation in the glycoxidation process (reflected by inhibition of the formation of AGE and AOPP) was found. In our experiment, the ability of vitamin C to inhibit albumin oxidation (99% at a concentration of 100 mM) as well as glycation was revealed. It should be noted that a similar concentration-dependent inhibitory effect was observed in the case of aminoguanidine. Vitamin C inhibits the formation of AGE and AOPP in the glycoxidation process at its lowest concentration (10 mM) by 58% and 91%, respectively. The results clearly indicate that, like aminoguanidine, in the case of combined glycation and oxidation, VC antioxidant properties increased, while the ability to inhibit glycation decreased, as compared to separate processes.

Quercetin, similarly to vitamin C, is known for its antioxidative properties and is applied mainly as a substance inhibiting the oxidation process. Its antioxidative activity was investigated in a wide range of concentrations, but AOPP formation was not examined. Zhang *et al.* [22] examined quercetin at the concentration range of 6.25–200 μg/ml and confirmed its antioxidant abilities in the inhibition of DPPH and ABTS free radical activity in the range of 50–95%, depending on the concentration used. Other studies have also demonstrated its ability to inhibit synthetic DPPH radical activity even by 98% [24], and in some research the antioxidant strength of quercetin is compared to the potency of vitamin C [28]. In turn, the level of quercetin's ability to inhibit glycation differs, depending on the experiment. In the range of 40% up to even 80%, lowering of the AGE level was observed [26, 29]. There is no research verifying the ability of Q to inhibit the formation of AGE and AOPP in the glycoxidation process. In our study, we confirmed its abilities to inhibit both glycation and oxidation processes. We observed the strongest inhibitory effect of glycation at the highest concentration of quercetin (50 mM) and the strongest inhibitory effect on oxidation at its medium concentration. Thus, we decided to use a Q concentration of 50 mM for the glycoxidation process. In the glycoxidation process, AGE formation was inhibited by 61% and AOPP by 88%. These results are opposite to those obtained for previously analyzed substances (aminoguanidine and vitamin C), indicating that in the case of glycoxidation, quercetin has increased the ability to inhibit glycation but reduced the ability to inhibit oxidation as compared to the results obtained for independently conducted processes of glycation and oxidation.

Green tea is often described as a main edible antioxidant [30]. Its antioxidant effects on inhibiting synthetic DPPH radical activity and reducing Fe^{2+} ions is very strong – up to 92% in some studies [30, 31] – and its ability is compared to vitamin C [32]. In studies on glycation, some researchers did not confirm the ability of GT to inhibit this process [33], but others have shown such properties, but emphasized the need for further research [34, 35]. The study by Babu *et al.* [36] on rats demonstrated the ability of green tea to lower the fluorescence of AGE at 50%. No research has been done on the inhibition of formation of AGE and AOPP in the process of glycoxidation. In our study, strong antioxidant properties of GT and inhibition of the glycation process was confirmed. In glycoxidation, GT inhibited the formation of AOPP by 92% and AGE by almost 50%. This shows that in the glycoxidation process the dependence was different from those of all previously tested substances – the inhibitory potential of green tea was lower, as compared to the measurements taken in separately performed processes.

Our research though, is of preliminary character. It is important and will be continued using other concentrations of the tested substances and measurement of the most effective pharmacokinetic parameters. The next step will be to confirm the results in studies on rats with induced diabetes, to be able to recommend the most effective substances to diabetic patients.

CONCLUSION

In conclusion, it should be emphasized that all examined substances of natural origin have both the ability to inhibit separately ongoing glycation and oxidation processes of proteins, as well as simultaneously ongoing glycoxidative modification, reflected by lowering the formation of advanced glycation end-products and advanced oxidation protein products. However, their properties depend on the concentration of the examined substances – all substances showed inhibitory effects on glycation, oxidation and glycoxidation, but in different degrees. However, we observed changes in the inhibition in the processes occurring separately in contrary to their simultaneous occurrence, which can make the recommendation of these substances to patients problematic, especially to those suffering from diabetes. Based on our results, vitamin C seems to be the mostly recommended natural substance because of its strong inhibitory properties toward AGE and AOPP formation, its widespread and most common presence in daily diet, as well as its occurrence in non-prescriptive dietary supplements.

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Conflict of interest: Authors declare no conflict of interest.

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SUBSTANCJE POCHODZENIA NATURALNEGO O NAJSILNIEJSZYCH WŁAŚCIWOŚCIACH HAMUJĄCYCH PROCES GLIKOOKSYDACJI

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S t r e s z c z e n i e

Wstęp: Zaawansowane końcowe produkty glikacji (AGE) i zaawansowane produkty utleniania białek (AOPP) są głównymi produktami glikooksydacji u pacjentów z cukrzycą. **Cel:** Celem pracy było zidentyfikowanie substancji naturalnego pochodzenia o najsilniejszych właściwościach hamujących proces glikooksydacji spośród suplementów diety i leków dostępnych bez recepty na polskim rynku farmaceutycznym. **Metody**: Albumina wołowa (BSA), witamina C (VC), aminoguanidyna (A), kwercetyna (Q) i zielona herbata (GT) były badane *in vitro* podczas procesów glikacji, utleniania i glikooksydacji. Zmniejszenie stężenia AGE i AOPP w porównaniu z kontrolą dodatnią przyjęto jako marker efektywności zachodzących procesów. **Wyniki**: Tworzenie AGE było zredukowane w 72% przez VC i w co najmniej 43% przez pozostałe substancje w procesie glikacji. Stężenie AOPP było obniżone o 99% przez VC i w przynajmniej 40% przez pozostałe substancje badane w procesie utleniania. W przebiegu glikooksydacji tworzenie AGE/AOPP było obniżone odpowiednio w 61% przez Q w 97% przez A i przynajmniej o 49%/88% przez pozostałe substancje. **Wnioski**: Badane substancje wykazały zdolności hamujące glikację, utlenianie i glikooksydację białek w różnym stopniu w zależności od zastosowanego stężenia.

Słowa kluczowe: *modyfikacje utleniające białek, modyfikacje glikujące białek, modyfikacje glikooksydacyjne białek, AGE, AOPP, inhibitory pochodzenia naturalnego, badania in vitro*