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Carotenoids-antioxidants of *D. radiodurans* stimulate regeneration in mice

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Abstract
In express tests on recombinant strains of *Escherichia coli*, we have studied antioxidant and DNA-protective activity of carotenoid extract of radioresistant bacterium *Deinococcus radiodurans* and antimutagenic potential of this preparation in the test on chemically induced mutagenesis of *E. coli.* In experiments on streptozotocin-induced diabetic mice, the studied preparation has shown the ability to stimulate regeneration more efficiently compared to the other carotenoid – lycopene. The obtained results indicate the possibility to predict systemic biological activity of the compounds based on the results of express tests on the antioxidant and DNA-protective activity.

Keywords: Antioxidants; regeneration; biosensors; carotenoids.

Introduction

It is known that the active formation of reactive oxygen species (ROS) occurs during the early stages of regeneration. ROS have both bactericidal and regulatory function [1]. However, in wounds in case of chronic inflammation there is no decrease in ROS which is one of the main causes of poor wound healing in patients with diabetes, atherosclerosis, as well as in the elderly ones. The development of topical medications with antioxidant activity, which would allow to eliminate inflammation and to create conditions for normal regeneration of chronic wounds, is extremely relevant today due to the high prevalence of diabetes mellitus (DM). The formation of nonhealing wounds is one of the most dangerous complications of DM. In some cases carotenoids promote healing of wounds [2,3].

A promising source of carotenoid antioxidants is *Deinococcus radiodurans.* Natural strains of these bacteria unlike colorless (unpigmented) mutants are 30 times more resistant to ionizing radiation than *E. coli* and 1,000 times more resistant than the human cells [4]. Carotenoid complex of *D. radiodurans* is based on deinoxantin and astaxanthin [5].

Genetic apparatus is one of the main cellular targets of ROS. Therefore, one should assess the ability of antioxidants to inactivate ROS as well as DNA-protective activity when performing the screening.

The aim of this study was to compare the antioxidant and antimutagenic activity of the carotenoid fraction of *D. radiodurans* in express tests involving bacteria and the ability to stimulate the healing of superficial wounds in mice with streptozotocin-induced diabetes.

Methods

Carotenoids
The protocol of preparation and chemical composition of the carotenoid fraction of *D.radiodurans* is described in [5].

Bioluminescence test
Antioxidant and DNA-protective activity of the carotenoid preparation was investigated by

recombinant biosensor strains of *E. coli* MG1655 (pSoxS-lux), *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pRecA-lux). Biosensors with promoters PkatG and PsoxS detect the presence of oxidants that are able to form hydroperoxides and superoxide anion radicals in the cell. Biosensor with plasmid pRecA detects the presence of factors that cause DNA damage [6]. Testing protocol and the formula to calculate the protective activity are specified in Ref. [7].

We used paraquat (1,1′-dimethyl-4,4-bipyridylium dichloride), dioxidine (1,4-dioxide 2,3-quinoxalinedimethanol) and hydrogen peroxide as an oxidative stress inducers.

Determination of the frequency of spontaneous and induced mutagenesis

To study an antimutagenic activity, we determined the frequency of mutants resistance to rifampicin (100 mg/l) in the presence of potential protectors and without them. Dioxidine was used as an inducer of mutagenesis. An overnight culture of *E. coli* was grown at 37°C in the presence of an inducer for 18-20 h (with the same volume of sterile saline in the control culture). On Day 2, the strain culture was diluted with fresh LB medium (Luria-Bertani) up to 1-2 McFarland units (3-6 $\times$ 10^8 CFU). An optical density of the solution was measured using a densitometer DEN-1B. Then we made serial dilutions of culture 1:10 in saline (taking the original culture as 1). Diluted culture was applied on plates with LB agar with and without antibiotic by a surface seeding. A count of colonies was performed after 48 h.

Experiments to study the process of regeneration in mice

Type I DM was induced in 10-11 week old male mice (CD-1 outbred stock) by intraperitoneal administration of streptozotocin [8]. The disease was diagnosed in mice with glucose concentration of 14 mmol/l and polyuremia. After 3-4 weeks, we made full-thickness skin wound by removing the skin flap of 7-12 mm in diameter with an area of 1.5% relative to the surface area of the mouse body. Carotenoid fraction of *D. radiodurans* dissolved in olive oil was applied to the wound surface once a day. In case of oral administration, we used a probe. The control animals received olive oil that has passed all the processing steps used to obtain a preparation of carotenoids in similar forms and doses. We used three methods of drug administration – on-skin (0.5 μg/day), oral (250 μg/kg/day) and their combination. Measurement of the wound surface area (planimetric study) in the process of wound healing was performed using digital macro photography on Days 1, 3, 7, 14, and 21 after wounding.

To assess the dynamics of wound healing on the background of DM, we have calculated an overall percentage of reduction in wound area between consecutive measurements using the ImageJ program (National Institutes of Health (NIH) http:/rsb.info.nih.gov/ij/). The extent of spontaneous and metal-catalyzed oxidation of proteins in the serum was determined by the method of Levine [9] in the modification of Dubinina [10]. We used a similar dose of lycopene as a positive control. Totally 96 mice were used. The statistical significance was determined by Student *t*-test for independent samples with p-value < 0.05.

Experiments on animals were performed in compliance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Results and Discussion

Antioxidant and DNA-protective activity of carotenoids of D. radiodurans *using bioluminescent assay*

Antioxidant and DNA-protective concentrations for carotenoid fraction of *D. radiodurans* were registered in the range from 0.001 to 10 μg/ml. The data on the maximum protective activities in this range are shown in Table 1. The results obtained with α-tocopherol in the similar series of tests are also specified.

As can be seen from the presented data, the mixture of carotenoids of *D. radiodurans*, in contrast to α-tocopherol, is able to reduce the level of generation of superoxide anion radicals by the action of hydrogen peroxide and dioxidine and considerably enhance the work of cellular mechanisms that provide decomposition of hydrogen peroxide when it is administered exogenously. At the same time, the mixture of carotenoids of *D. radiodurans* shows a lower level of DNA activity when compared to α-tocopherol.

Further experiments showed that carotenoids of *D. radiodurans* considerably reduce the frequency of mutations induced by dioxidine: concentration of 15.5 mg/l is able to reduce the frequency of mutations by 85.9%; concentration

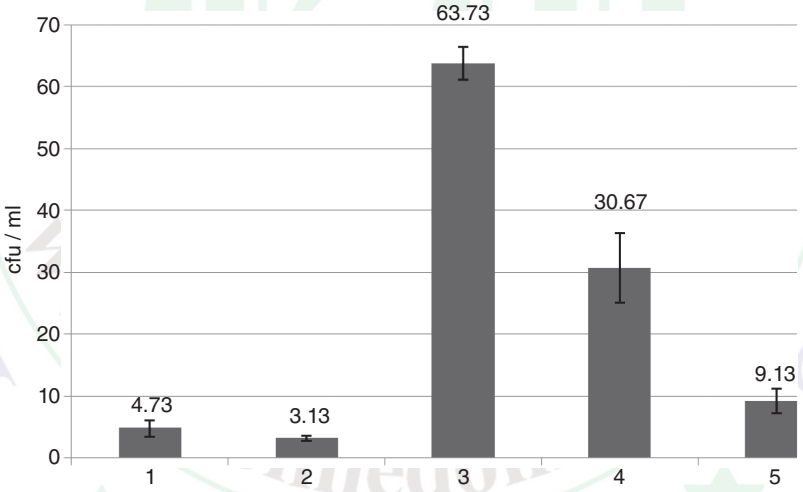
Table 1: Protective activity (P) of *D. radiodurans* carotenoids and tocopherol in the biosensors.

Antioxidants	Biosensors	Inductors of oxidative stress						Types of activity
		Hydrogen peroxide		Paraquat		Dioxidine		
		P (%)	Concentration (μg/ml)	P (%)	Concentration (μg/ml)	P (%)	Concentration (μg/ml)	
A mixture of carotenoids of *D. radiodurans*	*E. coli* MG1655 (pSoxS-lux)	23	10^{-3}	0	–	64.9	1	Removal of superoxide anion radical
	E. coli MG1655 (pKatG-lux)	100	10^{-3}	37	1	–	–	Removal of hydrogen peroxide
	E. coli AB1157 (pRecA-lux)	54	1	28	10^{-1}	36	10^{-1}	DNA protection
Tocopherol	*E. coli* AB1157 (pRecA-lux)*	49.3	10^{-5}	0	–	70.4	10^{-5}	DNA protection

*No statistically significant effects were found for tocopherol in the experiments on the remaining strains.

Figure 1: Quantity of Rif^r mutants per 1 ml of culture (10^8 cells) in the presence of dioxidine and carotenoids of *D. radiodurans.*

1 – control group; 2 – in the presence of 15.5 mg/l of carotenoids of *D. radiodurans*; 3 – in the presence of dioxidine 2.25×10^{-5} M; 4 – in the presence of dioxidine and carotenoids of *D. radiodurans* 1.55 mg/l; 5 – in the presence of dioxidine and carotenoids of *D. radiodurans* 15.5 mg/l.



of 1.55 mg/l – by 53.2% if compared to the control group. In Figure 1, the results of a quantitative comparison of the number of mutants in a culture treated with various concentrations of carotenoids, and in the control group, are demonstrated.

Thus, there is a direct connection between the antioxidant and antimutagenic effects of carotenoid fraction of *D. radiodurans.* This gives reason to assume that the antimutagenic activity of the preparation may be partially or entirely based on the ability of its components to neutralize ROS.

In experiments on mice, it was found that on-skin administration of extract of bacterial carotenoid improves the rate of regeneration of dermal wounds more effectively compared to the control compound – lycopene (see Figure 2). Maximum effect was obtained with combined administration (healing rate was improved by 21% ($p < 0.05$) in comparison to the control group, Figure 3).

On-skin administration of carotenoids of *D. radiodurans* caused statistically significant reduction in wound area during both formation and maturation of the granulation tissue and

Figure 2: The dynamics of wound area reduction, an average value per week during the administration of the protective compound on-skin in the dose of 0.5 mg/day.

1 – control group; 2 – carotenoids of *D. radiodurans*; 3 – lycopene. *$p < 0.05$ (*t*-criterion).

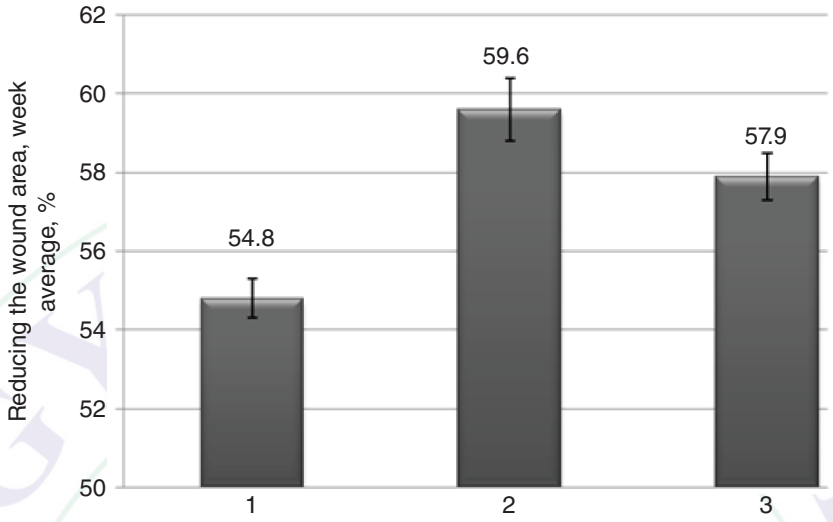
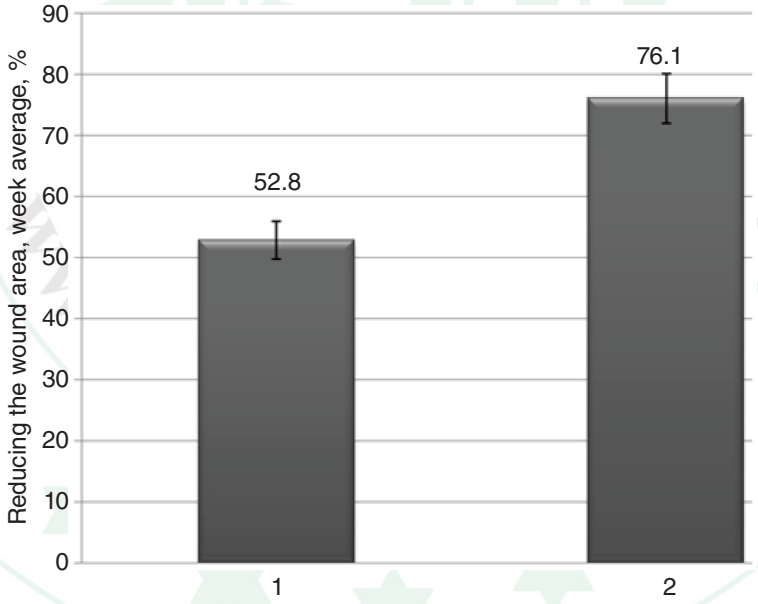


Figure 3: The dynamics of wound area reduction, an average value per week during combined administration of the protective compound (on-skin and oral administration).

1 – control group; 2 – carotenoids of *D. radiodurans*. *$p < 0.05$ (*t*-criterion).



scarring (Figure 4). Histological studies demonstrate the ability of carotenoids of *D. radiodurans* to reduce neutrophil infiltration of the wound. This process accelerates the first (exudative) regeneration phase and increases the rate of wound healing in diabetic mice.

The results of biochemical tests suggest that this is an antioxidant effect that causes the observed acceleration of the regeneration process. Thus, during the analysis of spontaneous and metal-catalyzed degradation of serum proteins of mice with type 1 DM, we have found that the intensity of oxidative modification of proteins was decreased by more than 30% ($p < 0.05$) during the combined administration of carotenoids of *D. radiodurans* (Table 2).

Figure 4: The dynamics of changes in the wound surface in streptozotocin-induced diabetic CD I mice during the combined administration of carotenoid fraction of *D. radiodurans* (on-skin and oral administration): A – Administration of the processed olive oil *per os* (300 μl) + applying on the wound surface (500 μl), once a day; B – Administration of lycopene through the processed olive oil *per os* (300 μl in the dose if 250 μg/kg/day) + applying to the wound surface (500 μl in the dose of 0.5 μg per day); C – Administration of the carotenoid fraction of *D. radiodurans* through the processed olive oil (300 μl in the dose if 250 μg/kg/day) + applying to the wound surface (500 μl in the dose of 0.5 μg per day).

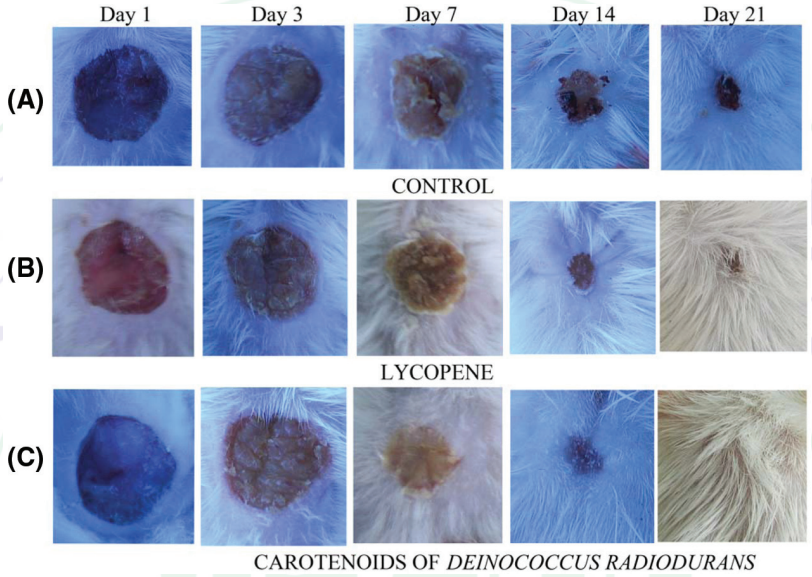


Table 2: Oxidative modification of proteins (arbitrary units/ml) of the serum of CD I mice during the administration of carotenoids of *D. radiodurans* or lycopene (M ± m, *n* = 2-6) *per os*.

Groups	Spontaneous oxidative modification of proteins	Metal-catalyzed oxidative modification of proteins
Control, olive oil *per os* and *per os* + on-skin, 21 days	5.89 ± 0.33	31.46 ± 0.80
Carotenoids of *D. radiodurans per os* (125 μg/kg/day) and on-skin (0.05 μg) + *per os* (125 μg/kg/day), 21 days	4.76 ± 0.64	29.92 ± 2.37
Carotenoids of *D. radiodurans per os* (250 μg/kg/day) and on-skin (0.5 μg) + *per os* (250 μg/kg/day), 21 days	4.03 ± 0.31*	27.82 ± 1.42*
Lycopene *per os* (125 μg/kg/day), 21 days	5.06 ± 0.87	30.13 ± 2.08
Lycopene *per os* (250 μg/kg/day), 21 days	4.90 ± 0.97	30.42 ± 2.18

*Differences from the control are statistically significant, *t*-test, $p < 0.05$.

Conclusion

Thus, the experimental results show that the carotenoids extract of *D. radiodurans* has high antioxidant activity that promotes healing of chronic wounds in experimental animals.

Furthermore, we demonstrated the possibility to predict antimutagenic activity based on express test using bacterial biosensors, responding to DNA damage and an increase in the level of ROS in a cell. The use of this technique for express screening in the study of

newly synthesized or extracted compounds and pharmaceutical preparations seems to be very promising.

Acknowledgment

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References

1. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF (2007) Impaired wound healing. Clinics in Dermatology 25(1): 19-25.

2. Chan KC, Pen PJ, Yin MC (2012) Anticoagulatory and antiinflammatory effects of astaxanthin in diabetic rats. Journal of Food Science 77(2): 76-80.

3. Rao AV, Rao LG (2007) Carotenoids and human health. Pharmacological Research 55(1): 207-216.

4. Slade D, Radman M (2011) Oxidative stress resistance in *Deinococcus radiodurans*. Microbiology and Molecular Biology Review 75(1): 133-191.

5. Lysenko VS, Chistyakov VA, Zimakov DV, Soier VG, Sazykina MA, *et al*. (2011) Separation and mass spectrometry identification of carotenoid complex from radioresistant bacteria *Deinococcus radiodurans.* Journal of Analytical Chemistry 66(13): 1281-1284.

6. Zavilgelsky GB, Kotova VY, Manukhov IV (2007) Action of 1,1-dimethylhydrazine on bacterial cells is determined by hydrogen peroxide. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 634(1): 172-176.

7. Chistyakov VA, Prazdnova EV, Gutnikova LV, Sazykina MA, Sazykin IS (2012) Superoxide scavenging activity of plastoquinone derivative 10-(6′-plastoquinonyl) decyltriphenylphosphonium (SkQ1). Biochemistry (Moscow) 77(7): 776-778.

8. Kunjathoor VV, Wilson DL, LeBoeuf RC (1996) Increased atherosclerosis in streptozotocin-induced diabetic mice. The Journal of Clinical Investigation 97: 1767-1773.

9. Levine RL, Garland D, Oliver CN, Amici A, Climent I, *et al.* (1990) Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymology 186: 464-478.

10. Dubinina EE, Burmistrov SO, Hodov DA, Porotov GE (1995) Oxidative modification of proteins of the human serum. Methods for its determination. Issues of Medical Chemistry 41(1): 24-26.