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QUERCETIN POTENTIATES ANTIRADICAL PROPERTIES OF EPIGALLOCATECHIN-3-GALLATE IN PERIODONTIUM OF RATS UNDER SYSTEMIC AND LOCAL ADMINISTRATION OF LIPOPOLYSACCHARIDE OF SALMONELLA TYPHI

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ABSTRACT

Introduction: There has been demonstrated that pharmaceutical effect of epigallocatechin-3-gallate (EGCG), a polyphenol, which is found in green tea (*Camellia sinensis*), is implemented through the activation of Nrf2 (Nuclear Factor Erythroid 2-Related Factor 2). The importance of Keap1 / Nrf2 / antioxidant response element (ARE) system is determined by the fact that the state of NF- κ B- and AP-1-associated pathways depends on its activity. Recent studies have demonstrated the property of quercetin to suppress ubiquitin-dependent proteolysis of complex of NF- κ B and its inhibitory protein I κ B. All this provides preconditions to eliminate the potentiality of NF- κ B-dependent expression of the number of genes of pro-oxidant and pro-inflammatory proteins. However, co-effect produced by quercetin and EGCG on the oxidative nitrosative stress markers in the periodontal tissues is still unclear.

The aim: To investigate the co-effect produced by quercetin and an inducer of the Keap1 / Nrf2 / ARE epigallocatechin-3-gallate on markers of oxidative-nitrosative stress in rats' periodontium under the systemic and local administration of *Salmonella typhi* lipopolysaccharide (LPS).

Materials and methods: The studies were conducted on 30 white rats of the Wistar line, divided into 5 groups: the 1st included intact animals, the 2nd was made up of animals after their exposure to combined systemic and local LPS administration, the 3rd and 4th groups included animals, which were given injections with water-soluble form of quercetin (corvutin) and EGCG respectively, and the 5th group involved rats, which were injected with co-administered corvutin and EGCG. The formation of superoxide anion radical (O_2^-) was evaluated by a test with nitro blue tetrazolium using spectrophotometry of the periodontal soft tissue homogenate. The total activity of NO-synthase and concentration of peroxynitrite in the homogenate of the soft components of periodontium were evaluated spectrophotometrically.

Results: Co-effect produced by corvutin and EGCG under systemic and local LPS administration is accompanied with reduced O_2^- production by NADPH-dependent electron transport chains (microsomal and NOS) by 20.0 % ($p < 0.05$) compared with values for the animals received separate corvutin during the experiment. O_2^- generation by the mitochondrial respiratory chain yielded to comparable data of the 3rd and 4th groups by 27.6 % ($p < 0.01$) and 23.8 % ($p < 0.05$) respectively. No differences were found between the groups exposed to combined or separate action of the above mentioned agents in the experiment when assessing O_2^- generation by leukocyte NADPH-oxidase. Combined effect of corvutin and EGCG during systemic and local LPS administration showed the decrease in NOS activity and peroxynitrite concentration in periodontal tissues by 53.3 % ($p < 0.001$) and 27.0 % ($p < 0.02$) compared with the findings in the 3rd group, and by 42.0 % ($p < 0.01$) and 22.3 % ($p < 0.01$) in the 4th group.

Conclusions: the co-administration of water-soluble form of quercetin and epigallocatechin-3-gallate under systemic and local introducing of lipopolysaccharide *Salmonella typhi* has been proven to be more effective means for preventing and correcting oxidative-nitrosative stress in the periodontal tissues than this occurs at separate administration of each of the polyphenols.

KEY WORDS: signal pathway Keap1 / Nrf2 / ARE, epigallocatechin-3-gallate, quercetin, lipopolysaccharide-induced systemic inflammatory response, oxidative-nitrosative stress, periodontium

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INTRODUCTION

It has been known that impairment of the periodontium structure and functions result from a number of somatic diseases (metabolic syndrome, atherosclerosis, chronic pyelonephritis, traumatic disease, etc), whose development includes lipopolysaccharide (LPS)-induced systemic inflammatory response as a key component of pathogenesis [6]. Free-radical processes, associated with prolonged activation of certain redox-sensitive transcription factors (NF- κ B, AP-1), contribute much to this process [2, 27]. This leads to the expression of genes of inflammatory cytokines,

metalloproteinases, inducible nitric oxide synthase (NOS), cellular adhesion molecules, cyclooxygenase-2, etc. that can induce production of reactive oxygen and nitrogen species [16, 19, 29].

The redox-sensitive signaling pathway Keap1/Nrf2 plays a key role in the highly regulated cell systems. It activates gene expression through the interaction Nrf2 (Nuclear Factor Erythroid 2-Related Factor 2) and cis-acting enhancer sequence, known as antioxidant response element (ARE) [21, 24].

It has been reported that Nrf2 signalling is important in controlling oxidative stress in healthy periodontium

that helps to maintain integrity of periodontium despite of its constant exposure to aggressive factors as bacteria, neutrophils, and macrophages [6].

Recently there has been demonstrated that pharmaceutical effect of epigallocatechin-3-gallate (EGCG), a polyphenol, which is found in green tea (*Camellia sinensis*) [12], is implemented through the activation of Nrf2 due to proteolysis of an inhibitory protein Keap1 [10, 22].

We have demonstrated that the introduction of EGCG in the lipopolysaccharide-inducible systemic inflammatory response is an effective mean to correct oxidative and nitrosative stress in the tissues of periodontium of the rats: it reduces the generation of superoxide anion radicals, in particular, its production by mitochondrial electron transport chain, microsomal and NOS electron transport chains, leukocyte NADPH-oxidase, as well as reduces the total activity of NOS and peroxy-nitrite concentration [30]. The administration of EGCG under modeled systemic inflammatory response is an effective means of preventing and correcting the disruption of connective tissue of periodontium in rats: it reduces collagenolysis and depolymerization of proteoglycans and glycoproteins [28].

The importance of Keap1 / Nrf2 / ARE system is determined by the fact that the state of NF- κ B- and AP-1-associated pathways depends on its activity. In this way Nrf2 inducers phenethyl isothiocyanate, sulforaphane and curcumin suppress the NF- κ B activation [15].

Recent studies have demonstrated the property of quercetin to suppress ubiquitin-dependent proteolysis of complex of NF- κ B and its inhibitory protein I κ B that impairs the I κ B degradation due to the proteasome activity [9]. All this provides preconditions to eliminate the potentiality of NF- κ B-dependent expression of the number of genes of pro-oxidant and pro-inflammatory proteins (cytokines, matrix metalloproteinases, inducible NOS, etc.) [18].

However, co-effect produced by quercetin and EGCG on the oxidative and nitrosative stress markers in the periodontal tissues is still unclear. Solving this problem will enable to evaluate the role of this polyphenols as a potential means of pathogenetic therapy for inflammatory and dystrophic diseases of periodontal tissues.

THE AIM

The aim of the present study was to investigate the co-effect produced by quercetin and an inducer of the Keap1 / Nrf2 / ARE epigallocatechin-3-gallate on markers of oxidative and nitrosative stress in rats' periodontium under systemic and local administration of *S. typhi* LPS.

MATERIALS AND METHODS

The studies were conducted on 30 white rats of the Wistar line weighing 180-220 g, divided into 5 groups: the 1st included intact animals, the 2nd was made up of animals after the combined systemic and local LPS administration, the 3rd and 4th groups included animals, which were given in-

jections with water-soluble form of quercetin (corvutin) and EGCG respectively, and the 5th group involved rats, which were injected with co-administered corvutin and EGCG.

For systemic administration, *S. typhi* LPS (pyrogenalum, "Medgamal", Russia) was injected intraperitoneally in a dose, which stimulated rise in temperature by 1.5 °C according to the scheme [30]: during the first week, 4 minimum pyrogenic doses (MPD) of 0.4 μ g / kg of rat body weight were given 3 times a week. During the following 7 weeks of the experiment, rats were given 4 MPD / kg of body weight once a week.

For local administration, LPS *S. typhi* was performed once in a dose of 1 μ g / kg, equally divided into four injections into the gum at the level of the second molars 7 days prior to the decapitation (acute gingivitis model).

Water-soluble form of quercetin (corvutin, "Borshchahivskiy CPP", Ukraine) was administered intraperitoneally in a daily dose of 10 mg / kg recalculated for quercetin [11], and EGCG (Sigma-Aldrich, Inc., USA) was administered in a dose of 21.1 mg / kg [28] 3 times a week, starting on the 30th day of the systemic LPS administration.

The research was conducted in compliance with the standards of the Convention on Bioethics of the Council of Europe 'European convention for the protection of vertebrate animals used for experimental and other scientific purposes' (Strasbourg, 18.III.1986). The animals were decapitated with ethereal anesthesia. Soft components of periodontium (gingiva and periodontal ligament) served as the objects of the study.

The formation of superoxide anion radical (O_2^-) was evaluated by a test with nitro blue tetrazolium using spectrophotometry of the periodontal soft tissue homogenate by the following inducers: NADH was used to evaluate O_2^- production by the mitochondrial electron transport chain, NADPH was used to evaluate O_2^- production by endoplasmic reticulum and NOS, and *S. typhi* LPS was used to assess O_2^- production by NADPH oxidase of white blood cells [13].

The activity of NOS was determined by the difference in the concentration of nitrite ions before and after the incubation of homogenate into the medium containing L-arginine (NOS substrate) and NADPH. The concentration of nitrite ions was assessed by the formation of diazo-compounds in the reaction with sulfanilic acid, and then we carried out the reaction with α -naphthylethylenediamine, resulting in the production of red color derivatives [1]. The concentration of peroxy-nitrite in the homogenate was evaluated spectrophotometrically by wavelength of 355 nm [1].

The findings obtained were statistically processed. To verify the normality distribution, the Shapiro-Wilk test was applied. If they corresponded to the normal distribution, then the Student's t-test was used to compare independent samples. When the results ranges were not subject to normal distribution, statistical processing was performed using a nonparametric method, the Mann-Whitney test. Statistical calculations were performed using the "StatisticSoft 6.0" program.

RESULTS AND DISCUSSION

Systemic and local LPS co-administration led to significant changes in the O₂ generation in the tissues of periodontium (Table I). Thus, the ·O₂ production by NADPH-dependent electron transport chains (microsomal and NOS) increased by 71.0 % (p<0.001), while the ·O₂ production by the mitochondrial respiratory chain increased by 85.5 % (p<0.001). Generation of ·O₂ by NADPH oxidase of white blood cells also elevated by 85.4 % (p<0.001).

According to the literature, the largest amount of ·O₂ is produced by the mitochondrial electron transport chain as a result of the one-electron reduction of oxygen at the level of the following complexes: NADH:ubiquinone oxidoreductase, ubiquinol:cytochrome c oxidoreductase and cytochrome bc1 [5].

Recent studies have demonstrated the activation of NF-κB and AP-1 enhances O₂ generation by NADH- and NADPH-dependent electron transport chains (mitochondrial, microsomal and NOS) in the periodontal tissues homogenate [14, 27]. This process is accelerated by the formation of peroxynitrite in the tissues [3, 14].

At the same time, a powerful level of ·O₂ production under the conditions of LPS-inducible systemic inflammatory response is provided by NADPH-dependent electron transport chains: in the reactions of microsomal oxidation (with cytochrome P450) [7] and by NOS itself, which is capable of switching from NO to ·O₂ under unfavourable conditions [20]. When the bacterial LPS are introduced into the mammalian body, the ·O₂ generation by leukocyte NADPH oxidase naturally increases [4]. In addition to LPS, proinflammatory cytokines, whose synthesis depends on the activation of NF-κB [8, 23], are also known as effective stimulants of ·O₂ synthesis.

Combined systemic and local LPS administration increased NOS activity and peroxynitrite concentration (Table II) in periodontal tissues in three times (p<0.001) and 85.5 % (p<0.001) respectively. This can be explained by

the ability of LPS to provide NF-κB-dependent activation of inducible NOS [23].

It was found that the separate administration of both corvitin and EGCG reduced ·O₂ production by NADPH-dependent electron transport chains by 27.0 % (p<0.001) and by 31.0 % (p<0.001) (Table I). Under the same conditions, O₂ production by the mitochondrial respiratory chain declined by 31.2 % (p<0.01) and 34.6 % (p<0.001) respectively. Generation of O₂ by leukocyte NADPH-oxidase in periodontal tissues was lessened by 34.5 % (p<0.001) and 37.9 % (p<0.001) respectively to comparable data of the 2nd group.

Under these conditions, NOS activity in periodontal tissues decreased by 32.8 % (p<0.01) and 45.9 % (p<0.001), peroxynitrite concentration declined by 35.1 % (p<0.001) and 39.0 % (p<0.001) respectively to comparable data of the 2nd group (Table II).

Co-effect produced by corvitin and EGCG under systemic and local LPS administration is accompanied with reduced O₂ production by NADPH-dependent electron transport chains (microsomal and NOS) by 20.0 % (p<0.05) compared with values for the animals received separate corvitin during the experiment (Table I). O₂ generation by the mitochondrial respiratory chain yielded to comparable data of the 3rd and 4th groups by 27.6 % (p<0.01) and 23.8 % (p<0.05) respectively.

No differences were found between the groups exposed to combined or separate action of the above mentioned agents in the experiment when assessing O₂ generation by leukocyte NADPH-oxidase.

Combined effect of corvitin and EGCG during systemic and local LSP administration showed the decrease in NOS activity and peroxynitrite concentration in periodontal tissues by 53.3 % (p<0.001) and 27.0 % (p<0.02) compared with the findings in the 3rd group, and by 42.0 % (p<0.01) and 22.3 % (p<0.01) in the 4th group.

The results obtained point out the synergic effect produced by water-soluble form of quercetin and EGCG on

Table I. Co-effect produced by corvitin and epigallocatechin-3-gallate on generation of superoxide anion-radical in periodontium soft tissues under local and systemic administration of *S. typhi* lipopolysaccharide (M+m, n=35)

Groups of the animals studied	Inductors of superoxide anion radical production, nmol / g · s		
	NADPH	NADH	LPS
Control group I (intact animals)	12.47±0.87	15.41±1.08	1.58±0.12
Control group II (combined systemic and local LPS introduction)	21.32±0.41 *	28.59±1.41 *	2.93±0.12 *
+ water-soluble form of quercetin (corvitin)	15.57±0.85 */***	19.68±1.32 */***	1.92±0.13 **
+ EGCG	14.72±0.81 **	18.69±1.31 **	1.82±0.14 **
+ corvitin + EGCG	12.45±0.83 **/***	14.24±0.90 **/****/*****	1.72±0.10 **

Note (in table I-II):

* – p<0.05 compared with values in the control group I (intact rats);

** – p<0.05 compared with values in the control group II (combined systemic and local LPS administration);

*** p<0.05 compared with values in the animals received corvitin only under systemic and local LPS administration;

**** p<0.05 compared with values in the animals received EGCG only under systemic and local LPS administration

Table II. Co-effect produced by corvutin and epigallocatechin-3-gallate on nitrosative stress markers in periodontium soft tissues under local and systemic administration of *S. typhi* lipopolysaccharide (M+m, n=35)

Groups of the animals studied	Total activity of NO-synthase, $\mu\text{mol} / \text{min} \cdot \text{g}$ -of protein	Concentration of peroxynitrite- ions, $\mu\text{mol} / \text{g}$, of homogenate
Control group I (intact animals)	4.20±0.22	0.83±0.04
Control group II (combined systemic and local LPS administration)	12.53±0.61 *	1.54±0.06 *
+ water-soluble form of quercetin (corvutin)	8.42±0.64 */**	1.00±0.08 **
+ EGCG	6.78±0.54 */**	0.94±0.05 **
+ corvutin + EGCG	3.93±0.48 **/****/*****	0.73±0.03 **/*** /*****

the correction of oxidative and nitrosative stress markers in the periodontal tissues.

Recent studies have demonstrated the potential of Keap1 / Nrf2 / ARE system to control other redox-sensitive elements, including NF- κ B and AP-1 [15, 25]. We have found out the activation of NF- κ B is an important component in the mechanism of free radical damage to the periodontal tissues during systemic inflammatory response. The use of an inhibitor of the nuclear translocation of NF- κ B ammonium pyrrolidine dithiocarbamate in these conditions limits the manifestations of oxidative and nitrosative stress in the periodontium [29].

Previously it was shown that LPS-induced NF- κ B activation could be attenuated by certain Nrf2 activators, such as phenethyl isothiocyanate, sulforaphane and curcumin [15]. The administration of these compounds significantly inhibits phosphorylation in the site of I κ B kinase (IKK) / I κ B and p65 NF- κ B subunit nuclear translocation, consequently alleviating NF- κ B signaling [26].

Co-administration of water-soluble form of quercetin and EGCG allows to limit NF κ B-dependent mechanisms of oxidative and nitrosative stress more effectively as well as to enhance antioxidant potential of periodontal tissues associated with ARE-dependent gene expression.

Non-toxicity of quercetin and EGCG is a benefit, which distinguishes them from most NF- κ B activation inhibitors, whose applying is known to cause a number of adverse reactions [17].

Thus, the co-administration of water-soluble form of quercetin and epigallocatechin-3-gallate under systemic and local introducing of *S. typhi* lipopolysaccharide has been proven to be more effective means for preventing and correcting oxidative and nitrosative stress in the periodontal tissues than this occurs at separate administration of each of the polyphenols.

REFERENCES

- Akimov O Ye., Kostenko VO. Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. Ukr Biochem J. 2016;88(6):70-5.
- Ambili R, Janam P. A critique on nuclear factor-kappa B and signal transducer and activator of transcription 3: The key transcription factors in periodontal pathogenesis. J Indian Soc Periodontol. 2017 Sep-Oct;21(5): 350-6.
- Bohdanov AV, Hryshko YuM, Kostenko VO. Mechanisms of nitroxide-ergic dysregulation in periodontal tissues of rats under combined excessive sodium nitrate and fluoride intake. Wiad Lek. 2016;69(3, pt 2):457-61. (In Russian).
- Cachat J, Deffert C, Hugues S, Krause KH. Phagocyte NADPH oxidase and specific immunity. Clin Sci (Lond). 2015 May 1;128(10):635-48.
- Dröse S, Brandt U. Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. Adv Exp Med Biol. 2012; 748:145-69.
- Hasturk H, Kantarci A, Van Dyke TE. Oral inflammatory diseases and systemic inflammation: role of the macrophage. 2012;3:118.
- Im SC, Waskell L. The interaction of microsomal cytochrome P450 2B4 with its redox partners, cytochrome P450 reductase and cytochrome b(5). Arch Biochem Biophys. 2011;507(1):144-53.
- Kaidashev IP. NF- κ B Activation as a molecular basis of pathological process by metabolic syndrome. Fiziol Zh. 2012;58(1):93-101. (In Ukrainian).
- Kang CH, Choi YH, Moon SK et al. Quercetin inhibits lipopolysaccharide-induced nitric oxide production in BV2 microglial cells by suppressing the NF- κ B pathway and activating the Nrf2-dependent HO-1 pathway. Int Immunopharmacol. 2013 Nov; 17(3):808-13.
- Kanlaya R, Khamchun S, Kapincharanon C, Thongboonkerd V. Protective effect of epigallocatechin-3-gallate (EGCG) via Nrf2 pathway against oxalate-induced epithelial mesenchymal transition (EMT) of renal tubular cells. Sci Rep. 2016 Jul 25;6:30233.
- Khmil' DO, Kostenko VO. Combined effect of L-arginine and water-soluble form of quercetin on markers of oxidative-nitrosative stress in skin of rats exposed to excessive sodium nitrate. Fiziol Zh. 2017; 63(6):53-9. (In Ukrainian).
- Kim HS, Quon MJ, Kim JA. New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biol. 2014 Jan 10;2:187-95.
- Kostenko VO, Tsebrzhins'kii OI. Production of superoxide anion radical and nitric oxide in renal tissues sutured with different surgical suture material. Fiziol Zh. 2000;46(5):56-62. (In Ukrainian).

14. Kostenko VA, Yelins'ka AM, Ljashenko LI et al. NO- and peroxynitrite-dependent changes in superoxide anion-radical production in rats' organs under modeled metabolic syndrome. *Zhurn. Grodnenskogo gos. med. univ.* 2014;(2):74-7. (In Russian).
15. Li W, Khor TO, Xu C et al. Activation of Nrf2-antioxidant signaling attenuates NF-kappaB-inflammatory response and elicits apoptosis. *Biochem Pharmacol.* 2008 Dec 1;76(11):1485-9.
16. Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. *Matrix Biol.* 2002 Apr;21(3):251-62.
17. Lin Y, Bai L, Chen W, Xu S. The NF-kappaB activation pathways, emerging molecular targets for cancer prevention and therapy. *Expert Opin Ther Targets.* 2010 Jan;14(1):45-55.
18. Liu X, Lin R, Zhao B et al. Correlation between oxidative stress and the NF-kB signaling pathway in the pulmonary tissues of obese asthmatic mice. *Mol Med Rep.* 2016 Feb;13(2):1127-34.
19. Ljashenko LI, Denisenko SV, Kostenko VA. Role of transcription nuclear factor kB in mechanisms of free radical processes impairment and connective tissue disorganization in periodontium under modeled metabolic syndrome. *Aktual'ni problemy suchasnoyi medytsyny: Visn Ukr med stomatol akad.* 2014; 14(1):97-100. (In Ukrainian).
20. Luo S, Lei H, Qin H, Xia Y. Molecular mechanisms of endothelial NO synthase uncoupling. *Curr Pharm Des.* 2014;20(22):3548-53.
21. Ma Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol.* 2013;53:401-26.
22. Sun W, Liu X, Zhang H et al. Epigallocatechin gallate upregulates NRF2 to prevent diabetic nephropathy via disabling KEAP1. *Free Radic Biol Med.* 2017 Jul;108:840-857.
23. Tornatore L, Thotakura AK, Bennett J et al. The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation. *Trends Cell Biol.* 2012 Nov;22(11):557-66.
24. Vomhof-Dekrey EE, Picklo MJ Sr. The Nrf2-antioxidant response element pathway: a target for regulating energy metabolism. *J Nutr Biochem.* 2012 Oct;23(10):1201-6.
25. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-kB response pathways. *Biochem Soc Trans.* 2015 Aug;43(4):621-6.
26. Xu C, Shen G, Chen C et al. Suppression of NF-kappaB and NF-kappaB-regulated gene expression by sulforaphane and PEITC through IkkappaBalpha, IKK pathway in human prostate cancer PC-3 cells. *Oncogene.* 2005 Jun 30;24(28):4486-95.
27. Yelins'ka AM, Akimov OYe, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. *Ukr Biochim J.* 2019;91(1):80-5.
28. Yelins'ka AM, Shvaykovs'ka OO, Kostenko VO. Epigallocatechin-3-gallate prevents disruption of connective tissue in periodontium and salivary glands of rats during systemic inflammation. *Wiad Lek.* 2018;71(4):869-73.
29. Yelins'ka AM, Shvaykovs'ka OO, Kostenko VO. Influence of ammonium pyrrolidine dithiocarbamate on production of reactive oxygen and nitrogen species in tissues of periodontium and salivary glands of rats exposed to systemic administration of Salmonella typhi lipopolisaccharide. *Fiziol Zh.* 2018;64(5):63-9. (In Ukrainian).
30. Yelins'ka AM, Shvaykovs'ka OO, Kostenko VO. Sources of production of reactive oxygen and nitrogen species in tissues of periodontium and salivary glands of rats under modeled systemic inflammation. *Probl ekol med.* 2017;21(3-4):51-4.

Authors' contributions:

According to the order of the Authorship.

Conflict of interest:

The Authors declare no conflict of interest.

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