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Research Article

Green Tea Suppresses the Radiation-Induced Increase of the Angiotensin-Converting Enzyme & Reactive Oxygen Species in the Aorta of Rats

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ABSTRACT

The consumption of green tea reduces the risk of cardiovascular diseases and suppresses the development of atherosclerosis. The main factor for the initiation and progression of atherosclerosis is an increase in the production of reactive oxygen species (ROS) in vessels. A significant contribution to the increase in ROS production is made by increased concentration of angiotensin II, a product of the angiotensin-converting enzyme (ACE). The effect of green tea on the level of ROS and ACE activity in blood vessels *in vivo* has not yet been studied. The activity of ACE in aorta sections of rat was determined by measuring the hydrolysis of hippuryl-L-histidyl-L-leucine, and the production of ROS was estimated from the oxidation of dichlorodihydrofluorescein. Green tea inhibited the radiation-induced activation of the ACE in the aorta of rats on intraperitoneal (i.p.) and peroral administration. Six hours after the administration of tea, the activity of ACE in irradiated rats decreased to the control level, and by 24 h after administration, the tea did not almost affect the ACE activity. On i.p. administration, effective doses were lower than on peroral administration. The concentration of orally administered tea that inhibited the ACE activation in irradiated rats by 50% (IC_{50}) was 1 ml of an extract of 2.1 g of tea brewed per 100 ml of water. One milliliter of i.p. administered green tea (1 g per 100 ml of water) completely suppressed the increased ROS production in the aorta of irradiated rats.

Introduction

Cardiovascular diseases (CVD) associated with atherosclerosis are currently the main cause of mortality [1]. The major factor of the initiation of atherosclerosis is oxidative stress in vessels, and angiotensin II, a product of the angiotensin-converting enzyme (ACE), makes a significant contribution to this process [2-4]. Angiotensin II activates NADPH oxidase, which leads to an increase in the production of reactive oxygen species (ROS), a stimulation of cell proliferation, enhanced expression of molecules of adhesion to monocytes in the endothelium, inflammation, and the development of atherosclerosis [5-9].

Ionizing radiation, which has been widely used for the treatment of oncological diseases, initiates atherosclerosis. Upon irradiation, not only tumors but also normal tissues fall within the irradiation zone, which leads to the development of inflammatory processes, fibrosis, and dysfunction of normal tissues [10, 11]. The main reasons for the destruction and dysfunction of normal tissues after irradiation are the damage to capillaries, microvascular occlusion, and fibrosis [12]. In large vessels that fall within the irradiation zone during radiotherapy, atherosclerosis followed by thromboembolism and stenosis develop, which manifests itself on the organism level as CVD [13, 14]. It was shown that irradiation at doses used in cancer treatment (2-2.5 Gy) induces an increase in ACE activity and ROS production in the aorta of rats [15, 16]. Some flavonoids: flavones, flavonols, and flavanonols suppressed these radiation effects [16-18] but other, e.g., catechin increased ACE activity and ROS production in the aorta of unirradiated rats [19].

Population studies show that green and black teas significantly reduce the risk of CVDs [20]. Some investigations in Asian countries have shown that green tea is more effective in diminishing the risk of CVDs

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[21]. Green tea contains various flavonoids, including catechins and flavonols [22]. The effect of green tea on the activity of ACE in vessels in vivo has not yet been studied. The goal of the present work was to examine the effect of green tea on the activity of ACE and the formation of ROS in the aorta of normal and irradiated rats.

Materials and Methods

I Animals, Methods of Preparation and Introduction of Green Tea, and Irradiation Conditions

Male Wistar rats weighing 300-320 g at an age of 9-10 weeks (N=66, the animal collection at the Institute of Theoretical and Experimental Biophysics, Pushchino, Russia) were used. Rats were maintained in facilities with free access to water and standard chow. All experiments on animals were conducted under protocols approved by the Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences (Protocol number 3 of 17.2.2020). Research was conducted in compliance with the principles stated in the Directive 2010/63/EU of the European Parliament of 22 September 2010 on the protection of animals used for scientific purposes. Green tea (1-4.5 g per 100 ml of water) was brewed for 5 min at 100ºC. Tea was administered to rats in three ways: 1) 1 ml (1 g/100 ml) was administered intraperitoneally; 2) 1 ml of tea was administered per os (1-4.5 g/100 ml); and 3) water in the drinking bowl was replaced by tea for 1 and 3 days at a ratio of 1 g/100 ml and for 1 day at a ratio of 2 g/100 ml. Before the intraperitoneal administration, tea was supplemented with 0.14 M NaCl to attain the physiological osmolarity of the solution. The rats consumed 100 ± 5 ml of drinking water per 1 kg of body weight each day. In the case that water in the drinking bowl was replaced by tea brewed at a ratio of 1 g/100 ml, rats drank 129 ± 6 ml per kg of body weight during the day and at a brewing ratio of 2 g/100 ml, they consumed 86 ±7 ml.

Thus, at a tea ratio of 1 g/100 ml, the amount of tea drunk by rats was by 29% greater than that of water, and at a brewing ratio of 2 g/100 ml, it was by 14% less. Presumably, the decrease in the consumption of tea in the latter case is due to the bitter taste of the drink. Whole-body irradiation was performed at room temperature with a single 2.5 Gy dose of 200-kV X-rays (RUT-250-15-1, Institute of Cell Biophysics, Russia; 20 mA, 1 mm Al, 1 mm Cu filtering) at a dose rate of 1 Gy/min. Rats were sacrificed 2 h after irradiation.

II Aorta Preparation

The aorta was prepared as described in [23]. At the end of the treatment protocols, animals were anaesthetized by ether, and the thorax was dissected before injection of heparin (pharmaceutical preparation, 500 units) into the heart to prevent blood clotting. The procedure from the beginning of operation to the removal of the aorta took less than 3 min. The rats were under anaesthesia during this period and died shortly after the injection of heparin. A greater part of the adventitial fat adherent to the aorta was cleaned in situ. Then, the aorta was removed, rinsed with cold (4ºC) 10 mM Hanks'-HEPES solution, pH 7.4, and placed to the same solution. The residuary fat was carefully cleaned; care was taken not to damage the endothelium. The aorta was cut into eight 4-5-mm sections beginning with the point at which the aorta became parallel to the vertebral column. The aortic sections were numbered from 1 to 7, starting with the section near to the aorta arc. Sections 1-6 were in the thorax aorta and section 7 was in the abdominal aorta. The aortic sections were cut lengthwise, turned inside out with the endothelium outside, and attached to the tip of a plastic pipette with a polyester thread. After the measurements of ACE or ROS, the aortic sections were taken away from the pipette, flattened, and their linear dimensions were determined using a slide gage to an accuracy of 0.1 mm.

III Measurements of ACE Activity in the Aorta

The ACE activity was determined by measuring the hydrolysis of hippuryl-L-histidyl-L-leucine (Hip-His-Leu) using the method of Ackermann *et al.* with a modification of Myamoto *et al.* [24, 25]. Briefly, isolated rat aorta sections were placed in Hanks'-HEPES solution (450 µl), pH 7.4, and incubated for 10 min at 37ºC with shaking (25 Hz, amplitude 1 mm) for adaptation before the addition of the ACE substrate. The reaction was started by the addition of 10 mM Hip-His-Leu (50 µl). After 30 min of incubation at 37˚C, the reaction was stopped by the addition of 1000 µl of 0.1 N NaOH. After stirring the reaction mixture, aorta sections were taken out of the solution, and their dimensions and weight were determined. A 200-µl aliquot of the remaining solution was incubated with 50 µl of o-phthaldialdehyde (20 mg/ml in dimethyl sulfoxide) for 30 min at 37˚C, and the reaction was stopped by the addition of 2 ml of 0.8 N HCl. The samples were centrifuged at 3000 g at 4˚C for 5 min, and fluorescence was measured using an MF44 Perkin-Elmer fluorimeter at excitation and emission wavelengths of 360 and 500 nm, respectively. For determining the ACE activity, a standard curve was constructed using His-Leu. The ACE activity was expressed as picomoles of Hip-His-Leu hydrolyzed per min per mm^2 of the inner aorta surface (the endothelium surface). The ACE activity in the aorta was determined by averaging the ACE activities of all eight sections for each rat, after which these values were averaged for all rats used in the experiment.

IV Measurement of ROS in the Aorta

The amount of ROS was determined by the method of Korystov *et al.* [23]. Aorta segments 4 to 5 cm long were placed in a Hank's solution supplemented with HEPES (Hank's-HEPES) (2.5 ml, pH 7.4) and incubated for 30 min at 37˚C on a shaker (25 Hz, amplitude 1 mm). Then, 2'7-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) at a final concentration of 20 µM was added, and the mixture was incubated for an additional 20 min at 37˚C on a shaker. After the termination of incubation, the solution containing $DCFH_2$-DA was decanted, and aorta segments were washed with a cold Hank's-HEPES solution, pH 7.4. Then, the segments were placed in citrate buffer (2.5 ml) (pH 4.0) containing a 0.02% digitonin, and the mixture was incubated for 20 min at 37ºC under shaking to extract the resulting dichlorofluorescein (DCF) from the aorta. The fluorescence of DCF was measured in extracts at pH 7.0 on an MF44 Perkin Elmer fluorimeter at excitation and emission wavelength 475 and 535 nm, respectively.

V Drugs

Original Ceylon green tea (Leoste tea, Link) was used in the work. Digitonin, dichlorodihydrofluorescein diacetate ($DCFH_2$-DA), Hank's solution, HEPES, Hip-His-Leu acetate salt, His-Leu, o-phthalaldehyde were obtained from Sigma (USA); heparin was a pharmaceutical preparation. A 10 mM $DCFH_2$-DA stock solution was prepared in ethanol, stored at -20˚C, and diluted in Hanks' solution before use.

VI Statistical Analysis

The results are expressed as the means ± SEM. The numbers of rats (N) used in the experiments are given in figure legends. The significance of differences in multiple comparisons was determined using the ANOVA and Tukey post-hoc tests. P values less than 0.05 were considered significant.

Results

I Dependence of the ACE Activity in the Aorta on the Time of Action of Tea Administered Intraperitoneally Before Irradiation

Figure 1 shows changes in the ACE activity in the aorta depending on the duration of action of tea i.p. administered before irradiation. It is seen that, if tea was administered 1-6 h before irradiation, the ACE activity in the aorta of irradiated rats progressively decreased and reached the level of activity in control rats (Figure 2) exposed to radiation 6 h after the injection of tea.

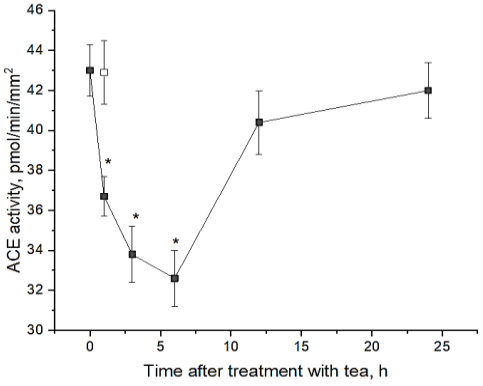

Figure 1: The dependence of ACE activity in the aorta of irradiated rats on the time of i.p. injection of 1 ml of tea brewed 1 g/100 ml before irradiation. The open square is the ACE activity after the i.p. injection of the physiological saline (1 ml) 1 h before the radiation exposure. N = 3-6 for each experimental point. * P < 0.05 vs. the ACE activity in the aorta of irradiated rats without tea injection.

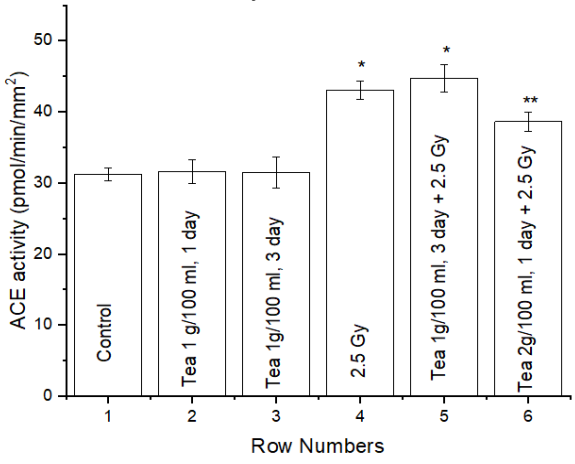

Figure 2: Effect of consumption of tea instead of water on ACE activity in the aorta of control and irradiated rats. N = 3-6 for each experimental point. *P < 0.05 vs. the ACE activity in the aorta of control rats. ** P < 0.05 vs. the ACE activity in the aorta of irradiated rats that did not receive tea.

Consequently, tea administered 6 h before the irradiation almost completely eliminated the effect of irradiation on ACE. It is also seen in (Figure 1) that the i.p. injection of the physiological saline (1 ml) 1 h before the exposure (open squares) does not affect the ACE activity. As the interval between the administration of tea and irradiation increases (more than 6 h), its effect decreases; by 12 h the ACE activity increases to a value that is only by 10% lower than the level in irradiated rats, and after 24 h the effect of tea is completely lost.

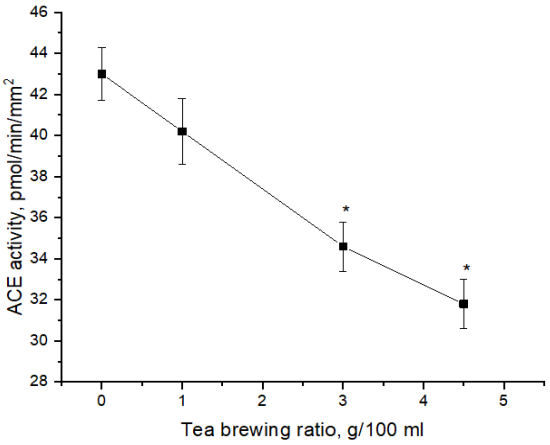

Figure 3: Dependence of suppression of radiation-induced ACE activation on the tea brewing ratio. Tea was administered to rats per os in a volume of 1 ml 3 h prior to irradiation. IC_{50}= 1 ml of 2.1 g/100 ml extract. N = 3-6 for each experimental point. * P < 0.05 vs. the ACE activity in the aorta of irradiated rats without tea injection.

II Dependence of the Effect of Tea Administered per os on the Radiation-Induced Increase in ACE Activity on the Tea Brewing Ratio

Figure 3 shows how the effect of tea on the radiation-induced increase in the ACE activity in the aorta depends on its brewing ratio. Tea brewed at a ratio of 1, 3, and 4.5 g per 100 ml was administered to rats per os in a volume of 1 ml 3 h prior to irradiation. It is seen that the effect of tea on the activation of the ACE increases with increasing brewing ratio. The IC_{50} value for the suppression of the irradiation-induced increase in ACE activity is 1 ml of tea brewed at a rate of 2.1 g/100 ml. At a tea ratio of 4.5 g/100 ml, the effect of irradiation on ACE activity is completely abolished.

III Effect of Tea on the Radiation-Induced Increase in ACE Activity After Replacing Water by Tea in the Drinking Bowl

From (Figure 3) it is seen that 1 ml of teat brewed at a ratio of 1 g/100 ml administered per os 3 h prior to irradiation insignificantly (by about 10%) reduces the ACE activity. It would be logical to expect that, if rats would constantly consume tea of this concentration instead of water, its effect should increase. However, the consumption of this tea for both one and three days does not decrease the ACE activity in the control, and the consumption of the tee for three days before irradiation does not affect it (Figure 2). Only the consumption of a tea extract at a ratio of 2 g/100 ml for 24 h decreased the ACE activity by 12% (Figure 2, column 6). The amount of tea at a ratio of 2 g/100 ml consumed by rats was by 33% less than of tea brewed at a ratio of 1 g/100 ml (see Materials and Methods). Thus, the twofold increase in tea concentration leads to an increase in the daily consumption of tea only by 34%.

IV Effect of Tea on the Radiation-Induced Increase in ROS Production in the Aorta of the Rat

As was noted in the Introduction section, the activation of the ACE induces an increase in ROS production through the angiotensin II-mediated stimulation of NADPH oxidase. In the aorta of rats exposed to ionizing radiation, the generation of ROS also increases [16]. Figure 4 shows the effect of tea brewed at a ratio of 1 g/100 ml (1 ml i.p. 3 h prior to irradiation) on ROS production in the aorta of control and experimental rats. It is seen that the tea does not affect the ROS generation in the aorta of control rats and completely eliminates the effect of irradiation on this process in the aorta of irradiated rats.

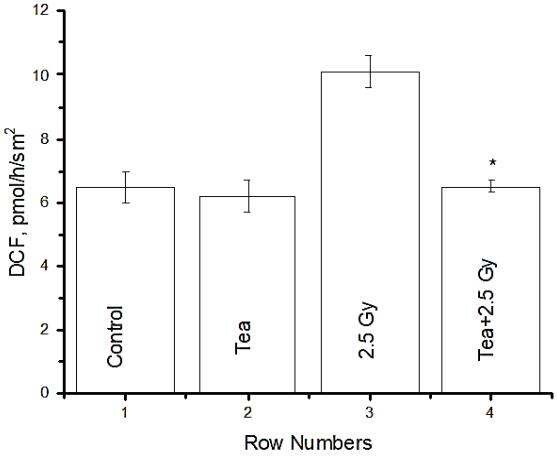

Figure 4: Effect of green tea on ROS production in the aorta of control and irradiated rats. Tea was brewed at a ratio of 1 g/100 ml, and 1 ml was injected i.p. 3 h prior to irradiation. N=3 for each experimental point. * $P < 0.05$ vs. the ACE activity in the aorta of irradiated rats not injected with tea.

Discussion

The results of the study showed that green tea administered into the body by different ways inhibits an increase in ACE activity and ROS generation in the aorta of rats exposed to radiation. Tea produces the most pronounced effect on intraperitoneal administration and is least effective when it is constantly consumed (apparently in small amounts) instead of water. The lower efficiency of tea consumed per os is due to the fact that tea flavonoids are ejected by transport systems of enterocytes, and only an insignificant portion of flavonoids enter the blood [26]. Presumably, constant consumption of tea instead of water activates both the systems of transport of flavonoids into the intestinal lumen and the enzymes of their metabolism; therefore, this way of administration is least effective.

A man consumes tea brewed usually at a ratio of 1 g/100 ml in portions, two to three times daily, 230 ml (8 OZ) a portion. Taking the average weight of a man to be 70 kg, we obtain that, with one portion, the amount of tea received by a man is 3.3 ml/kg of body weight. Rats used in our experiments weighed on the average 310 g; consequently, with 1 ml of tea administered per os, they received a dose of 3.2 ml/kg of body weight, which is comparable with the amount the man receives with one cup of tea. The IC_{50} value for the suppression of ACE activation in rats is 1 ml of tea brewed at a ratio of 2.1 g/100 ml, which is equivalent to the amount of 6.7 ml/kg of tea brewed at a ratio of 1 g/100 ml. Considering that the rate of metabolism and excretion of various agents in a man is much lower, as estimated by conversion factors (7.5 for a rat weighing 0.3 kg and 39 for a man weighing 70 kg), we obtain that IC_{50} for a man is (6.7 ml/kg×7.5)/39 = 1.3 ml/kg of tea brewed at the 1 g/100 ml ratio [27]. This value is about threefold lower than the amount of tea consumed with one cup and is even greater than the dose completely suppressing the activation of ACE by irradiation. Thus, one cup of tea is sufficient to completely eliminate the effect of irradiation on the aorta.

Green tea contains two types of flavonoids: flavan-3-ols, which are presented almost exclusively (about 99%) by catechin and its gallo derivatives (gallates) and flavonols (quercetin, kaempferol, myricetin) [22]. It has been shown earlier that catechin, epigallocatechin, and epigallocatechin gallate (unpublished data) increase the ACE activity in the aorta of rats, and flavonols reduce the radiation-induced increase in ACE activity [18, 19]. It is known that flavonols are much better absorbed in the intestine than flavan-3-ols and stay much longer in the blood plasma ($T_{1/2}$ of flavonols 8 h and $T_{1/2}$ of flavan-3-ols 0.5-1 h) [28-30]. Probably, the difference in the kinetics of flavonols and flavan-3-ols in the blood plasma determines the kinetics of the effect of green tea on the ACE activity in the aorta. Green tea is a complex mixture of organic compounds; along with flavonoids, it contains proteins, carbohydrates, organic acids, lignin, amino acids, caffeine, and lipids [31]. Some of these compounds can also contribute to the inhibition of ACE activity by green tea, but their effect on the ACE activity has not yet been examined.

The study revealed the capacity of green tea to normalize the elevated activity of the ACE and the generation of ROS in the aorta after irradiation, which may be a factor that inhibits the development of atherosclerosis and decreases the risk of CVDs at radiotherapy of tumors.

Conflicts of Interest

None.

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