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INFLUENCE OF DIET WITH KALE ON LIPID PEROXIDES AND MALONDIALDEHYDE LEVELS IN BLOOD SERUM OF LABORATORY RATS OVER INTOXICATION WITH PARAQUAT

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ABSTRACT

Background. Organism's lipid peroxidation is one of the most often examined and known physiological process evoked by free radicals. It concerns oxidation reaction of unsaturated fatty acid and/or other lipids leading to lipid oxidation products (LOP), which as a result of further changes generate among others the malondialdehyde molecules. The aim of the work was an estimation if raw or cooked kale addition to rat's diet influences antioxidant defense efficiency in their organisms in comparison to rats fed with standard AIN-93G diet.

Material and methods. The experiment was conducted with 36 Wistar strain, male rats over 21 days. The rats were divided into 3 groups (each 12 stuck) which were fed with: standard diet AIN-93G (2 groups), AIN-93G diet with 10% addition of raw kale (2 groups), and AIN-93G with 10% addition of cooked lyophilised kale. The total content of polyphenols (FC method) and antioxidant activity (ABTS++) were previously determined in raw and then in cooked kale. On the 20th day of experiment, half of rats (6 stuck) of each kind of the diet were injected intraperitoneally by the solution of paraquat (PQ) in physiological salt to evoke the oxidative stress. The next day animals were stunned and blood from their hearts was sampled. In the obtained serum, the levels of lipid oxidation products (LOP) and malondialdehyde (MDA) were assessed.

Results. It was observed that in blood serum of rats fed with modified diet with raw and cooked lyophilised kale addition the lipid oxides level was lower in comparison to control group fed with standard diet (p < 0.05). It was found that intoxication with paraquat caused growth of MDA and LOP levels in blood serum of all rats in comparison to not intoxicated groups but that growth was the lowest in group fed diet with cooked kale addition.

Conclusion. Diet with kale, both raw and cooked, efficiently inhibited the lipid peroxidation process in rats' organisms, ongoing during natural metabolism and during evoked oxidative stress.

Key words: kale, paraquat, malondialdehyde, lipid peroxides

INTRODUCTION

Many biochemical processes ongoing in organisms lead to development of free radicals. Although small amounts of radicals are necessary for proper cells functioning, in abundance cause real threat. Lipid membranes and enzymes of a living cell are the main

objective of free radicals, what results in weakening of all living processes and consequently can cause the early cell's death. The cell membrane is built in a great share from unsaturated fatty acids and is especially susceptible to cohesiveness loss and impairment

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of the active transport and receptor characteristics because of oxygen hyperactive form (ROS) toxic activity. Free radicals also cause collagen degradation, disturb synthesis and inactivation of proteoglycans, inhibit enzymes, damage chromosomes, inhibit oxidative phosphorylation in mitochondria, modify cells' abilities (characteristics) and disturb intracellular calcium homeostasis. The oxidative stress accompanies almost 100 illnesses units, commonly occurred. Most important are: arteriosclerosis, hypertension, diabetes, cancers, inflammations caused by bacteria or other pathogenic factors can be included [Bandyopadhyay et al. 1999, Waris and Ahsan 2006].

As a defense system against oxidative damages the living organisms generated many highly specialised antioxidative boarders during evolution, which are necessary for proper functioning of the organism [Halliwell and Gutteridge 1990, 1995].

Since now scientific researchers have documented, that one of the ways to enlarge the effectiveness of the antioxidative defense system in human being is a consumption of food rich in high antioxidative abilities components. Their basic source is mainly plant origin products, especially, vegetables, fruits and their products that is why the nutritionists advise to consume them 5 times a day in different forms [Czapski 2001, Prior 2003, Grajek 2004, Vinson et al. 2005, Nantz et al. 2006, Paterson et al. 2006, Sikora and Bodziarczyk 2012].

Organism's lipid peroxidation is one of the most often examined and known physiological processes evoked by free radicals. It concerns oxidation reaction of unsaturated fatty acid and/or other lipids leading to lipid oxidation products (LOP), which as a result of further changes generate among others the malondial-dehyde molecules.

The aim of this experiment was a comparison between rats fed with modified AIN-93G diet containing raw or cooked lyophilized kale addition and control groups in their ability to fight against induced stress. As biomarkers of ongoing processes the lipid oxidation products and MDA levels in animals' blood serum were estimated.

MATERIAL AND METHODS

Plant material

The experimental material consisted of kale (*Brassica oleracea* L. var. *acephala*) of Winterbor F₁ variety, which was grown in accordance to GAP at the "Polan" Plant and Horticultural Seed Production Centre Ltd. (Krakow, Poland) – Experimental Station. The harvested plants were washed up with water and dried in a room temperature, then cut into small pieces; mixed and average laboratory sample was obtained. One part of the average sample was cooked in a stainless steel vessel for 15 min at 100°C, in the small amount of water. After cooking, the material was left of sieves and cooled in stream of cold air.

Analytical method of plant material

From raw and cooked leaves of tested kale, the methanol extracts were obtained. The leaves were homogenized in laboratory homogenizer. Three grams of raw or five grams of cooked homogenized material were extracted by 80 cm³ of 70% methanol at room temperature for 2 hours.

The total phenolic content of kale extracts was determined by using the Folin-Ciocalteu colorimetric method [AOAC 1995]. The methanol extract (5 ml) of the tested vegetable were diluted 20-fold with distilled water. To 5 ml of this solution was added 0.5 ml of Folin-Ciocalteau reagent (FLUKA), diluted with distilled water 1:1 v/v) and 0.25 mL of 25% Na₂CO₃. The mixture was incubated for 20 min at room temperature in a dark place. After this time the tube was centrifuged for 10 min (1250 rev/min). The absorbance was measured at 760 nm. The results were expressed in milligrams of chlorogenic acid equivalents per 100 g of fresh and dry matter of raw and cooked vegetable.

Antioxidant capacity (TEAC) as well as the ABTS⁺⁺ were determined by the spectrophotometric method [Re et al. 1999]. To a 10 ml-flask was transferred: 1 ml of potassium persulfate (66.2 mg of crystalline potassium persulfate (Sigma) in 10 ml distilled water) and 38.4 mg of crystalline ABTS⁺⁺ (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (SIGMA). The whole was made up to mark with distilled water and thoroughly mixed. The flask was wrapped with aluminum foil and left at room temperature for 14-16 h to generate free radicals. After this time the mixture

was diluted with methanol about 80 times so that the absorbance measured at 734 nm was in the range 0.740-0.750. The absorbance was monitored periodically during the analysis and if necessary adjusted to the previously agreed level. Into test tubes containing methanol extracts of tested vegetable in the amounts: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 and 0.8 ml and filled to 1 ml with methanol. Then 2 ml of free radicals ABTS⁺⁺ solution was added, mixed in mixer and the whole was incubated in the incubator at 30°C for 6 minutes. The absorbance was measured at 734 nm. The result was expressed as the Trolox equivalent antioxidant capacity (TEAC) in μM of Trolox/g of fresh matter.

Animals and experimental groups

Raw and cooked kale parts were lyophilised with Christ Ralpha 1-4 apparatus. The lyophilisates after grinding constitute as parts of the diets applied in a feeding experiment with Wistar rats. The 3-month-old male with an initial body weight of about 250-350 g (36 pieces) came from the Institute of Pharmacology, Polish Academy of Sciences in Krakow. All treatments were performed on living organisms in accordance with the consent of the Local Ethical Committee d/s Experiments on Animals (Resolution No. 89/2006 of 30 November 2006) and in accordance with its requirements.

The experiment was performed over 21 days. The animals were divided into 3 groups (each 12 stuck) obtaining the original, semi synthetic diet AIN-93G recommended by American Institute of Nutrition [Reeves et al. 1993] for growing animals (I group) and the diet modified with raw (II group) or cooked (III group) lyophilized kale addition. The amount of lyophilsate used standed for 10% of AIN-93G typical diet. The basic chemical composition of vegetable added to the diet was estimated at first and then the diet composition was modified with equivalent exchange of nutritive fibre of AIN-93G original diet with proper amount of fibre originating from kale lyophilsate. The average diet consumption was established at 10% of rat body mass level (25-35 g/day) and it was subsequently corrected during everyday feeding (weighing of animals). Every day, a portion of diet was weighed and served as mix with water, as socalled raw "cake". The animals have unlimited access to water. On 20th day of experiment, 6 rats fed with

each type of diet were intraperitionealy injected with paraquat (PQ, dichloro[1,1'-dimethyl-4,4'-bipirydyne]) dissolved in physiological salt solution. The dose for one rat was 35 mg/kg rat's body mass. The amount of PQ dose was established basing on bibliography, where that dose had not been found as lethal [Melchiorri et al. 1996]. The chemical was applied for evoking the oxidative stress in organism of animals. After intoxication, all rats were placed in numbered metabolic cages for 24 hours. During this, animals were supplied with water (ad libitum). At the last day of experiment animals were anaesthetized with sodium thiopentate (Biochemie, Vienna, Austria) that was injected to peritraneum at 25 mg/100 g of rat's body mass. Then the euthanasia was performed by full blood evacuation from heart (according to Local Ethic Commission demands). All the experiments were performed accordingly with Local Ethic Commission regulations.

Analysis of blood serum

Blood serum was separated by centrifugation at 4000 rpm for 10 min. and stored in screw capped vials in the freezer (–80°C) until lipid oxidation and malon-dialdehyde (MDA) analyses. The estimations were performed with enzymatic sets LOP-CC (catalogue no CC-004) of Kamiya Biomedical Company (Japan) and OXI-TEK TBARS Assay Kit (catalogue no: #850-287-KI01) of Alexis Biochemicals. All analyses were result of duplicate analyses and results were estimated by using 2-factor analysis of variance (p < 0.05) and tested by Duncan test.

Statistical analyses

Statistical analysis was performed by using twoway analysis of variance (ANOVA) followed by Duncan Test at p < 0.05. Results are expressed as mean $\pm SD$ from six rats in each group.

RESULTS AND DISCUSSION

The sum of polyphenols estimated in raw kale was 675.50 mg/100 g fresh matter (Table 1). In the research by Łata and Wińska-Krysiak [2006], the total polyphenols content in extracts (methanol: formic acid and distillated water) of kale Winterbor F_1 assessed spectrophotometically, calculated as gallic acid, was

Table 1. Total polyphenols compounds and antioxidant capacity of raw and cooked kale

	Raw		Cooked	
Compound	mg/100 g f.m.	mg/100 g d.m.	mg/100 g f.m.	mg/100 g d.m.
Total phenolic compounds	676.50 ±11.21 ^b	3 890.2 ±64.47 ^B	154.90 ±5.20 ^a	1705.90 ±23.70 ^A
TAEC μM Trolox/g	30.25 ± 2.25^{b}	173.95 ±12.93 ^B	11.18 ± 1.40^{a}	108.44 ±13.57 ^A

a, b, A, B – mean with different letters in rows are statistically different at $p \le 0.05$, n = 3.

202.1 mg/100 g. Manach et al. [2004] found the sum of polyphenols in kale at the level of 30-60 mg/100 g.

Research of Heimler et al. [2006] showed that total polyphenols in Italian kale, estimated with FC reagent, was 138 mg/g of d.m. Korus and Lisiewska [2011] reported 240.44 mg of total polyphenol compounds in 100 g of kale Winterbor F_1 variety. Ninfali and Bacchiocca [2003] claimed that the plant genotype and its variety are meaningful factors influencing polyphenols content and antioxidant activity of vegetable at the same time. The significant influence may also have the term of plant harvesting and climatic factors [Hagen et al. 2009, Zietz et al. 2010].

The mean antioxidant activity measured as ability to scavenge the cation radical ABTS⁺⁻ was 30.25 mM Trolox/g. Cao et al. [1996] assessed 22 vegetables and showed a large antioxidant activity of kale (17.7 mM Trolox/g for ORAC_{ROO} and 6.2 mM Trolox/g for ORAC_{OH}.). Their results allowed placing kale at first place among assessed vegetables, including other *Brassica* (broccoli, cabbage, Brussels sprout and cauliflower). A lower antioxidant activity of kale Witerbor variety found Zietz at al. [2010] (49 μ M Trolox/g d.m.)

and Korus and Lisiewska [2011] (11.17 μ M Trolox/g f.m.), while Sharique and Seerat [2009] determine the antioxidant activity (FRAP) in the range of 43-86 μ M/g f.m. in six varieties of kale.

In the cooked kale, a much smaller content of total polyphenols was observed, which was 154.9 mg/100 g f.m. (loss of 56%). Korus and Lisiewska [2011] detected in cooked kale the similar decrease (about 70%) of total polyphenol compounds. As a result of polyphenols losses, the antioxidant activity was also decreased (loss of 60%). In the investigation of the cited authors losses amounted 45%.

Animal experiments

The mean level of lipids peroxides in blood serum of rats fed with standard diet was 6.86 nmol/ml (Table 2). In animals group, in which the diets were supplied in cooked or raw lyophilized kale, the lower level of LOP was observed in comparison to groups fed with standard diet. The level of LOP in blood serum of rats fed with diet supplied with cooked kale (4.76 nmol/ml) was the lowest in comparison to control group.

Table 2. Concentration of lipid oxidation products (LOP) in serum, nmol/ml

Group	I	II	III
Diet	AIN-93 ^G	AIN-93G + lyophi- lized raw kale	AIN-93G + lyophi- lized cooked kale
Non-intoxicated with PQ	6.86 ± 1.19^{bc}	5.75 ± 0.31^{ab}	$4.76\pm\!0.37^a$
Intoxicated with PQ	15.49 ±0.31°	10.29 ± 1.01^{d}	7.30 ± 0.38^{c}

a, b, c, d, e – the averages with different letter scripts differed significantly at $p \le 0.05$, n = 6.

 Table 3. MDA concentration in blood serum of rats, nmol/ml

Group	I	II	III
Diet	AIN-93G	AIN-93G + lyophi- lized raw kale	AIN-93G + lyophi- lized cooked kale
Non-intoxicated with PQ	12.53 ± 1.08^{b}	7.44 ± 0.84^{a}	8.48 ± 0.99^a
Intoxicated with PQ	23.33 ± 2.33^d	$15.75 \pm 1.17^{\circ}$	$15.43 \pm 1.10^{\circ}$

a, b, c, d – the averages with different letter scripts differed significantly at $p \le 0.05$, n = 6.

In group of animals intoxicated with PQ, the highest average concentration of assessed parameter was found for animals fed with standard diet (15.49 nmol/ml) and the lowest for the group fed with modified diet with raw lyophylised kale (10.29 nmol/ml) or with lyophylised cooked kale (7.30 nmol/ml). The differences between groups were significant (p < 0.05).

It was observed that the level of lipid peroxides for all (3) groups with PQ injection was significantly higher than that for group without intoxication. The highest growth of LOP concentration (126%) was found in rats serum fed with standard diet AIN-93G and the lowest (53%) in group fed with diet supplemented with lyophilized cooked kale. There were significant differences within each experimental group.

In groups fed with diets enriched with lyophylisates of kale lower concentrations of malondialdehyde (MDA) in comparison to groups fed with standard diets and those changes were significant for both experimental cases were also observed (p < 0.05; Table 3).

At the same time small differences between results obtained for animals fed with modified kale were not significant (p > 0.05).

The level of MDA in rats blood serum intoxicated was significantly higher in comparison to groups without intoxication (p < 0.05). The strongest growth of MDA level, up to 111% was noted in group fed with diet enriched with raw lyophylised kale, and in the rest of group that increase was similar, at average 84%. All those differences were significant (p < 0.05).

Significant increase of LOP and MDA concentrations in all experimental groups intoxicated with PQ in comparison to not toxicated groups proved that in the fed experiment the level of oxidative stress was obtained. Parallely, the influence of PQ on the tested parameters (LOP, MDA) was pronounced at different level depending on the applied diet. The highest increase of LOP concentration observed in control group can confirm not effective way of defense system against free radicals in those organism with diet of the antioxidant compounds deficiency.

Definitely lower growth of LOP concentrations was estimated in groups fed with cooked or raw lyophylised kale supplemented diets. At the same time was shown that the kale additive acted protectively against strengthen lipids peroxidation. The diet supplemented with kale also showed naturally occurring lipids peroxidation process in groups without PQ and that was manifested in lower concentration of lipids peroxides compared to control AIN-93G group (Table 2). It was emphasized more pronouncedly in rats fed with diet with lyophylised cooked kale. More effective influence of diet enriched with cooked kale was probably caused by higher accessibility of antioxidant compounds than in raw plant.

It was also documented a positive and similar influence of applied modified diets with lyophylised kale on MDA level, originating from both regular metabolism and as a result of PQ activity. The method of MDA estimation (TBARs) has many limitations and often is perceived as not specific. Despite of this it was applied in different research experiments [Atli et al. 2004, Mateos et al. 2005, Barzdo et al. 2005, Del Rio et al. 2005].

In the accessible bibliography, studies concerning the influence of kale consumption on lipids peroxidation process were not found. However, the results of research on other plant material are known. In Venkatesan [2000], the content of TBARs in rats intoxicated with peritraneum PQ and fed with the diet with curcumin addition was balanced like in control group. Leontowicz et al. [2002] observed significant decrease

of MDA concentration in animals plasma fed with hypercholesterolemic diet supplemented with apple.

In brain tissue of ICR mice parallely intoxicated with N-methyl-d-asparginic acid and consuming extracts obtained from red cabbage (Brassica oleracea L. var. capitala) and from asters (Aster scaber L.) the TBARS content was lower than that obtained in control not toxicated, fed with not modified diet [Lee et al. 2002]. Gamal el-Din et al. [2005] performed experiment with mice injected with single dose of PQ (30 mg/kg body mass) what intensified process of lipid peroxidation in lungs. After 5 days of diet enriched in arabic gum, the significant lowering of MDA level was showed in assessed tissue. According to studies by Rajagopal et al. [2003] protracted ethanol application in Wistar rats caused significant increase of TBARs level in tissues (liver, brain, kidneys). This impact was partially abolished by diet supplementation with Cassia auriculata plant, rich in tannins and antioxidant compounds. Similarly, Wistar rats intoxicated with single injection of carbon tetrachloride, increase of TBARs in liver tissue homogenate was shown (p < 0.01). This was higher in rats fed with not modified diet than in group fed with lyophilised extract Adhadota vasica protection [Pandit et al. 2004]. The similar results were obtained by Rajesh et al. [2004], who observed that albinotic Sprague-Dawley rats eating powdered Glycyrrhiza glabra L. plant overcame the process of lipids peroxidation in liver and kidney tissues initiated with CCl₄. The marker of stress was TBARS level which was lowered to values obtained in a control group. Melchiorri et al. [1996] showed that PQ injected intraperitoneally at 50 mg/rat's body mass resulted in increase of MDA level in serum and lung tissue both in Wistar and Sprague-Dawley rats and its influence was partially abolished by melathonine (5 mg/kg body mass) application.

In other research, a reduction of oxidative stress was shown evoked by *per os* application of PQ with soy bean proteins presence in rats' diet [Aoki et al. 2002]. In exposition on isoproterenol the protective effect of garlic oil (*Allium sativum*) was reported. The consuming of it with diet, within 60 days, lowered MDA concentration in serum to level similar as for control group [Saravanan and Prakash 2004]. The assessment of MDA level in human erythrocytes membranes showed that water extracts of powdered plants

(ginger, onion, and chili) inhibited lipids peroxidation process [Sujatha and Srinivas 1995].

Literature data revealed that kale is a rich source of antioxidant compounds, first of all of polyphenols, α,β --carotene, and also of vitamins C and E. The antioxidative potential of ascorbic acid is connected to its ability to regenerate other antioxidants such as α -tocopherol, glutathione, α,β -carotene. Also β -carotene in addition reaction of lipid peroxide radical scavenges it giving carotene adduct able for further reactions with radicals. Blanking of singleton oxygen by b-carotene breaks the propagation reactions and the lipid peroxide do not rise up. Research of Liao and Yin [2000] also proved that parallel presence of β-carotene and of polyphenols led to better protection of erythrocytes membranes' lipids against oxidation catalysed by Fe²⁺ ions. The protective role for lipids peroxide is played also by polyphenolic compounds which donating, in an easy way, the hydrogen of hydroxyl group, are ongoing oxidation with peroxides reduction. In Mateos et al. [2005] experiment the significant lowering of MDA level in Wistar rats serum fed witch AIN-93M original diet supplemented with powder obtained from different fruit (strawberries, cacaoyer, plum) rich in polyphenols (antocyanins, flavonols, procyanidins, hydroxycynnamic acid). That effect was held also after application of hypercholesterolemic diets.

Also polyphenols presented in green tea lowered effectively the level of MDA in rats blood serum fed with hypercholesterolemic diet [Yokozawa et al. 2002]. Juśkiewicz et al. [2002] applied the diet enriched in flavonols from grapefruit in rat feeding and obtained a lowery of MDA in serum after 10 days, what was explained by increasing serum antioxidative potential by flavonoids.

CONCLUSION

The performed experiment showed that diet with kale, both raw and cooked, efficiently inhibited the lipid peroxidation process in rats' organisms, ongoing during natural metabolism and during evoked oxidative stress. It confirms antioxidant properties of this vegetable and prohealthy advantages originating from its consumption and also confirms the stimulating influence of diet rich in antioxidants on organism's antioxidative defense effectiveness.

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WPŁYW DODATKU LIOFILIZATU JARMUŻU DO DIETY NA POZIOM NADTLENKÓW LIPIDOWYCH I DIALDEHYDU MALONOWEGO (MDA) W SUROWICY KRWI SZCZURA W WARUNKACH WYINDUKOWANEGO STRESU OKSYDACYJNEGO

STRESZCZENIE

Cel. Peroksydacja lipidów ustrojowych jest najczęściej badanym i najlepiej poznanym procesem ustrojowym wywoływanym przez wolne rodniki. Obejmuje ona reakcje utlenienia nienasyconych kwasów tłuszczowych lub innych lipidów, prowadząc przede wszystkim do powstawania nadtlenków tych związków (LPO), które następnie w wyniku dalszych przemian generują m.in. cząsteczki dialdehydu malonowego (MDA). Celem pracy było poznanie możliwości antyoksydacyjnych organizmów szczurów żywionych dietą z dodatkiem jarmużu surowego i gotowanego w porównaniu ze szczurami żywionymi dietą standardową AIN-93G.

Materiał i metodyka. Doświadczenie przeprowadzono z udziałem 36 szczurów rasy Wistar (samce) przez okres 21 dni. Zwierzęta podzielono na 6 grup (po 6 sztuk), które żywiono: dietą standardową AIN-93G (2 grupy), dietą AIN-93G z 10-procentowym dodatkiem liofilizatu jarmużu surowego (2 grupy), dietą AIN-93G

z 10-procentowym dodatkiem liofilizatu jarmużu gotowanego. W przedostatnim dniu doświadczenia, zwierzętom z jednej z poszczególnych grup żywieniowych wstrzyknięto dootrzewnowo roztwór parakwatu w soli fizjologicznej, w celu wywołania stresu oksydacyjnego. W następnym dniu zwierzęta doświadczalne uśpiono i pobrano krew z serca. W uzyskanej surowicy oznaczono poziom nadtlenków lipidowych i dialdehydu malonowego (MDA).

Wyniki. Zaobserwowano, że w surowicy krwi szczurów żywionych dieta zmodyfikowaną dodatkiem liofilizatu jarmużu surowego lub gotowanego poziom nadtlenków lipidowych był istotnie niższy w porównaniu z grupą kontrolną żywioną standardowo, przy czym zróżnicowanie było większe po diecie z dodatkiem jarmużu gotowanego. Stwierdzono, że intoksykacja parakwatem spowodowała prawie dwukrotny wzrost poziomu MDA i nadtlenków lipidowych w surowicy szczurów w porównaniu z grupami nieintoksykowanymi, ale był on istotnie mniejszy u szczurów, którym podawano dietę z dodatkiem jarmużu.

Wnioski. Dieta z dodatkiem jarmużu surowego, a w szczególności gotowanego, skutecznie hamowała w organizmach szczurów proces peroksydacji lipidów ustrojowych, przebiegający zarówno w toku metabolizmu naturalnego, jak i w warunkach wywołanego stresu oksydacyjego.

Słowa kluczowe: jarmuż surowy, parakwat, dialdehyd malonowy, nadtlenki lipidowe

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