Cigarette Smoke Exposure Intensifies Ventricular Remodeling Process following Myocardial Infarction

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OBJECTIVE

To evaluate the role of cigarette smoke exposure (CSE) on ventricular remodeling following acute myocardial infarction (AMI).

METHODS

Rats were submitted to myocardial infarction and divided into two groups: C (control, n = 31) and F (CSE: 40 cigarettes/day, n = 22). After 6 months, the survivors were submitted to echocardiogram, functional study with isolated heart, and morphometric analysis. For comparison purposes, we used the t test (mean ± standard deviation) or the Mann-Whitney test (with median and 25th and 75th percentiles).

RESULTS

The CSE animals tended to have larger diastolic (C = 1.5 ± 0.4 mm^2, F = 1.9 ± 0.4 mm^2; p = 0.08) and systolic (C = 1.05 ± 0.3 mm^2, F = 1.32 ± 0.4 mm^2; p = 0.08) left ventricular(LV) areas. The systolic function of the LV, assessed according to the fractional area change, tended to be impaired in CSE animals (C = 31.9 ± 9.3%, F = 25.5 ± 7.6%; p = 0.08). The - dp/dt values for CSE animals were statistically lower (C = 1474 ± 397 mmHg, F = 916 ± 261 mmHg; p = 0.02) than for control animals. The CSE animals presented higher right ventricle (RV) weight adjusted for body weight (C = 0.8 ± 0.3 mg/g, F = 1.3 ± 0.4 mg/g; p = 0.01), higher content of water in lungs (C = 4.8 (4.3-4.8)%, F = 5.4 (5.1-5.5); p = 0.03), and larger LV myocyte cross-sectional areas (C = 239.8 ± 5.8 μm^2, F = 253.9 ± 7.9 μm^2; p = 0.01).

CONCLUSION

Cigarette smoke exposure intensifies ventricular remodeling following acute myocardial infarction.

KEY WORDS

Ventricular function, ventricular dilatation, ventricular hypertrophy.

After acute myocardial infarction (AMI), complex changes in ventricular architecture may take place involving the infarcted and the non-infarcted region alike. After coronary occlusion, acute ventricular dilatation may occur, characterized by the thinning and distension of the infarcted region. This change is called infarction expansion, and results from the slippage of necrotic muscle bundles as a consequence of the disintegration of interfibrillar collagen[1]. In the late phase, different degrees of cavity dilatation were observed. This phenomenon results from the process of hypertrophy that involves both ventricles and apparently occurs as a response to increased wall stress. At the same time, we observed an abnormal accumulation of collagen (fibrosis) in the viable areas of the myocardium, both in the infarcted ventricle and in the other one. This set of adaptations, which includes changes in the composition, mass, volume and geometry of the heart, is known as myocardial remodeling[2-4].

The intensity of the ventricular remodeling process is directly associated with worse prognosis, due to the higher incidence of aneurysm formation, ventricular rupture and arrhythmia, and is also associated with the progression of ventricular dysfunction. Therefore, a number of strategies have been employed to prevent or mitigate the process of ventricular remodeling following AMI[5-7].

Coronary atherosclerotic disease and AMI are closely related to smoking. This risk factor is a major cause independent of morbidity and mortality[8,9]. However, while the vascular effects of exposure to cigarette smoke are well known, the effects of smoking on the heart have been less studied. Some experimental and clinical evidence suggest that smoking may be associated with changes in the function and morphology of the heart[10-19]. However, the effects of smoking on cardiac variables after AMI have not been studied to date. The objective of this study therefore was to analyze the effects of cigarette smoke exposure on the process of ventricular remodeling in rats.
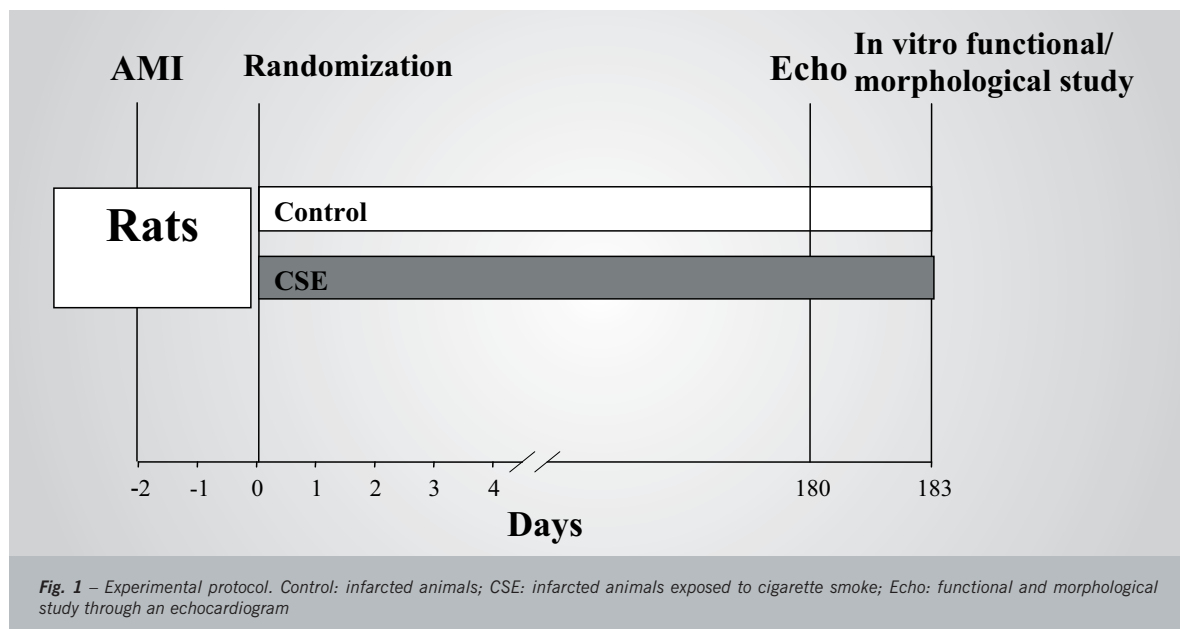
METHODS

Experimental groups

The experimental protocol of this paper was approved by the Ethics Committee for Experiments with Animals of our institution, and complies with the Ethical Principles for Experiments with Animals adopted by the Brazilian College of Experiments with Animals.

We used male Wistar rats, with weights between 200 and 250 grams. The first stage of the study was the induction of experimental infarction (fig. 1). Acute infarction was produced using a method previously described[20]. In summary, the rats were anesthetized with ether, and submitted to left side thoracotomy. After the exteriorization of the heart, the left atrium was pushed aside and the left coronary artery was ligated with a 5-0 mononylon treads between the exit of the pulmonary artery and the left atrium. The heart was then put back into the thorax, the lungs were inflated with positive pressure and the thorax was closed with 10 cotton sutures.

The animals were kept in cages for recovery. They were fed with standard commercial feed and had free access to water, with controlled light cycles – 12-hour cycles, temperature of approximately 25ºC and controlled humidity.

The second phase of the protocol comprised the formation of experimental groups. Forty-eight hours after the infarction, the surviving animals were randomly divided into two groups: Group C (n = 31), formed by infarcted animals which received no therapeutic intervention and Group F (n = 22), formed by infarcted animals exposed to cigarette smoke.

Fig. 1 – Experimental protocol. Control: infarcted animals; CSE: infarcted animals exposed to cigarette smoke; Echo: functional and morphological study through an echocardiogram

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Cigarette smoke exposure

The third stage of the study comprised the 6-month observation period. For the animals of Group F, we used the method proposed by Wang et al.[21], which has been standardized in our laboratory[22], to expose the animals to smoke in a modified incubator. The rats were placed in the transparent chamber, with a volume of approximately 95x80x65 cm, which was connected to the smoking device. Smoke puffs were drawn from the cigarette through vacuum by the smoking device and then fed into the chamber for a period of thirty minutes. After this period, the smoke was exhausted and the procedure was repeated. During the first week, the smoke was released at a rate of 5 cigarettes, twice a day, in the afternoon, with a rest period of 10 minutes. The number of cigarettes was increased to ten cigarettes/thirty minutes, twice in the morning and twice in the afternoon through the end of the study. At the end of the study the animals were exposed to the smoke of forty cigarettes/day. The cigarette used was a commercial brand with the following composition: 1.1 mg of nicotine, 14 mg of tar and 15 mg of carbon monoxide.

Morphological and functional assessment through an echocardiogram

The fourth stage of the protocol comprised the echocardiogram-based morphological and functional assessment of all surviving animals. After six months of treatment, the surviving animals (C, n = 13 and F, n = 11) were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg) and xylidine hydrochloride (1 mg/kg), for the echocardiographic study. Following the trichotomy of the anterior part of the thorax, the animals were put in the left lateral decubitus position for the test. The test was carried out using a Hewlett-Packard (Sonos 2000) piece of equipment, with a 7.5 MHz electronic transducer. The assessment of the mitral and aortic transvalvular flow was carried out using the same transducer operating at 5.0 MHz. The measurements of the heart structures were taken on the monodimensional images obtained with the ultrasound beam oriented according to the bidimensional image, in the parasternal short-axis view. The image of the left ventricle cavity was obtained by positioning the cursor in the M-mode between the papillary muscles, right below the mitral valve plane. The images of the aorta and of the left atrium were obtained from the parasternal short-axis view, with the M-mode cursor positioned at the level of the aortic valve. The monodimensional image (velocity: 100 mm/s) was printed on a Sony Co.'s UP-890MD printer.

At a later stage, the heart structures were manually measured with a precision pachymeter according to the recommendations of the American Society of Echocardiography[23] which have been validated for the model of infarcted rats[24]. The heart structures were measured in at least five consecutive heart cycles. The left ventricle diastolic diameter (LVDd) and the left ventricle posterior wall thickness (LVPWd) were measured at the time when the cavity reached its maximum diameter. The left ventricle systolic diameter (LVSd) was measured at the maximum systolic excursion of the posterior wall of the cavity. The diastolic areas (DA) and systolic areas (SA) were measured in the bidimensional mode, by means of planimetry. The systolic function of the left ventricle was assessed by calculating the fractional area change (FAC=DA-SA/DA x 100)[24]. The transmitral diastolic flow (E and A waves) was obtained with the transducer positioned at the apical four-chamber view. The measurements relating to the flows were calculated directly on the monitor of the echocardiography device.

In vitro functional analysis

The fifth stage of the protocol comprised the *in vitro* functional analysis. Within one and three days after the echocardiographic study, the same animals (C, n = 13 and F, n = 11) received intraperitoneal injections of sodium pentobarbital (50 mg/kg) and heparin (1,000 UI) and were submitted to positive pressure ventilation and oxygen at 100%. Then their thorax was cut open and the aorta was catheterized with a number-15 metal cannula. The retrograde myocardial perfusion began, using Krebs-Henseleit nutrient solution with the following composition, in mmol/l: 115 NaCl; 5.4 KCl; 1.2 MgSO4; 1.25 CaCl2; 1.15 NaH2PO4; 25 NaHCO3; 11 glucose. To this solution we added 10UI/l of insulin and mannitol, at a concentration of 8 mmol/l, to ensure greater preservation of the myocardium[25]. The hearts were removed from the compages thoracis and placed in a device to study the isolated heart, size 3 type 830 (Hugo Sacks Electronic-Germany), with a constant perfusion pressure of 75 mmHg. The nutrient solution was continuously oxygenated with a gaseous mixture of 95% of oxygen and 5% of CO_2, in that oxygen partial pressure was maintained between 500-600 mmHg, temperature was maintained at 37°C and the pH was maintained between 7.3 and 7.4. The left atrium was cut open and the apex of the left ventricle was punctured with a needle to drain the ventricular cavity thus preventing the collection of fluid in its interior.

A latex balloon attached to a PE 90 polyethylene tube was placed in the ventricular cavity. The other end of the polyethylene tube was connected to a three-way tap, in that one of the outlets was coupled to a pressure transducer (Stathan P23 XL) and the other to a 1 ml-syringe, which allowed the variation of the volume of the intracavity balloon. The muscles of the right atrium, comprising the sinoatrial node, were extirpated and the electrode of an artificial pacemaker was placed in the myocardium of the right ventricle to artificially maintain the heart frequency between 200-250 bpm. By means of the preparation described above, Starling curves were obtained with the infusion of liquid into the balloon which allowed us to vary the diastolic pressure of the

left ventricle from 0 to 25 mmHg, in gradual 5-mmHg increments, and record the systolic pressure associated with each variation in volume. In this preparation, in which the heart operates in isochoric fashion, we recorded the systolic pressure (SP), and the maxima positive derivative (dp/dt) and negative derivative (-dp/dt) [26].

Morphometric study

The sixth stage of the protocol comprised the *in vitro* morphometric assessment of the hearts. After the *in vitro* functional study, the hearts of the same animals (C, n = 13 and F, n = 11) were removed, dissected, and the right and left ventricle, including the interventricular septum, were separated and weighed. The wet weight of the LV and of the RV, adjusted for the final body weight of the rat (BW), was used as an index of ventricular hypertrophy. The water content of the lungs was evaluated according to the ratio between the wet weight and the dry weight of tissues.

Samples of heart tissue were fixed with a 10% formol solution for a period of 48 hours, according to a method previously described [27]. After fixation, the tissue was embedded into paraffin blocks. Four-micron coronal histological sections were obtained. The histological sections were stained on slides with Hematoxylin – Eosin (HE) solution or Masson's stain to check the cross-sectional areas of myocytes with a LEICA DM LS microscope coupled with a video camera that sends digital images to a computer equipped with Image Pro-plus, an image analysis program (Media Cybernetics, Silver Spring, Maryland, EUA). We measured between fifty and seventy cells per ventricle analyzed. The selected myocytes were cross-sectioned, and had a round shape, the nucleus was visible in the center of the cell. The myocytes were located in the subendocardial layer of the muscle wall of the LV. This was done this way to ensure the greatest possible uniformity among the set of myocytes from different groups. The average cross-sectional areas obtained in each group were used as an indicator of the size of cells.

Slides with 6-micron coronal sections, stained with the Picro-Sirius red technique, specific for visualizing collagen, were made to assess the interstitium of myocardium of the LV. The reading was done with a LEICA DM LS microscope coupled with a video camera that sends digital images to a computer equipped with Image Pro-plus, an image analysis program (Media Cybernetics, Silver Spring, Maryland, EUA). Between thirty to forty fields per ventricle were analyzed, using a 40X objective. The fields chosen were located far from the infarcted area and from the perivascular area.

Statistical analysis

The comparisons between the C and F groups were carried out using Student's *t* test, for normal distribution data. For non-normal distribution data, the comparisons between the groups were carried out using the Mann-Whitney U test. The data were expressed as mean ± standard deviation (for normal distribution) or median with the 25th and 75th percentiles (for non-normal distribution). The results were considered statistically significant if p < 0.05. The statistical analyses were carried out with the SigmaStat for Windows v2.03 program (SPSS Inc, Chicago, IL, EUA).

RESULTS

During the observation period, the difference in mortality in the groups was not statistically significant (C = 58%, F = 50%; P = 0.67).
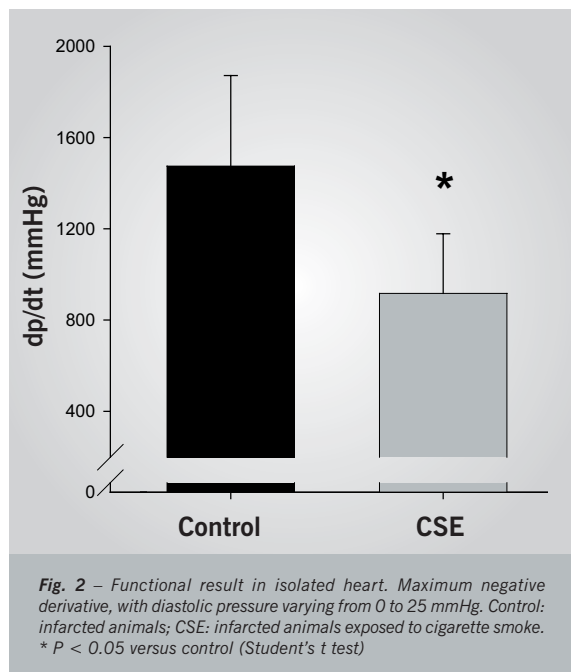
The results of the echocardiographic study are shown in table. The heart frequency was statistically higher in animals exposed to cigarette smoke (C = 248 ± 31 bpm, F = 302 ± 39 bpm; p = 0.001). This group tended to present larger LV diastolic areas (C = 1.5 ± 0.4 mm², F = 1.9 ± 0.4 mm²; p = 0.08) and systolic areas (C = 1.05 ± 0.3 mm², F = 1.32 ± 0.4 mm²; p = 0.08) as compared to control animals. The systolic function of LV, evaluated according to the fractional area change, also tended to be lower in animals exposed to cigarette smoke (C = 31.9 ± 9.3%, F = 25.5 ± 7.6%; p = 0.08). Considering the other morphometric and functional variables, no difference between the two groups was observed.

Table 1 - Echocardiographic study

Variables	Control (n = 13)	CSE (n = 11)	p
HF (bpm)	248 ± 31	302 ± 39	0.001
LA (mm)	6.6 ± 1.7	6.8 ± 1.4	0.69
LA/BW (mm/g)	12.2 ± 3.6	14.3 ± 3.2	0.16
LVDd (mm)	11.1 ± 1.1	11.1 ± 1.2	0.94
LVDd/BW (mm/g)	20.5 ± 3.4	23.4 ± 5.3	0.12
DTPW (mm)	1.4 ± 0.2	1.3 ± 0.3	0.55
DT/LVDd	0.13 ± 0.03	0.12 ± 0.03	0.69
E/A	2.7 ± 2.5	4.5 ± 2.7	0.15
DA (mm²)	1.57 ± 0.4	1.90 ± 0.4	0.08
SA (mm²)	1.05 ± 0.3	1. 32 ± 0.3	0.08
FAC (%)	31.9 ± 9.3	25.5 ± 7.5	0.08

Control: infarcted animals; CSE: infarcted animals exposed to cigarette smoke; BW: rat's body weight; LA: diameter of left atrium; LVDd: diastolic diameter of left ventricle; DTPW: diastolic thickness of the posterior wall; DT/LVDd: ratio between diastolic thickness and diastolic diameter of the left ventricle; E/A: ratio between the E and A-waves evaluated in transmitral flow; DA: diastolic area; SA: systolic area; FAC: fractional area change. The data were expressed as mean ± standard deviation

As regards the results of the study of isolated hearts, exposure to cigarette smoke did not change SP values (C = 113 ± 19 mmHg, F = 111 ± 30 mmHg; p = 0.89) and the maximum dp/dt values (C =1887 ± 367 mmHg/s, F = 2177 ± 1017 mmHg/s; p = 0.56) as compared to control animals. However, the - dp/dt values for animals exposed to cigarette smoke were statistically lower (C = 1474 ± 397 mmHg, F = 916 ± 261 mmHg; p = 0.02) than the values found for control animals (fig. 2).

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Fig. 2 – *Functional result in isolated heart. Maximum negative derivative, with diastolic pressure varying from 0 to 25 mmHg. Control: infarcted animals; CSE: infarcted animals exposed to cigarette smoke. * P < 0.05 versus control (Student's t test)*

The results of the morphometric study are shown in Table 2. Exposure to cigarette smoke was associated to higher RV weight, adjusted for body weight (C = 0.8 ± 0.3 mg/g, F = 1.3 ± 0.4 mg/g; p = 0.01), higher content of water in the lungs (C = 4.8 (4.3-4.8)%, F = 5.4 (5.1-5.5); p = 0.03) and larger myocyte cross-sectional area in the LV (C = 239.8 ± 5.8 μm^2, F = 253.9 ± 7.9 μm^2; p = 0.01). We did not observe other differences with respect to the other variables analyzed (p > 0.05).

Table 2 – Morphometric data

Variables	Control (n = 13)	CSE (n = 11)	p
BW (g)	548 ± 68	487 ± 74	0.05
LV/BW (mg/g)	2.9 ± 0.9	2.8 ± 0.5	0.86
RV/BW (mg/g)	0.81 ± 0.3	1.31± 0.4	0.01
W/D Lungs	4.8 (4.3-4.8)	5.1 (5.1-5.4)	0.03
MA (μm^2)	239.8 ± 5.8	253.9 ± 7.9	0.01
IC (%)	4.05 ± 0.4	3.4 ± 0.8	0.29
% AMI	46.3 ± 4.1	47.7 ± 4.9	0.45

Control: infarcted animals; CSE: infarcted animals exposed to cigarette smoke; BW: rat's body weight; LV: weight of left ventricle; RV: weight of right ventricle; W/D: ratio between wet and dry weight; MA: myocyte cross-sectional area; IC: interstitial collagen fraction. % AMI: size of infarction. The data were expressed as mean ± standard deviation (for normal distribution) or median with 25th and 75th percentiles (for non-normal distribution)

DISCUSSION

The objective of the study was to evaluate the effects of exposure to cigarette smoke on the process of ventricular remodeling after AMI in rats. Our results indicate that chronic exposure to smoke results in changes in the function and morphology of the heart. Therefore the habit of smoking after an infarction could result in the intensification of the process of ventricular remodeling.

The effects of smoking on the heart have been studied

in recent years, with somewhat inconsistent results. In an experimental study, it was verified that chronic exposure to carbon monoxide, a major component found in the vapor phase of cigarette smoke, brought about an increase in the genetic expression of endothelin 1 and induced heart hypertrophy[11]. Houdi et al. exposed rats to cigarette smoke for four days and verified an increase in blood pressure and a reduction in cardiac output. This effect was mitigated by a vasopressin antagonist[12]. In spontaneously hypertensive rats, exposure to smoke for eight weeks resulted in increased blood pressure and decreased heart frequency as compared to control groups[14]. Other authors observed that exposure to cigarette smoke for six months resulted in the enhanced expression of messenger RNA for endothelin 1 in the heart tissue of rats[15].

In a previous paper, we showed that exposure to cigarette smoke for thirty days was enough to impair the function of the LV[22]. Likewise exposure to cigarette smoke for four months resulted in ventricular dilatation, with a reduction in the systolic function. Interestingly enough these changes were accompanied by a slight increase in blood pressure[28]. Therefore, although the mechanisms have not yet been sufficiently explained, the set of evidences presented suggests that the activation of neurohormonal factors could play at least a partial role in the mechanisms associated with the effects of tobacco. Brooks et al., however, used the model to evaluate contractility in isolated papillary muscle preparations and found no differences in the heart function of rats exposed to smoke for 180 days as compared with control animals[13]. In the dog model, the administration of nicotine and the exposure to cigarette smoke for 22 months did not change the ejection fraction or the end-diastolic pressure of the left ventricle, as compared with the controls[16].

Some clinical studies also analyzed the effects of smoking on the heart. Therefore, in patients with coronary artery disease, the acute inhalation of cigarette smoke was accompanied by changes in the diastolic function[17,18]. In the CARDIA observational study, smokers presented greater left ventricular mass as compared to nonsmokers on echocardiogram assessment[19]. Therefore, despite some controversy the data set suggests that exposure to cigarette smoke may result in changes in the heart and its function. However, the effects of chronic exposure to cigarette smoke on the process of remodeling after AMI remain unknown.

One of the most important findings of this paper was that exposure to cigarette smoke caused morphological changes in the left ventricle. This phenomenon was characterized by an increase in the cross-sectional area of the myocytes, indicative of heart hypertrophy. This change was associated with increased ventricular diameters, both diastolic and systolic, which suggests the intensification of the process of left ventricle remodeling with exposure to smoke. In agreement with our results, acute treatment with nicotine in rats with myocardial infarction resulted in ventricular cavity increase, with the thinning of the infarcted wall, which suggests that nicotine also had a

damaging effect on post-infarction remodeling[10].

A widely accepted concept is that heart remodeling invariably brings about a progressive decrease in ventricular function. Initially, as a result of cell growth, remodeling may contribute to maintain or restore heart function. In the long term, however, there are biochemical, genetic and structural changes which will result in progressive ventricular dysfunction[2-4]. In agreement with this concept, the process of remodeling in rats exposed to cigarette smoke was accompanied by a decrease in the variables that reflect the systolic function, the fractional area change, the diastolic fuction and the negative derivative of pressure alike. As a consequence of this dysfunction, "smoking" animals presented signs of pulmonary congestion and right ventricle hypertrophy.

In conclusion, our data set suggest that chronic cigarette smoke exposure intensifies ventricular remodeling and worsens post-infarction heart function.

No potential conflict of interest relevant to this article was reported.

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