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MECHANOKINETICS OF THE SPONTANEOUS CONTRACTIONS OF SMOOTH MUSCLES IN THE STOMACH AND LARGE INTESTINE OF RATS UNDER CHRONIC EFFECT OF TiO_2 NANOPARTICLES

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Background. Currently, nano-sized materials of titanium dioxide (TiO_2) have wide industrial uses, particularly in the food industry and pharmacology. Therefore, the problem of TiO_2 toxicity to living organisms in case of their chronic *in vivo* intake needs thorough investigation. As nanoparticles enter the internal environment of the body, they spread with the bloodstream to tissues and organs, where they partially accumulate. Thus studying the state of the pacemaker mechanisms of regulation of smooth muscle spontaneous contractions in the stomach and large intestine under chronic exposure to TiO_2 nanoparticles is relevant and interesting. The purpose of this research work was to study the spontaneous contractile activity of the gastric and large intestine smooth muscles of rats under chronic (for 6 months) intake of an aqueous suspension of TiO_2 nanoparticles.

Materials and Methods. Wistar rats were used in the experiments. Rats of the experimental group were daily intragastrically administered an aqueous suspension of TiO_2 nanoparticles for six months at a dose of 0.1 mg/kg. The study of the mechanokinetics of the contraction-relaxation process of muscle preparations was carried out according to the method (Kosterin *et al.*, 2021) with the calculation of the mechanokinetic parameters of the contraction-relaxation cycle: force (F_{max}, F_C and F_R), time ($τ_0$, $τ_C$ and $τ_R$), impulse (I_{max}, I_C and I_R) and velocity (V_C and V_R).

Results. A comprehensive mechanokinetic analysis of spontaneous contractions of the *antrum* and *caecum* circular smooth muscles was carried out in control and under chronic *in vivo* exposure to TiO_2 nanocolloids (0.1 mg/kg/day) for 6 months.

It was found that the chronic action of TiO_2 nanocolloids significantly inhibits the contractile activity of the antrum smooth muscles accompanied by a decrease in all mechanokinetic parameters of time, force, velocity and impulse.

Under the same conditions, inhibition of the spontaneous contractions of the large intestine smooth muscles was observed. However, the time (τ_0, τ_C and τ_R) and impulse (I_{max}, I_C and I_R) parameters increased for these muscles against the background of the decrease in the force and velocity mechanokinetic parameters.

Conclusions. Modulation of the mechanokinetic parameters of the spontaneous contractile activity of the stomach and large intestine smooth muscles of rats under chronic intragastric administration of TiO_2 nanocolloids suggests that the functioning of pacemakers changes significantly under these conditions. Since the parameters of the contraction and relaxation phases of both the stomach and the large intestine smooth muscles do not differ by the magnitude of the inhibitory effects, it can be assumed that the effects of TiO_2 are not specific for particular Ca^{2+} transport systems involved in contractile responses.

***Keywords**:* smooth muscles of the stomach and large intestine, spontaneous contractions, chronic action of TiO_2 nanocolloids, mechanokinetic analysis

INTRODUCTION

The regulation of tone and contractile activity of visceral smooth muscles (SMs) is controlled by the autonomic nervous system with the participation of neurotransmitters. At the same time, even in the absence of neurotransmitters' and hormones' action, spontaneous contractile activity is registered in the digestive tract wall SMs based on the mechanisms of the pacemaker activity of the interstitial cells of Cajal (ICC). ICC cells initiate slow waves of depolarization in the digestive tract SMs, and mediate the enteric neurons effects on them. ICCs are classified into several cell populations and differ in morphology, location, and pacemaker activity parameters; they form gap junctions with myocytes (Foong *et al.*, 2020; Cretoiu, 2022; Tobias & Sadiq, 2022). In particular, two types of ICCs (ICC-IM and ICC-MY) have been identified in the SMs of the antral part of rat stomach (Wang *et al.*, 2005; Powley *et al.*, 2012). The stellate-shaped ICCs of the myenteric plexus (ICC-MY) are located next to the myenteric ganglia between the smooth muscle layers; they generate pacemaking potentials that produce high-amplitude, low-frequency contractile responses (Kito *et al.*, 2009). Bipolar ICCs, located inside the muscle layers (ICC-IM), are the main rhythm drivers in the stomach tissue, providing regular high-frequency, low-amplitude contractions (Kito *et al.*, 2009; Sanders, 2019). In the case of pathologies involving the violation of the gastrointestinal tract SM contractile function, significant changes in the number of ICC networks and, accordingly, their contribution to the overall pacemaking activity are observed. In the case of gastric motility disorders (gastropathy and gastroparesis), which develop in nearly half of patients with diabetes, the loss of ICCs in the gastric wall SMs has been proven by histochemical methods. Similar changes have also been detected in animal models of diabetes (den Braber-Ymker *et al.*, 2021; Huizinga *et al.*, 2021; Sukhotnik *et al.*, 2021; Bianco *et al.*, 2022; Biemans *et al.*, 2022).

Modern trends in the development of industrial production technologies involve, in particular, the creation of materials with fundamentally new characteristics. Nanomaterials (nanoparticles, nanotubes, quantum dots, etc.) are among the most industrially widespread modern materials (David *et al.*, 2022; Kozinetz *et al.*, 2022). Currently, a large number of technological processes use nano-sized materials of the titanium dioxide (TiO_2) amphoteric compound. In recent years, the world market of this material is estimated at approximately ten million tons per year, of which a large sector is occupied by the food, medical and pharmaceutical industries (Irshad *et al.*, 2021; Mansoor *et al.*, 2022). Thus, TiO_2 is widely used in food coloring, cosmetic and hygiene products, nanomedical reagents, and pharmaceutical products (titanium dioxide food additive is marked with E171 code). However, 36% of dietary TiO_2 size is <100 nm, which makes this xenobiotic easily permeable through histogematic barriers in human and animal organisms, especially in the case of the gastrointestinal tract walls (Hou *et al.*, 2019; Lama *et al.*, 2020). Although a significant amount of data on the interaction of TiO_2 nanoparticles with cells has been collected, the question of TiO_2 toxicity to living organisms remains open. The problem is further complicated by the high activity of TiO_2 towards other chemical substances, as well as the interaction with various physical factors due to its photocatalytic properties. Recent research has shown that such active groups of TiO_2 nanoparticles can interact with thiol, carboxyl, amino groups and groups of side chains of amino acids, as well as with peptide bonds of protein macromolecules, in particular, with receptors on the plasma membranes of cells, and nucleic acids (Rashid *et al.*, 2021; Malakootian *et al.*, 2021; Fei *et al.*, 2022).

When entering the body, nano-sized xenobiotics, in particular TiO_2 nanoparticles, easily pass through the intestinal endothelial barrier and enter the systemic circulation bloodstream through the portal vein. Nanoparticles, in particular nano-TiO_2, are transported by blood plasma proteins and can accumulate in tissues and organs (Disdier *et al.*, 2015; Naumenko *et al.*, 2016; Coméra *et al.*, 2020; Shamel *et al.*, 2021; He *et al.*, 2022).

Our previous studies showed that a long-term (for 1 and 3 months) intragastric intake of nano-TiO_2 by rats leads to its selective accumulation in tissues and organs, and is accompanied by changes in the motility of visceral SMs (Tsymbalyuk *et al.*, 2017; Tsymbalyuk *et al.*, 2019; Tsymbalyuk *et al.*, 2021; Tsymbalyuk & Kosterin, 2021; An *et al.*, 2022)

Since nanoparticles are known to enter the tissues of the digestive tract wall and partially accumulate there, studying the state of the pacemaker mechanisms of regulation of smooth muscle spontaneous contractions in the stomach and large intestine under chronic exposure to TiO_2 nanoparticles is relevant and interesting. Thus the aim of this research work was to study the spontaneous contractile activity of the gastric and large intestine SMs of rats under chronic (for 6 months) intake of an aqueous suspension of TiO_2 nanoparticles.

MATERIALS AND METHODS

Wistar rats with an average body weight of 200–250 g were used in the experiments. The rats were kept in standard vivarium conditions (at room temperature of 20 ± 2 °C, relative humidity of 50–70%, light-darkness cycle of 12:12 h, and free access to water and standard feed). All the manipulations with animals were conducted according to the International Convention for the Protection of Animals and the Law of Ukraine "On Protection of Animals from Cruelty" (the Minutes of the meeting of bioethics commission of SSC Institute of Biology and Medicine No. 3 dated May 2, 2019).

The studies of the action of TiO_2 nanoparticles lasted for six months, which corresponds to 10 years of human life (Andreollo *et al*., 2012). The animals were randomly divided into two groups: the control group – intact rats (7 individuals), and experimental rats that were daily intragastrically administered an aqueous suspension of TiO_2 nanoparticles for six months at a dose of 0.1 mg/kg (7 individuals). To ensure the correct calculation of the amount of TiO_2 nanomaterial, the weight of the animals was monitored weekly. The rats were euthanized by dislocation of the cervical vertebrae under ether anesthesia.

TiO_2 nanoparticles (PlasmaChem Berlin, Germany) were used in the form of nanopowder (a mixture of rutile and anatase), specific surface – 50 ± 10 m^2/g; purity >99.5%, Al_2O_3 content <0.3% by mass; SiO_2 <0.2% by mass. The nanopowder was resuspended in water; for the destruction of aggregates in the TiO_2 NP suspension, it was exposed to ultrasonic treatment.

Tensometric studies. After removing the stomach and *cecum*, they were cut along the lesser curvature and carefully washed in Krebs solution. In the case of the stomach, the muscles of its antral section were carefully cleaned from the mucous membrane and cut into 2×10 mm strips in the circular direction of the of muscle fiber orientation. In the case of the *caecum*, the muscles in its first third part near the ileocecal junction were carefully cleaned from the mucous tissue and cut into 2×10 mm strips in in the circular direction of the of muscle fiber orientation.

To store smooth muscle preparations in a functional state and study their contractile activity, the normal Krebs solution (mM): NaCl – 120.4; KCl – 5.9; $NaHCO_3$ – 15.5; NaH_2PO_4 – 1.2; $MgCl_2$ –1.2; $CaCl_2$ – 2.5; glucose – 11.5 (pH 7.4) was used.

To study spontaneous contractile activity, preparations of smooth muscles were placed in the working chamber (effective volume of 2.0 mL) of tensometric equipment with a flowing Krebs solution (flow rate – 5 mL/min). Contractile activity of SMs was recorded in an isometric mode using analog-to-digital converter.

Mechanokinetic analysis. The study of the mechanokinetics of spontaneous contractile activity of SM preparations was conducted using the method of multiparameter mechanokinetic analysis (Kosterin *et al.*, 2021; Tsymbalyuk *et al*., 2022). The analysis of the complete profile of single spontaneous contractions was based on their linearization in the coordinates where f and t are instant values of force and time at the level of the contraction cycle, while C and R indicate the contraction and relaxation phases, respectively). Further on, F_C and F_R will respectively indicate the value of the force at the inflection points of the mechanogram at the level of contraction phases (from the beginning of the increase in force to its maximum value F_{max}) and relaxation (from the maximum value of the force F_{max} at τ_0 time point till its return to the basal level), and Δt indicates the randomly chosen constant time interval.

Characteristic constants k and n were determined from the linearized graphs and further used to calculate the following parameters: time (τ_0, τ_C and τ_R), force (F_{max}, F_C and F_R), velocity (V_C and V_R) and impulse (I_{max}, I_C and I_R). Here, V_C and V_R refer to the maximum velocities of the contraction and relaxation phases, respectively, whereas I_{max}, I_C and I_R are the parameters of the force impulse at the level of the amplitude and maximum velocities of the contraction and relaxation phases, respectively.

Statistical analysis. The data were processed by variation statistics methods using the Origin 2018 software. The samples were checked in terms of belonging to normally distributed general populations according to the Shapiro–Wilk's test. An unpaired *t*-test was used to determine significant differences between mean values of the samples. The results were considered reliable if *p*-value was under 5% ($p < 0.05$). The validation analysis of data approximation by the linear function (linearization) was performed using Fisher's F-test; in all cases determination coefficients (R^2) were at least 0.96. The results are presented as the arithmetic mean ± standard error of the mean value, where *n* is the number of experiments.

RESULTS AND DISCUSSION

A comprehensive mechanokinetic analysis of the spontaneous contractile activity of the antrum and cecum of rats in the control group. The pacemaker activity of the SMs in the digestive tract walls is regulated by ICCs and modulated through neurotransmitters, paracrine regulators and hormones (Mañé & Jimenez, 2014; Foong *et al.*, 2020; Cretoiu, 2022; Tobias & Sadiq, 2022).

Firstly, we performed a comprehensive mechanokinetic analysis of individual spontaneous contractions of the gastrointestinal tract wall fragments – the *antrum* and *caecum* – of control rats (**Fig. 1*A*** and ***B***, respectively).

Individual contractions of the *antrum* in the control had an average amplitude of 10.1 ± 0.9 mN; the number of contractions of the preparations during 10 minutes was 22.3 ± 1.8 (n = 7). In the case of spontaneous contractions of the circular SMs of the *caecum*, the distribution of amplitudes was observed in the range from 0.9 mN to 12.6 mN (**Fig. 1*B***). The average frequency of SM contractions in the preparations of the *caecum* calculated in the control during 10 minutes was 45.3 ± 2.1 (n = 7).

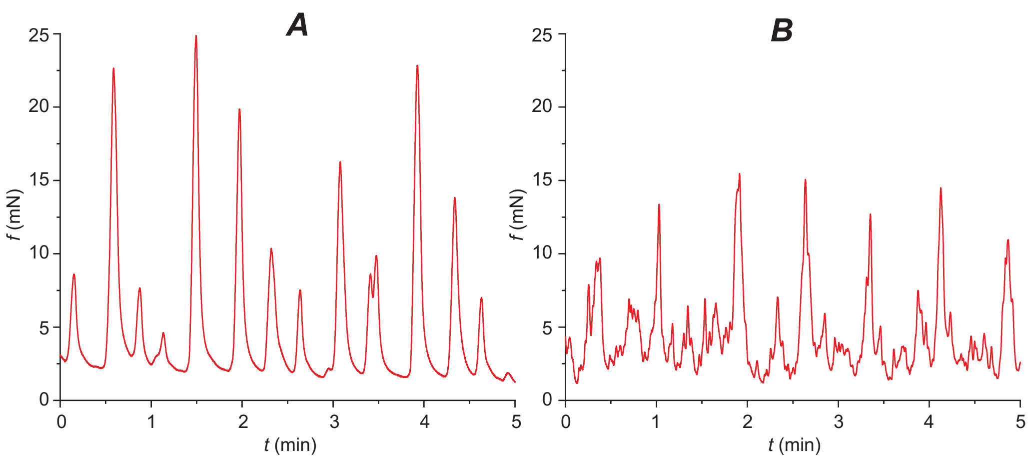

Fig. 1. Typical mechanograms of spontaneous contractions of the *antrum* (***A***) and *caecum* (***B***) circular smooth muscles of control rats

Using the method of multiparameter mechanokinetic analysis, we calculated the force (F_{max}, F_C and F_R), time (τ_0, τ_C and τ_R), impulse (I_{max}, I_C and I_R) and velocity parameters (V_C and V_R). These parameters have been systematized in **Table**.

Mechanokinetic parameters of spontaneous contraction-relaxation cycles of the circular smooth muscles of the *antrum* and *caecum* of rats (n = 7)

Mechanokinetic parameter	*antrum*	*caecum*
Time parameters:		
τ_0	10.69 ± 0.85	4.41 ± 0.39
τ_C	7.21 ± 0.74	2.35 ± 0.31
τ_R	14.16 ± 0.98	6.48 ± 0.51
Force parameters:		
F_{max}	10.08 ± 0.93	10.75 ± 0.74
F_C	5.55 ± 0.65	5.09 ± 0.46
F_R	6.68 ± 0.62	7.32 ± 0.48
Velocity parameters:		
V_C	2.31 ± 0.23	4.87 ± 0.49
V_R	1.46 ± 0.14	2.50 ± 0.26
Impulse parameters:		
I_{max}	105.8 ± 10.2	48.1 ± 6.6
I_C	37.1 ± 3.7	12.5 ± 2.5
I_R	93.3 ± 9.0	47.7 ±5.6

Notes: designations of mechanokinetic parameters: time parameters (τ_0 – time to reach maximum force, τ_C – time to reach maximum velocity at the contraction phase level, τ_R – time to reach the maximum velocity at the relaxation phase level); force parameters (F_{max} – maximum force, F_C – contraction force at the inflection point τ_C, F_R – relaxation force at the inflection point τ_R); velocity parameters (V_C – maximum velocity at the level of the contraction phase, V_R – maximum velocity at the level of the relaxation phase), impulse parameters (I_{max} – force impulse at the point of maximum force, I_C – force impulse at the inflection point at the contraction phase level τ_C, I_R – force impulse at the inflection point at the relaxation phase level phase τ_R)

Mechanokinetic characteristics of spontaneous contractions of circular gastric smooth muscles of rats under chronic action of TiO_2 nanocolloid. At the next stage, we investigated and analyzed the spontaneous contractile activity of isolated circular SMs of the *antrum* of rats daily exposed to an action of an aqueous suspension of TiO_2 nanoparticles (a mixture of rutile:anatase polymorphs = 1:4, average particle size 21 ± 5 nm) at a dose of 0.1 mg/kg of body weight for 6 months.

As mentioned above, the food additive E171 based on titanium dioxide – a white dye widely used in the food and pharmaceutical indusrties – contains about a third of nano-sized material with a particle size of <100 nm, which can easily penetrate the barrier of the gastrointestinal tract walls. For a model study of the chronic intake of nano-TiO_2 into the body, we chose a daily dose of 0.1 mg/kg of body weight taking into consideration the fact that this dose of TiO_2 nanoparticles is usual for the human body and corresponds to the average daily consumption of this nanomaterial in Great Britain with food products (Weir *et al*., 2012).

It was found that the chronic intake of TiO_2 nanomaterial causes significant changes in the mechanokinetics of spontaneous contractions of circular smooth muscles of the *antrum* in rats (**Fig. 2*A***). Thus, under the action of this xenobiotic, we observed a significant inhibition of gastric motility, which at the level of general characteristics of amplitude and frequency constituted: F_{max} – 9.8% on average (n = 7, p <0.001), the number of contractions in 10 min – 78.2% on average (n = 7, p <0.05) relative to the corresponding control indicators taken as 100%.

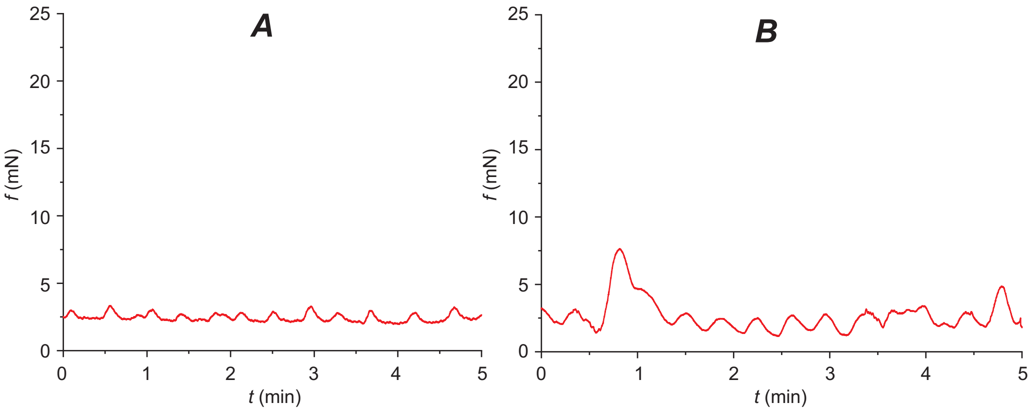

Fig. 2. Typical mechanograms of spontaneous contractions of the *antrum* (***A***) and *caecum* (***B***) circular smooth muscles of rats under chronic exposure (for 6 months) to an aqueous suspension of TiO_2 nanoparticles (0.1 mg/kg)

A comprehensive mechanokinetic analysis of individual spontaneous contractions revealed that under the chronic action of nano-TiO_2 the time parameters (τ_0, τ_C and τ_R) significantly decreased and averaged: 47.6% (n = 7, p <0.01), 26.0% (n = 7, p <0.001) and 58.6% (in all cases n = 7, p <0.01) relative to the corresponding control indicators taken as 100% (**Fig. 3*A***).

Besides, under these conditions, the force indicators, at which the inflection of the contraction and relaxation phases was observed, decreased significantly: the F_C indicator was on average 7.0% compared to the control and F_R was on average 10.5% compared to the control (in both cases n = 7, p <0.001) (**Fig. 3*B***).

Under chronic exposure to nano-TiO_2, the parameters of velocity at the inflection points of the contraction and relaxation phases (V_C and V_R, respectively) also significantly decreased (**Fig. 3*C***). Thus, in the experimental group, the maximum velocity V_C constituted on average 14.6% relative to the control (n = 7, p <0.001); in the case of the relaxation phase, the maximum velocity V_R decreased even more significantly (on average to 9.1% relative to the control, n = 7, p <0.001).

Another group of mechanokinetic parameters that were subject to the greatest suppression under the chronic exposure to nano-TiO_2, are impulse parameters (I_{max}, I_C, and I_R): they decreased by more than ten times compared to the control and averaged 4.78%, 2.06%, and 6.18% (n = 7, p <0.001) (**Fig. 3*D***).

Since the formulas used for calculation of the velocity parameters do not take into account the impacts of the change in force components, we calculated amplitude-independent normalized maximum velocities of the contraction and relaxation phases (Vn_C and Vn_R) (Burdyga & Kosterin, 1991; Tsymbalyuk & Vadzyuk, 2020). As in the case of V_C and V_R, the normalized Vn_C and Vn_R indicators significantly decreased: the Vn_C parameter was on average 29.6% and Vn_R – on average 17.1% relative to the corresponding control parameters (in both cases n = 7, p <0.001).

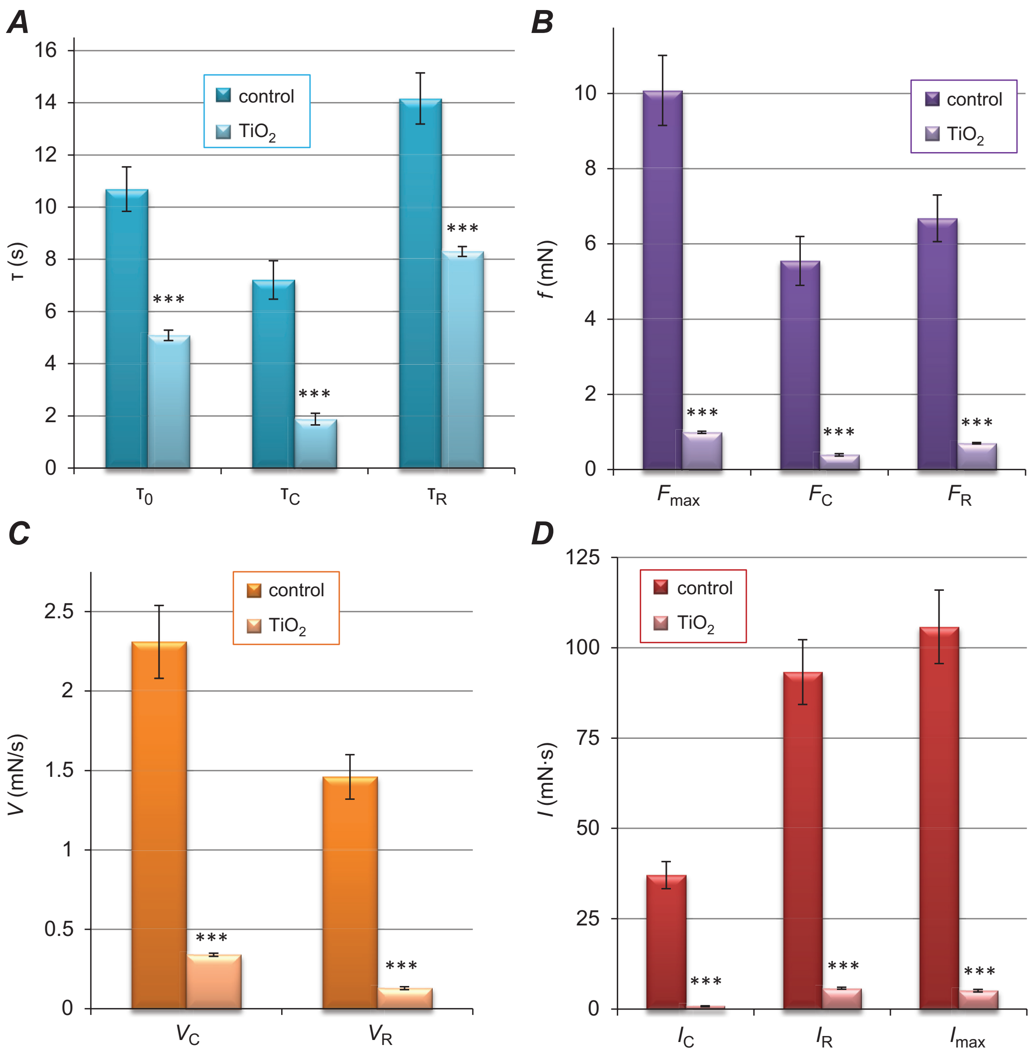

Fig. 3 Mechanokinetic parameters of spontaneous contraction-relaxation cycles of the circular smooth muscles of the *antrum* of rats in the control group and under chronic exposure (for 6 months) to an aqueous suspension of TiO_2 nanoparticles (0.1 mg/kg) (n = 7, M ± m): ***A*** – time parameters (τ_0, τ_C and τ_R); ***B*** – force parameters (F_{max}, F_C and F_R); ***C*** – velocity parameters (V_C i V_R); ***D*** – impulse parameters (I_{max}, I_C and I_R). *** – p<0.001 – significant difference compated with the control

Thus, the chronic intake of TiO_2 nanomaterial is accompanied by a significant inhibition of the contractile function of the gastric SMs. Since the parameters of the contraction and relaxation phases do not differ by the magnitude of the inhibitory effects, it can be assumed that TiO_2 effects are not specific for particular Ca^{2+} systems that participate in the generation of contractile responses (Taggart *et al*., 1997; Karakhim *et al*., 2021).

Mechanokinetic characteristics of spontaneous contractions of circular smooth muscles of the cecum of rats under chronic action of TiO_2 nanocolloid. The spontaneous contractile activity of isolated circular SM strips of the *caecum* of rats under chronic exposure to TiO_2 nanoparticles was also analyzed. It was found that chronic intake of TiO_2 nanomaterial causes significant changes in the mechanokinetics of spontaneous contractions of the circular SMs of the *caecum* of rats (**Fig. 2*B***). Under these conditions, cecal motility was inhibited at the level of F_{max} amplitude which decreased to 53.5% relative to the corresponding control values taken as 100% (n = 7, p <0.001).

Further, we conducted a comprehensive mechanokinetic analysis of individual spontaneous contractions. It was found that, contrary to the force parameters, the time parameters (τ_0, τ_C and τ_R) significantly increase under chronic exposure to nano-TiO_2, and on average constitute 286.8% (n = 7), 244.7% (n = 7), and 315.4% (n = 7) relative to the corresponding control values taken as 100% (in all cases n = 7, p <0.001) (**Fig. 4*A***).

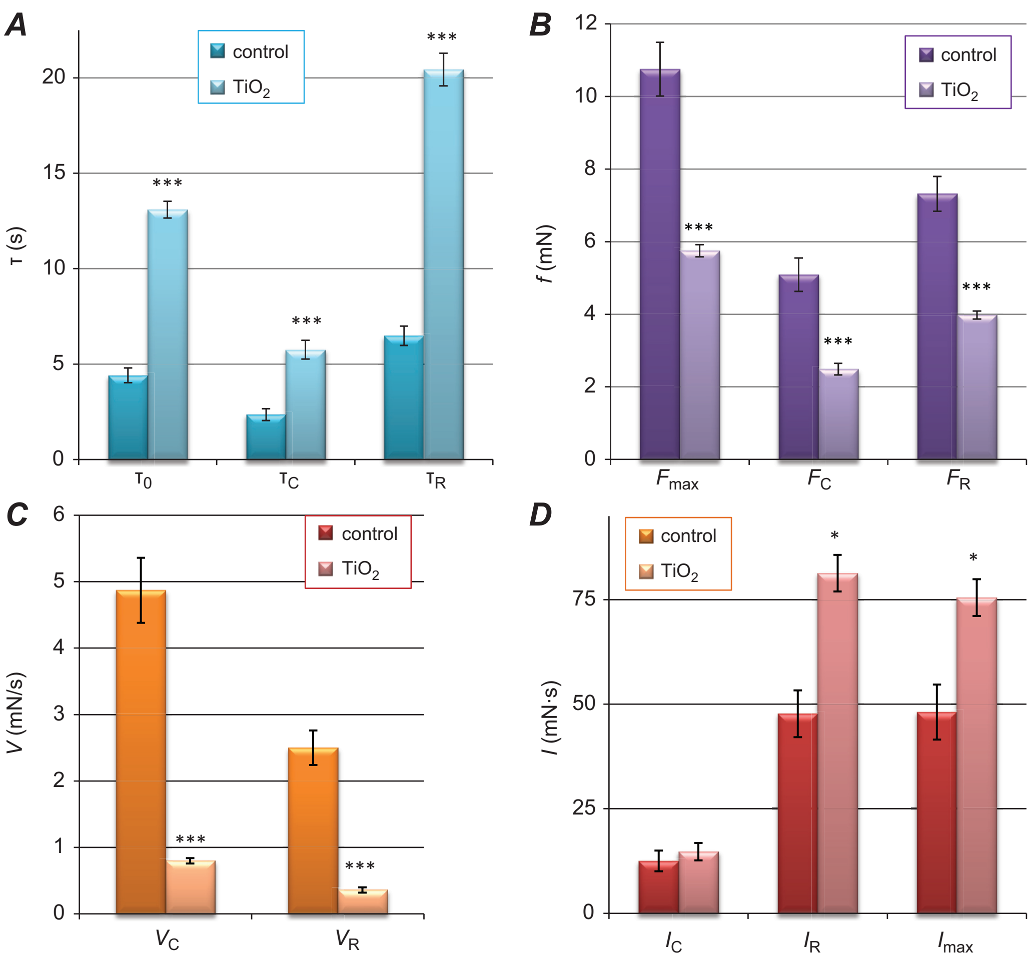

Fig. 4. Mechanokinetic parameters of spontaneous contraction-relaxation cycles of the circular smooth muscles of the *caecum* of rats in the control group and under chronic exposure (for 6 months) to an aqueous suspension of TiO_2 nanoparticles (0.1 mg/kg) (n = 7, M ± m): ***A*** – time parameters (τ_0, τ_C and τ_R); ***B*** – force parameters (F_{max}, F_C and F_R); ***C*** – velocity parameters (V_C and V_R); ***D*** – impulse parameters (I_{max}, I_C and I_R). * – p <0.05 and *** – p <0.001 – compated with the control

Besides, under the chronic action of nano-TiO_2, a significant decrease in force indicators corresponding to the inflection points in the contraction and relaxation phases was observed: the value of the F_C indicator was on average 48.9% compared to the control and that of F_R averaged 54.4% compared to the control (in both cases n = 7, p <0.001) (**Fig. 4*B***).

The study also revealed that under the chronic action of nano-TiO_2, the velocity parameters at the inflection points of the contraction and relaxation phases (V_C and V_R, respectively) significantly decrease (**Fig. 4*C***). Thus, the V_C value averaged 16.4% compared to the control (n = 7, p <0.001) while the V_R value averaged 14.4% compared to the control, n = 7, p <0.001). However, under these conditions, a significant increase in impulse parameters was observed. Thus, I_{max}, I_C and I_R values were on average 156.9%, 117.8% and 170.4% relative to the control (n = 7, p <0.05) (**Fig. 4*D***).

Thus, the conducted research showed fundamental differences in the numerical values of the mechanokinetic parameters of spontaneous contractions of the SMs in different parts of the gastrointestinal tract (stomach and large intestine) in the control and under the chronic action of TiO_2.

We can presume which cellular mechanisms underlie the detected effects of nano-TiO_2 on spontaneous contractions of the gastrointestinal tract. ICCs generate spontaneous slow waves of depolarization, at the peak of which action potentials occur that induce spontaneous contractions of this tissue. The frequency of slow waves determines the frequency of spontaneous contractions, while their amplitude correlates with the number of Ca^{2+} ions entering the cytoplasm of working myocytes during depolarization (Ward *et al.*, 2003; Hirst & Edwards, 2006; Sanders *et al.*, 2006; Sanders, 2019). The initial component of depolarization during the generation of a spontaneous wave is due to the release of Ca^{2+} ions through inositol-1,4,5-triphosphate-sensitive calcium channels of the sarcoplasmic reticulum, and these ions are then captured by mitochondria. In general, slow waves of depolarization are coordinated by a set of interconnected processes: an activation of potential-controlled L-type Ca^{2+} channels, and the release of Ca^{2+} ions from the sarcoplasmic reticulum and their sequestration in mitochondria (Ward *et al*., 2003; Sanders *et al*., 2006; Sanders, 2019). The process of mobilization of Ca^{2+} ions from the sarcoplasmic reticulum and their redistribution in mitochondria determines the frequency of spontaneous activity. The amplitude of spontaneous contractions is determined by Ca^{2+}-dependent (and partially Ca^{2+}-independent) processes in the working smooth muscle cells (Ward *et al*., 2003; Sanders, 2019).

Therefore, we have every reason to assume that TiO_2 nanoparticles, after penetrating the cells of the gastrointestinal tract smooth muscle wall, firstly, change the functioning of the pacemaker cells of Cajal, and, secondly, inhibit the processes of entry of Ca^{2+} ions and their sequestration in the mitochondria of working myocytes. Eventually, it can be presumed that chronic exposure to TiO_2 may cause the disruption of the coordination between pacemaker and working myocytes in the smooth muscle tissue.

CONCLUSIONS

In the study, a comprehensive mechanokinetic analysis of spontaneous contractions of circular smooth muscles of the antral part of the stomach and caecum was performed in control and under a chronic *in vivo* administration of a suspension of TiO_2 nanoparticles (0.1 mg/kg/day) for 6 months.

It was found that under the chronic nano-TiO_2 action, occurs a significant suppression of the contractile function of the smooth muscles of the *antrum*, which is accompanied by a decrease in all time, force, velocity, and impulse mechanokinetic parameters. Since the parameters of the contraction and relaxation phases of the stomach do not differ by the magnitude of the inhibitory effects, it is possible that the effects of TiO_2 are not specific for particular Ca^{2+} systems that participate in the generation of contractile responses.

The study revealed that the inhibition of spontaneous contractions of the large intestine of rats is also observed under the chronic *in vivo* action of nano-TiO_2. However, under these conditions, there is an increase in time (τ_0, τ_C and τ_R) and impulse (I_{max}, I_C and I_R) parameters against the background of a decrease in force (F_{max}, F_C and F_R) and velocity (V_C and V_R) mechanokinetic parameters. It can be assumed that upon penetrating the tissue of the large intestine, TiO_2 nanoparticles change the functioning of the pacemaker cells of Cajal, which is due to the suppression of the entry of Ca^{2+} ions and their sequestration in the mitochondria of myocytes.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: All international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [T.O.V.]; methodology, [T.O.V.; D.T.L.; V.I.S.; N.A.M.; S.V.A.]; investigation, [T.O.V.; G.L.A.; D.T.L.; V.I.S.; N.A.M.]; data analysis, [T.O.V.; G.L.A.; V.I.S.; S.O.V.]; writing – original draft preparation, [T.O.V.; D.T.L.; V.I.S.]; writing – review and editing, [T.O.V.; G.L.A.; D.T.L.; S.Kh.V.; K.M.S.]; visualization, [T.O.V.; G.L.A.; D.T.L.; S.Kh.V.; K.M.S.]; supervision, [T.O.V.; S.V.A.]; project administration, [T.O.V.; D.T.L.; V.I.S.; N.A.M.; S.V.A.]; funding acquisition, [-].

All authors have read and agreed to the published version of the manuscript.

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МЕХАНОКІНЕТИКА СПОНТАННИХ СКОРОЧЕНЬ ГЛАДЕНЬКИХ М’ЯЗІВ ШЛУНКУ І ТОВСТОГО УИШЕЧНИКА ЩУРІВ ЗА ХРОНІЧНОГО ВПЛИВУ НАНОЧАСТИНОК TiO_2

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Обґрунтування. Наразі нанорозмірні матеріали діоксиду титану (TiO_2) надзвичайно широко використовують у різних галузях промислового виробництва, зокрема, у харчовій промисловості та виробництві лікарських засобів. Відкритою до з’ясування залишається проблема токсичності TiO_2 для живих організмів у разі їхнього хронічного надходження *in vivo*. Відомо, що наночастинки потрапляють у внутрішнє середовище організму, розповсюджуються з кровотоком у тканини й органи і там частково накопичуються, тому є актуальним та цікавим дослідити стан пейсмекерних механізмів регуляції спонтанних скорочень гладеньком’язової тканини шлунку і товстого кишечника за умов хронічного надходження в організм нано-TiO_2. Метою роботи було дослідження спонтанної скорочувальної активності гладеньких м’язів шлунку і товстого кишечника щурів за хронічного (упродовж 6 місяців) надходження в організм водної суспензії наночастинок TiO_2.

Матеріали та методи. Експерименти проводили на щурах лінії Wistar. Експериментальній групі тварин щодня впродовж 6 місяців перорально вводили суспензію наночастинок TiO_2 із розрахунку 0,1 мг/кг. Дослідження механокінетики спонтанних процесів скорочення та розслаблення препаратів гладеньких м’язів здійснювали за методом Костеріна (Kosterin *et al.,* 2021) з розрахунком механокінетичних параметрів поодиноких циклів скорочення-розслаблення: силових (F_{max}, F_C та F_R), часових (τ_0, τ_C і τ_R), імпульсних (I_{max}, I_C та I_R), а також швидкісних (V_C та V_R).

Результати. У роботі здійснено повний механокінетичний аналіз спонтанних скорочень кільцевих гладеньких м'язів антрального відділу шлунку (*antrum*) і сліпої кишки (*caecum*) у контролі та за хронічної дії *in vivo* упродовж 6 місяців суспензії наночастинок TiO_2 (0,1 мг/кг/добу). Встановлено, що хронічне надходження в організм нано-TiO_2 спричиняє суттєве пригнічення скорочувальної функції гладеньких м'язів *antrum*, яке супроводжується зниженням усіх часових, силових, швидкісних та імпульсних механокінетичних параметрів. За тих самих умов також спостерігали інгібування спонтанних скорочень товстого кишечника щурів. Втім, для цих м'язів мало місце підвищення часових (τ_0, τ_C і τ_R) та імпульсних (I_{max}, I_C та I_R) параметрів на тлі зниження силових (F_{max}, F_C та F_R) і швидкісних (V_C і V_R) та механокінетичних параметрів.

Висновки. Модуляція механокінетичних параметрів спонтанної скорочувальної активності гладеньких м'язів шлунку і товстого кишечника щурів за хронічного перорального надходження суспензії наноматеріалу TiO_2 дає змогу зробити висновок про суттєву зміну функціонування пейсмекерів за таких умов. Оскільки за величиною інгібіторних ефектів параметри фаз скорочення і розслаблення як шлунку, так і товстого кишечника не відрізняються, ймовірно, ефекти TiO_2 не є специфічними для окремих Ca^{2+}-транспортувальних систем, які беруть участь у розвитку скорочувальних відповідей.

Ключові слова: гладенькі м'язи шлунку і товстого кишечника, спонтанні скорочення, хронічна дія наноколоїду TiO_2, механокінетичний аналіз

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