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ORIGINAL ARTICLE

Changes in redox processes in the cells of test objects – bull spermatozoa under the effect of naphazoline nitrate

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ABSTRACT

Naphazoline nitrate is a medicine with a number of side effects, which should be taken into account not only in therapeutic practice, but also when developing preventive measures in pharmaceutical production. The purpose of our work was to study the effect of naphazoline nitrate on redox processes and the duration of survival of bull spermatozoa. Bull semen samples were divided into groups: control – without adding naphazoline nitrate and experimental – with adding naphazoline nitrate. The respiratory activity of spermatozoa was determined by the polarographic method, reducing capacity – potentiometrically, succinate dehydrogenase activity – photometrically; sperm survival – visually. The respiratory activity of spermatozoa is significantly inhibited under the effect of all studied doses of naphazoline nitrate ($\eta = 0.762$ and 0.840) and the degree of effect for the highest dose reaches 85.5 %. The reducing capacity changes significantly, starting from a dose of 1/10 LD50 and above, the dose-indicator correlation is low, the dose of LD50 causes changes in the indicator by 229.0% with a change of charge of the medium. SDH activity significantly decreases under the effect of 1/10 LD50 and above, the dose-indicator correlation is moderate, the dose of LD50 causes changes in the indicator by 49.5 %. The substance affects the duration of survival only in the maximum dose, reducing the survival time by 18 %. Conclusion: The substance has the maximum effect on the respiratory activity of germ cells and on the reducing capacity, it has a lesser effect on the SDH activity and slightly affects the survival time. The dose-dependent nature of the effect of naphazoline nitrate on certain indicators of redox processes in spermatozoa confirms the prospects of using the latter as alternative test objects in studying the harmful effects of chemicals.

KEY WORDS: naphazoline nitrate; bull spermatozoa; redox processes

Introduction

Naphazoline nitrate is a sympathomimetic vasoconstrictor that has been actively used in medical practice for more than fifty years (Mortuaire *et al.*, 2013; Kuzminov *et al.*, 2018). At the same time, the substance has a number of side effects, such as mucosal irritation, hypersensitivity reaction, allergic skin reactions, elevation in blood pressure, and decreased body temperature (Kotliar, 2000; Wenzel *et al.*, 2004). These effects should be taken into account not only in therapeutic intervention, but also in the development of preventive measures in pharmaceutical production (Sargent *et al.*, 2013; Reichard *et al.*, 2016). Thus, the toxicity parameters obtained in pre-clinical drug studies are used to calculate permissible limits of exposure to substances for pharmaceutical workers, for example, Maximum permissible concentration (Ukraine), Occupational exposure limit (European Union), or institutional regulations – temporary or internal hygiene regulations (Zazulyak, 2019). The maximum permissible concentration of naphazoline nitrate in the air of the work area (Ukraine) is 0.1 mg/m^3, the hazard class marked "A" (allergen) and "+" (requires special protection of the skin and eyes) (Ministry of health of Ukraine, 2020). Available information sources do not contain data on regulations limiting the occupational exposure to naphazoline nitrate implemented in other countries.

A humane and quite informative method for studying chemically-induced toxicity is the use of alternative test objects (Trakhtenberh *et al.*, 2008; Vahdati *et al.*, 2015). Based on the assumption that the effect of chemicals on

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a living organism occurs at the cellular level, cell cultures or isolated cells are used as test objects (Kempa *et al.*, 1990; Ostapiv *et al.*, 2014; Kopylchuk&Voloshchuk, 2015; Lysenko, 2017; Kharchenko *et al.*, 2018). Tests on live bovine germ cells have a fairly high degree of correlation with the data of classic toxicological experiments. At the same time, it is the course of redox processes in the cells of living organisms, the activity of enzymes involved in the resynthesis of adenosine triphosphoric acid that can be sensitive biochemical markers of their physiological state, which determined the aim of our research.

Materials and methods

Materials

Freshly obtained bull ejaculates (n = 8) with the following physiological characteristics were used for the studies: volume – 3–4 ml, sperm concentration – 0.6–1.1×10^9 cells/ml, number of live germ cells – 80–85%. Semen samples were diluted with phosphate-buffered saline (PBS) of the composition: NaCl – 0.8 g, KCl – 0.02 g, Na_2HPO_4 – 0.11 g, KH_2PO_4 – 0.02 g, $MgCl_2$ – 0.01 g, distilled water – up to 100 ml) and divided into groups: control – without adding naphazoline nitrate and experimental – with adding naphazoline nitrate. Doses of the active substance in semen samples were selected taking into account the parameters of acute toxicity for warm-blooded animals, namely, the LD50 value for male rats intragastrically, which is equal to 1210.0 mg/kg. The effect of naphazoline nitrate was studied in two series of experiments: the I series of experiments – at doses of: 1/100 LD50 (0.01 mg/ml), 1/10 LD50 (0.1 mg/ml), LD50 (1.0 mg/ml) and the II series – at doses of: 1/200 LD50 (0.005 mg/ml), 1/20 LD50 (0.05 mg/ml), 1/2 LD50 (0.05 mg/ml).

Methods

The respiratory activity of spermatozoa (ng-atom O2/min×0.1 ml) was determined by the polarographic method in a temperature-controlled cell (at a temperature of 38.5 °C); the reducing capacity (mV/min×0.1 ml) – potentiometrically using closed microelectrodes placed in a polarographic cell (Shtolts *et al.*, 1980); succinate dehydrogenase activity (SDH, μm/min×L) using 2,3,5-triphenyltetrazolium chloride (Vlizlo *et al.*, 2012). Survival of spermatozoa (h) was established by recording the moment when rectilinear translational motion ceased after incubation of semen samples at a temperature of 2–5 °C.

Statistical analysis

The check for emissions was carried out using a graphical criterion – the construction of a box-and-whisker plot (ISO 16269-4:2010, 2010). Verification of the deviation of the probability distribution from the normal distribution was carried out using the Shapiro-wilk-test (W) criterion (ISO 5479:1997, 1997). The data distribution type could not be unambiguously established, so the data was presented as medians and interquartile ranges. The reliability of the difference between the control and experimental data was established using the Mann-Whitney criterion (U-test) (Habibzadeh, 2017). The correlation ratio (η) was used as a measure of the correlation between quantitative variables. P values < 0.05 were considered statistically significant. Mathematical processing was performed using Microsoft® Excel® 2016 and STATISTICA Base®.

Results

The results of the study of the effect of different doses of naphazoline nitrate on the intensity of oxidation processes and survival of bull spermatozoa are presented in Table 1 and Table 2. It was found that naphazoline nitrate in all the studied doses causes significant changes in the respiratory activity of spermatozoa. The changes had a pronounced dose-dependent nature (η = 0.840 and 0.762 for two experiments, respectively), and the most intense effect was observed at the maximum dose of the substance – LD50 (1.0 mg/ml).

Naphazoline nitrate in high doses (1/10 LD50 and above) has a significant effect on the redox potential of germ cells, namely, there is a decrease in the activity of electron transport up to a change of charge of the medium from negative to positive.

The succinate dehydrogenase activity moderately correlates with the change in the concentration of

Table 1. Intensity of oxidation processes and survival of spermatozoa with adding naphazoline nitrate at doses of 1/100 LD50, 1/10 LD50, LD50.

Names of indicators	Indicator values (n=8)				Correla-tion ratio, η
	Control	Samples with adding naphazoline nitrate			
		1/100 LD50	1/10 LD50	LD50	
Respiratory activity, ng-atom O_2/min×0.1 ml	2.10 (1.85–2.35)	0.81** (0.63–0.99)	0.49*** (0.38–0.60)	0.39*** (0.32–0.46)	0.840
Reducing capacity, mV/min×0.1 ml	–0.17 (–0.23 – –0.11)	–0.15 (–0.23 – –0.07)	–0.09* (–0.12 – –0.06)	0.22* (0.1–0.34)	0.230
SDH activity, μm/min×L	11.5 (10.1–12.9)	10.1 (9.5–10.67)	5.5** (4.24–6.76)	5.7* (4.18–7.22)	0.622
Survival, h	85.7 (79.6–91.8)	92.6 (85.04–100.16)	85.7 (79.55–91.85)	70.3* (64.15–76.45)	0.427

Note: the difference is statistically significant compared to the control (* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$)

Table 2. Intensity of oxidation processes and survival of spermatozoa with adding naphazoline nitrate at doses of 1/200 LD50, 1/20 LD50, 1/2 LD50.

Names of indicators	Indicator values (n=8)				Correla-tion ratio, η
	Control	Samples with adding naphazoline nitrate			
		1/200 LD50	1/20 LD50	1/2 LD50	
Respiratory activity, ng-atom O_2/min×0.1 ml	2.16 (1.84–2.51)	0.43*** (0.33–0.53)	0.41*** (0.28–0.54)	0.40*** (0.24–0.56)	0.678
Reducing capacity, mV/min×0.1 ml	–0.30 (–0.44 – –0.16)	–0.06 (–0.08 – –0.04)	0.07 (0.04–0.01)	0.09* (0.04–0.14)	0.427
SDH activity, μm/min×L	11.5 (9.99–13.1)	11.4 (10.3–12.5)	10.5 (9.66–11.3)	5.5** (4.37–6.63)	0.627
Survival, h	89.1 (83.32–94.88)	94.3 (89.81–98.79)	92.6 (85.04–100.16)	72.0 (65.14–78.86)	0.469

Note: the difference is statistically significant compared to the control (*– $p<0.05$; ** – $p<0.01$; *** – $p<0.001$)

naphazoline nitrate (correlation ratio at the level of 0.6) and when exposed to the substance in doses from 1/200 LD50 to 1/20 LD50, the SDH value does not change significantly, and for 1/10 LD50 and above, it significantly decreases compared to the control.

When studying the effect of naphazoline nitrate on spermatozoa survival, a moderate correlation was observed (η at the level of 0.43 and 0.47 in two series of experiments, respectively) between the amount of substance introduced and the obtained values of indicators, but significant changes between the indicators of the control and experimental groups were found only in one case – with exposure at the level of LD50 (Table 1, Table 2).

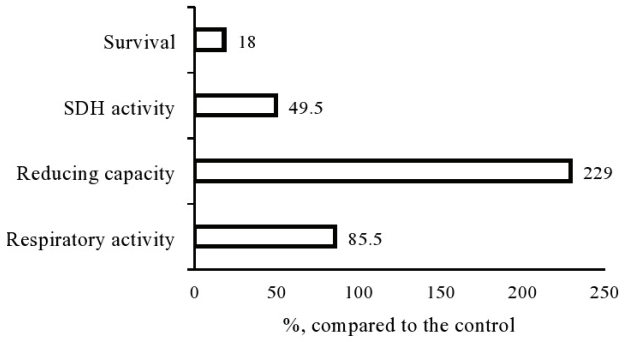

Figure 1. The degree of inhibition of redox processes and the survival ability of sperm under the effect of the maximum dose of naphazoline nitrate.

Discussion

Thus, it was found that naphazoline nitrate has the ability to inhibit the respiratory activity and reducing capacity of germ cells; the substance has a lesser effect on the SDH activity and their survival, which is especially clearly seen at the level of exposure to the maximum dose of the substance (Figure 1).

The molecular structure of naphazoline nitrate contains a nitrate ion and is capable of partial dissociation in water. As is known, nitrates in the body can be reduced to nitrites, which leads to the formation of methemoglobin, and probably contribute to the negative effect of naphazoline nitrate on the respiratory activity of cells established as a result of the above studies (Henig&Pierson, 2000; Weinberge *et al.*, 2001).

Succinate dehydrogenase is a mitochondrial enzyme involved in the electron respiratory chain, and in our case, the affinity of naphazoline nitrate to this enzyme is manifested only at the level of high doses with inhibition of enzymatic function (Table 1, Table 2). However, it is established that with an overdose of medicines, in particular, with a psychotropic effect (anxiolytics, sedative and sleep-inducing drugs), the level of this enzyme increases in the first hours of the toxicogenic period and further depletion of SDH function occurs due to possible damage to cell membranes (Beckers *et al.*, 2010, Huang&Millar, 2013). The latter indicates the difference in the mechanisms of toxic effect of substances on the body's metabolic processes depending on their chemical composition and cell type, as well as the feasibility of studying changes in the levels of mitochondrial enzymes in dynamics.

The ability to survive is an important criterion for assessing the degree of pathological changes in cells as a result of the action of chemical agents. Provided that there is a pronounced effect, it is possible to talk about the presence of a cytotoxic effect of compounds (Weil Marie-Theres&Werner Tobias, 2012; Aleksandrova *et al.*, 2018). Our findings do not clearly prove the ability of naphazoline nitrate to cause a cytotoxic effect, however, it is impossible to exclude the appearance of pathological changes in cells during the prolonged exposure to the substance, which can occur in practice both in the case of taking a dosage form based on naphazoline nitrate and when workers' bodies are exposed to the substance in production conditions.

The dose-dependent nature of the effect of naphazoline nitrate on certain indicators of redox processes in spermatozoa confirms the prospects of using the latter as alternative test objects in studying the harmful effects of chemicals.

Conclusions

Naphazoline nitrate in doses corresponding to 1/200 LD50 (0.005 mg/ml), 1/100 LD50 (0.01 mg/ml), 1/20 LD50 (0.05 mg/ml), 1/10 LD50 (0.1 mg/ml), 1/2 LD50 (0.05 mg/ml), LD50 (1.0 mg/ml), established in toxicological experiments for white male rats, can affect redox processes in bull spermatozoa.

Under the effect of naphazoline nitrate, the respiratory activity and succinate dehydrogenase activity are dose-dependently inhibited; high doses of naphazoline nitrate (1/10 LD_{50} and above) have a significant effect on the redox potential of germ cells, however, without correlation with doses.

Naphazoline nitrate has a small effect on the survival of spermatozoa – changes in indicators are reliably manifested only under the effect of the maximum dose of the substance.

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