



A.S. Smirnov, S.N. Smirnov, M.A. Mirzebasov

Effect of Echinacea Purpurea Extract and Thiotriazoline on the Muscular Membrane of the Pyloric Part of the Stomach of Rats under the Action of Epichlorohydrin

Luhansk State Medical University, Luhansk, Ukraine

Keywords:

stomach;
epichlorohydrin;
Echinacea
purpurea
extract;
thiotriazoline;
rats

Abstract.

During the experiments on white rats, the changes occurring in the muscular membrane of pyloric part of rats' stomach after a two-month inhalation exposure to epichlorohydrin, Echinacea purpurea extract and thiotriazoline administration were studied. It has been shown that one of the manifestations of epichlorohydrin action was decrease in the thickness of the muscular membrane in pyloric part of rats' stomach. The degree of reduction of the muscular membrane thickness during the period between the first on the sixtieth day after inhalation decreased. Introduction of Echinacea purpurea extract as well as thiotriazoline was accompanied by thickening of the muscular membrane of pyloric part of the stomach. Echinacea purpurea extract and thiotriazoline showed the ability to reduce the period of reduction of the muscle membrane thickness induced by epichlorohydrin from sixty to seven days.



Copyright © Author(s), 2016

Problem statement and analysis of the recent research

Chemical anthropogenic pollutants belong to the most important factors that determine the health of modern person [1, 7, 11]. In everyday life and under the conditions of chemical production a person contacts with the chemicals which include the epoxy group. Such compounds include epichlorohydrin. Its intake into the body causes disorders of respiratory tract, visual organs, skin, reproductive organs, and immune system [4, 6, 8]. Significant changes occur in the stomach wall under the influence of exogenous factors [2, 3, 5, 9, 10]. However, the characteristics of its changes under the influence of epichlorohydrin are understudied. Therefore, it is relevant to study the state of the wall of the stomach pyloric part of and its structural component – the muscle membrane – under the conditions of epichlorohydrin chronic inhalations as well as to consider possible approaches in correcting the upcoming changes.

The objective of the research was to study changes in the muscular layer of the pyloric part of the rats' stomach after epichlorohydrin inhalations, the introduction of Echinacea purpurea extract and Thiotriazoline.

Material and methods of the research

180 albino outbred sexually mature adult male rats were used in the experiment. The rats were divided into six experimental groups including thirty rats each. Rats of Group I constituted Control Group. Rats of Group II were administered epichlorohydrin in a dose of 10 MPC (10 mg / kg) by inhalation during two months, five days a week for five hours a day. Rats of Group III were given Echinacea purpurea extract in a dose of 200 mg per kg of body weight through gastric tube during two months, five days per week. Rats of Group IV were administered thiotriazoline in a dose of 117.4 mg per kg of body weight as an injection of 2.5% solution intraperitoneally within two months, five days a week. Rats of Group V were exposed to epichlorohydrin and extract of Echinacea purpurea, and rats of Group VI were exposed to epichlorohydrin and thiotriazoline according to the above described procedure.

After two-month administration of epichlorohydrin, the extract of Echinacea purpurea and thiotriazoline, six rats from each experimental group were taken out of the experiment on the first, seventh, fifteenth, thirtieth and sixtieth day. The stomach was fixed in 10% solution of neutral formalin. Histological processing was performed according to the standard method by dehydration in ethanol solution followed by removal of the alcohol with xylene. Preparations were filled into paraffin. In order to study the stomach wall, its cross sections obtained on sliding microtome were stained with hematoxylin and eosin, and according to Van Gieson method. The thickness of the muscular membrane of the stomach pyloric part was measured. Excel program was used for the statistical analysis of the results. Significance of differences was assessed using Mann-Whitney U test. Differences were considered significant at $p < 0.05$.

Results of the research and their discussion

Epichlorohydrin introduction decreased the thickness of the muscular membrane of pyloric part of the stomach in comparison with the corresponding index in the rats of Control Group by 12.6% ($p < 0.01$) on the first day, by 9.3% ($p < 0.05$) on the seventh day, by 6.2% ($p < 0.05$) on the fifteenth day, by 9.4% ($p < 0.05$) on the thirtieth day, by 7.5% ($p < 0.05$) on the sixtieth day after the inhalation cessation. It should be noted that the thickness of muscle membrane of the pyloric part of the stomach of rats undergoing the inhalation of epichlorohydrin increased by 6.1% ($p < 0.05$) for the period from the first to the sixtieth day of observation (Table 1).

The thickness of the structure increased by 3.7% ($p < 0.05$) in the rats affected by Echinacea purpurea extract in relation to the thickness of muscle membrane of the pyloric part of stomach of rats in the Control Group on the seventh day of observation. Muscular membrane thickness did not

undergo statistically significant changes ($p > 0.05$) during sixty days of observation in rats treated with the extract of *Echinacea purpurea* (Table 1).

The thickness of the muscle membrane of the pyloric part of the stomach was smaller by 9.1% ($p < 0.01$) and 9.9% ($p < 0.01$), respectively, in rats that underwent the impact of epichlorohydrin and *Echinacea purpurea* extract on the first and on the seventh day after its completion in comparison with that of the Control Group rats. The use of the extract of *Echinacea purpurea* caused an increase in the thickness of the muscle membrane of rats treated with epichlorohydrin inhalation by 8.0% ($p < 0.05$) on the thirtieth day of the research. The thickness of the muscular membrane of the pyloric part of the stomach increased by 14.7% ($p < 0.01$) from the first day to the thirtieth day of observation in the group of rats treated with epichlorohydrin and extract of *Echinacea purpurea*. However, the changes in the index were not statistically significant ($p > 0.05$) in the period between the thirties and sixties day (Table 1).

Table 1

The thickness of muscle membrane of the pyloric part of the stomach of rats after epichlorohydrin inhalation and exposure to *Echinacea purpurea* extract ($M \pm SLE$)

Day of observation	Number of rats per group	Thickness of muscle membrane of the pyloric part of the stomach (microns)			
		The control group	After epichlorohydrin inhalation	After exposure to <i>Echinacea purpurea</i> extract	After exposure to epichlorohydrin and <i>Echinacea purpurea</i> extract
1	n = 6	320.72±11.95	280.33±12.08*	332.88±12.71	291.53±13.33*
7	n = 6	313.88±11.62	284.64±14.99*	325.44±7.88*	282.76±8.98*
15	n = 6	323.06±16.31	303.12±11.62*	322.34±12.70	307.11±12.79
30	n = 6	330.13±19.36	299.15±19.27*	327.67±23.61	322.95±17.16 [#]
60	n = 6	321.66±16.70	297.53±11.79 ^{*x}	317.39±17.06	304.25±16.44

Note.

* - $p < 0.05$ in comparison with indices of rats in the Control Group;

[#] - $p < 0.05$ in comparison with indices of rats that underwent epichlorohydrin inhalation;

^x - $p < 0.05$ in comparison with the indices in the rats of the same experimental group at different periods of observation.

Thiotriazoline introduction was accompanied by changes in the thickness of the muscle membrane of the pyloric part of the stomach in relation to the corresponding index in the rats in the Control Group, manifested in the increase by 3.3% ($p < 0.05$) on the seventh day. Muscular membrane thickness changed statistically insignificant ($p > 0.05$) in rats treated with thiotriazoline in the period from the first to the thirtieth day of observation. However, its decrease by 6.0% was observed from the thirties to the sixtieth day ($p < 0.05$) (Table 2).

The combined use of epichlorohydrin and thiotriazoline led to a decrease in thickness of muscle membrane of the pyloric part of the stomach by 7.1% ($p < 0.05$) on the first day of observation, by 4.1% ($p < 0.05$) on the seventh day in comparison with the Control Group rats. A comparison of the index values in rats treated with epichlorohydrin inhalation and rats treated with epichlorohydrin accompanied by thiotriazoline injection showed that the thickness of the muscular membrane in the second case was greater by 6.3% ($p < 0.05$) on the first day, by 5.8% ($p < 0.05$) on the seventh day, by 6.5% on the fifteenth day, by 10.0% ($p < 0.05$) on the thirtieth day, by 7.8% ($p < 0.01$) on the sixtieth day. Changes in the thickness of the muscular membrane of the pyloric part of the stomach were not statistically significant ($p > 0.05$) from the first to the seventh day and from the fifteenth to the sixtieth day of observation in the experimental group of rats treated with epichlorohydrin and thiotriazoline.

However, an increase in index by 8.8% ($p < 0.05$) was noted in the period from the seventh to the fifteenth day (Table 2).

Table 2

The thickness of muscle membrane of the pyloric part of the stomach of rats after epichlorohydrin inhalation and exposure to thiotriazoline ($M \pm SLE$)

Day of observation	Number of rats per group	Thickness of muscle membrane of the pyloric part of the stomach (microns)			
		The control group	After epichlorohydrin inhalation	After exposure to thiotriazoline	After exposure to epichlorohydrin and thiotriazoline
1	n = 6	320.72±11.95	280.33±12.08*	331.60±13.16	297.88±15.39*#
7	n = 6	313.88±11.62	284.64±14.99*	324.34±8.71	301.12±12.10*#
15	n = 6	323.06±16.31	303.12±11.62*	329.15±18.47	327.51±14.25* ^x
30	n = 6	330.13±19.36	299.15±19.27*	332.35±16.49	324.23±15.98 [#]
60	n = 6	321.66±16.70	297.53±11.79* ^x	312.38±12.19	323.56±12.58 [#]

Note.

* - $p < 0.05$ in comparison with indices of rats in the Control Group;

- $p < 0.05$ in comparison with indices of rats that underwent the epichlorohydrin inhalation;

^x - $p < 0.05$ in comparison with the indices in rats of the same experimental group at different periods of observation.

The results of experimental research suggest that epichlorohydrin, Echinacea purpurea extract and thiotriazoline affect the thickness of the muscular membrane of the pyloric part of the stomach of rats.

Conclusions

1. Epichlorohydrin affect causes a decrease in the thickness of the muscle membrane of the pyloric part of the stomach of rats, the severity of which decreases during sixty days after the epichlorohydrin introduction.
2. Use of Echinacea purpurea extract and thiotriazoline shortens the duration of decrease in thickness of pyloric part of rats' stomach of induced with epichlorohydrin, from sixty to seven days.

Prospects for further research

Continued study of the nature of epichlorohydrin effect on the stomach will make it possible to discover profoundly the mechanisms of changes in the body in response to xenobiotics creating an experimental basis for the development of promising ways to prevent the emergence and correction of the upcoming changes.

References

1. Aminova AI, Minchenko EYu. Causes of chronic gastroduodenal and bone disorders in children living under the influence of man-made environmental factors (review). Eksperimentalnaya klinicheskaya gastroenterologiya. 2014; 1: 95-101.
2. Vasiliev YuV. Treatment and prevention of gastric and duodenum erosions associated with stressful situations. Russkiy meditsinskiy zhurnal. 2010; 18 (28): 1728-1731.
3. Sych VF, Voronova OS, Kondratenko YuN, Sanzhapova AF. Food consistency of postnatal morphogenesis of stomach musculature. Vestnik novykh meditsinskikh tekhnologiy. 2009; 2: 180-182.

4. Taranenko NA, Meshchakova NM, Zhurba OM, Telezhkin VV. Air pollution with organochloride hydrocarbons in the polyvinyl chloride and epichlorohydrin production. *Gigiena i sanitariya*. 2014; 4: 47-51.
5. Cao L, Yu J. Effect of *Helicobacter pylori* Infection on the Composition of Gastric Microbiota in the Development of Gastric Cancer. *Gastrointest Tumors*. 2015; 2(1): 14-25. <http://dx.doi.org/10.1159/000380893>
6. Luo JC, Cheng TJ, Kuo HW, Chang MJ. Decreased lung function associated with occupational exposure to epichlorohydrin and the modification effects of glutathione s-transferase polymorphisms. *J Occup Environ Med*. 2004; 46(3): 280-286. <http://dx.doi.org/10.1097/01.jom.0000116807.73005.97>
PMid:15091291
7. Mehra R, Sodhi RK, Aggarwal N. Memory restorative ability of clioquinol in copper-cholesterol-induced experimental dementia in mice. *Pharm Biol*. 2015; 9: 1-10. <http://dx.doi.org/10.3109/13880209.2014.974061>
8. Shin IS, Park NH, Lee JC, Kim KH, Moon C, Kim SH, Shin DH, Park SC, Kim HY, Kim JC. One-generation reproductive toxicity study of epichlorohydrin in Sprague-Dawley rats. *Drug Chem Toxicol*. 2010; 33(3): 291-301. <http://dx.doi.org/10.3109/01480541003734030>
9. Wang LL, Yu XJ, Zhan SH, Jia SJ, Tian ZB, Dong QJ. Participation of microbiota in the development of gastric cancer. *World J Gastroenterol*. 2014; 20(17): 4948-4952. <http://dx.doi.org/10.3748/wjg.v20.i17.4948>
10. Whary MT, Muthupalani S, Ge Z, Feng Y, Lofgren J, Shi HN, Taylor NS, Correa P, Versalovic J, Wang TC, Fox JG. Helminth co-infection in *Helicobacter pylori* infected INS-GAS mice attenuates gastric premalignant lesions of epithelial dysplasia and glandular atrophy and preserves colonization resistance of the stomach to lower bowel microbiota. *Microbes Infect*. 2014; 16(4): 345-355. <http://dx.doi.org/10.1016/j.micinf.2014.01.005>
11. Yi SW, Ohrr H. Agent Orange exposure and cancer incidence in Korean Vietnam veterans: a prospective cohort study. *Cancer*. 2014; 120(23): 3699-3706. <http://dx.doi.org/10.1002/cncr.28961>