Experimental Studies

Late ultrastructural changes in the rat chorioretinal complex following injection of mixture of 40% ethanol and 100% methanol

N. I. Molchaniuk

SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the NAMS of Ukraine";
Odesa (Ukraine)

E-mail: elmicroscop@gmail.com

Background: *Consumption of surrogate alcohol containing methanol may result in blindness and even death. The literature is scant on experimental and especially morphological studies on the effect of surrogate alcohol containing methanol and ethanol on organs (particularly, the eye) and tissues of experimental animals.*

Purpose: *To examine late ultrastructural changes in the interplay of cells of the rat chorioretinal complex (endothelial cells of the choriocapillaries, retinal pigment epithelium (RPE) cells, and photoreceptor cells) following a single intraperitoneal (IP) injection of alcohol mixture (40% ethanol and 100% methanol) at the proportion of 3:1, with a methanol dose of 2.5 g/kg.*

Material and Methods: *Twelve adult Wistar rats (weight, 250-300 g) were divided into two groups, each of 6 rabbits. Group 1 (the experimental group) received a single IP injection of alcohol mixture (40% ethanol and 100% methanol) at the proportion of 3:1, with a methanol dose of 2.5 g/kg, whereas group 2 (controls) received a single IP injection of 100% methanol at a dose of 2.5 g/kg. The LD_{50} value for IP administration of methanol in rats is reported as 9.5 g/kg body weight. The ultrastructure of endothelial cells of the choriocapillaries, RPE cells, and photoreceptor cells was examined on a PEM-100-01 Transmission Electron Microscope (Selmi, Sumy, Ukraine) at 1 and 3 months after IP injection of the above alcohol mixture in rats.*

Results: *At one month after IP injection of the alcohol mixture, the lumen of the choroidal capillaries and ground substance of the Bruch's membrane appeared osmiophilic, indicating increased lipid levels. In most choroidal capillaries, endothelial cells exhibited signs of hydropic degeneration. The RPE cells showed polymorphic changes; some of them showed severe degeneration of organelles, sometimes with total loss of cytoplasm and damage to the plasmalemma at the basal and apical surfaces; some other RPE cells showed signs of compensatory and restorative processes aimed at intracellular repair processes. There were signs of intercellular and intracellular edema and degeneration of membrane structure in the photoreceptor cell layer. At 3 months after IP injection of the alcohol mixture, signs of hydropic degeneration in the examined structures of the chorioretinal complex were somewhat less severe than at the previous time point. A long-term toxic effect of consuming a small dose of methanol was characterized by severe pathologic changes in and poor reserve potential of the cells of the chorioretinal complex, which was reflected in slow repair processes during the period from 1-month to 3-month time points. This likely explains the reported cases of a prolonged serious condition of individuals after consuming surrogate alcohol.*

Conclusion: *A single IP injection of the alcohol mixture with a methanol dose of 2.5 g/kg body weight resulted both in signs of hydropic degeneration and slow repair processes in the cells of the rat chorioretinal complex during the period from 1-month to 3-month time points. A single IP injection of pure methanol (2.5 g/kg) resulted in uniform and more severe changes in the cells of the rat chorioretinal complex. Methanol can be attributed a leading role in the development of pathological changes in the examined structures of the chorioretinal complex after injection of the alcohol mixture.*

Keywords:
ultrastructure, degenerative changes in the cells of the chorioretinal complex, choriocapillaries, retinal pigment epithelium, toxic effect of ethanol and methanol mixture

Introduction

Consumption of surrogate alcohol containing methanol may result in blindness and even death. Methanol first affects the optic nerve, retina and brain tissues [1-5]. Our comprehensive research on the mechanisms of toxic effects of various doses of methanol on the rat uvea and retina is being conducted for 12 years [6, 7]. We found that a single intraperitoneal (IP) injection of methanol (0.75 g/kg, 2.5 g/kg, 5.0 g/kg, 7.0 g/kg body weight) produced primarily changes in endothelial cells of choroidal vessels and capillaries and retinal pigment epithelium (RPE) cells, with the severity of damage depending on the injected dose of methanol.

The literature is scant on experimental and especially morphological studies on the effect of surrogate alcohol containing methanol and ethanol on organs and tissues of experimental animals [8, 9]. Ethanol, on the one hand, produces toxic effect on the human body when consumed in large amounts or chronically, and, on the other hand, is an antidote for methanol toxicity in humans [1, 10, 11]. Some studies on toxic and kinetic interactions of ethanol and methanol in white rats found that, compared to methanol, ethanol had higher absorption rate during transport of substances from the gastrointestinal tract to the blood stream [12]. Others found that ethanol, when used as an antidote in acute methanol intoxication (LD_{50}), increased immunotoxic effects in white rats [8]. We have demonstrated previously that a single intraperitoneal (IP) injection of alcohol mixture containing methanol (2.5 g/kg) produced ultrastructural changes in endothelial cells of choroidal vessels and capillaries and RPE cells that as early as 70 minutes after injection, with these changes practically maintained for a two-week follow up [13].

The purpose of the current study was to examine late ultrastructural changes in cells of the chorioretinal complex (endothelial cells of the choriocapillaries, RPE cells, and photoreceptor cells) in rats after a single IP injection of alcohol mixture (40% ethanol and 100% methanol) at the proportion of 3:1, with a methanol dose of 2.5 g/kg.

Material and Methods

Twelve adult Wistar rats (weight, 250-300 g) were divided into two groups, each of 6 rabbits. Group 1 (the experimental group) received a single IP injection of alcohol mixture (40% ethanol and 100% methanol) at the proportion of 3:1, with a methanol dose of 2.5 g/kg, whereas group 2 (controls) received a single IP injection of 100% methanol at a dose of 2.5 g/kg. The LD_{50} value for IP administration of methanol in rats is reported as 9.5 g/kg body weight.

All manipulations were performed in compliance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Tissues were processed for ultrastructural examination according to routine electron microscopy procedures. The ultrastructure of endothelial cells of the choriocapillaries, RPE cells, and photoreceptor cells was examined on a PEM-100-01 Transmission Electron Microscope (Selmi, Sumy, Ukraine) at 1 and 3 months after IP injection of the above alcohol mixture in rats.

Results

At one month after IP injection of the alcohol mixture, the lumen of the choroidal capillaries and ground substance of the Bruch's membrane appeared osmiophilic, indicating increased lipid levels in the lumen. In most choroidal capillaries, endothelial cells had electron-transparent hyaloplasm and edematous inner mitochondrial matrix and exhibited destruction of some mitochondrial cristae and dilation of solitary endoplasmic reticulum cisternae, indicating hydropic degeneration of these cells. In addition, these cells showed reduced abundance of free rybosomes and polysomes. In the rest choroidal capillaries, endothelial cells showed almost normal ultrastructure or increased abundance of their typical organelles, which reflected active cell metabolism and was a sign of compensatory and restorative processes aimed at restoration of normal ultrastracture after damage from ethanol and methanol, which was confirmed by similar changes in samples from the control rats. The RPE cells showed polymorphic changes; some RPE cells showed severe degeneration of organelles, sometimes with total loss of cytoplasm and damage to the plasmalemma at the basal and apical surfaces of the cells; some other RPE cells showed signs of compensatory and restorative processes, with an increased abundance of such organelles as mitochondria, polysomes and free rybosomes, which indicated energy producing and protein synthesis functions aimed at intracellular repair processes, but at that time point these changes were less apparent than during the first two weeks of the follow-up period [13]. Of note was a significant abundance of lysosomes in all RPE cells. There were signs of intercellular and intracellular edema and degeneration of membrane structure in the photoreceptor cell layer. Accumulation of photoreceptor outer segment debris with either disorganized or normally organized photoreceptor membranes was seen beneath RPE cells. Of note that pathological changes in the photoreceptors corresponded to those in the RPE cells. In addition, there was almost no RPE phagocytosis of shed outer segment fragments.

At 3 months after IP injection of the alcohol mixture, signs of hydropic degeneration in the examined structures of the chorioretinal complex observed were somewhat less severe than at one month after IP injection (Figs. 1, 2). The lumen of some choroidal capillaries and the Bruch's membrane appeared more transparent, and RPE phagocytosis of shed outer segment fragments more active than at the previous time point. There were, however, signs of intracellular repair processes similar to the previous time point (Figs. 1, 2).

In the cells of the chorioretinal complex in the control group, the changes were unidirectional and more severe than in the study group. At the same time, however, restoration of intracellular infrastructure was observed in the cells of the chorioretinal complex in both groups (Fig. 3).

Discussion

At months 1 and 3 after a single IP injection of alcohol mixture (40% ethanol and 100% methanol) at the proportion of 3:1, with a methanol dose of 2.5 g/kg, the changes in cooperation of cells of different types of the choreoretinal complex were similar in type to those observed at day 14 [13], i.e., there were both degenerative changes of various degrees and slow repair processes. These changes were more pronounced in RPE cells, likely due to their high regenerative capacity. It is worthy of note that we found that the severity of damage to RPE cells depended on the state of endothelial cells of the choriocapillaries. The outer retinal layers in rats and humans are known to be supplied with blood from the choroidal capillaries. The pathological processes in endothelial cells of the choriocapillaries and plasma induced by the alcohol mixture (especially, methanol) result in impaired transport from the capillaries to RPE cells, leading to nutrient deficiency in RPE cells and, subsequently, in photoreceptor cells. Previous studies have reported on the negative effects of alcohol on cell membranes and methanol-induced effects on rheological characteristics of blood and damage to red and white blood cells and platelets [3, 11]. In addition, one cannot exclude direct alcohol-induced (particularly, methanol-induced) damage to endothelial cells of the choriocapillaries, RPE, and, subsequently, to other retinal cells. This may be the reason why RPE phagocytosis of shed outer segment fragments appeared to be abruptly discontinued in the first hours after injection of the alcohol mixture or pure methanol [13]. Such changes were observed over the course of 3 months. This results in the deficiency of retinal, which is released in RPE cells from the shed outer segment disk membranes during their digestion by lysosomes. In addition, vitamin A deficiency ensures; normally, this vitamin is delivered to the RPE cells from the liver through the blood stream, but alcohol causes damage also to the structure of the liver [10, 14]. Loss of these substances makes impossible the formation of a visual pigment (rhodopsin) that is involved in transforming light energy to the energy of nerve impulses, leading to partial or (if a large dose of methanol is consumed) complete loss of sight. And altogether, it results in retinal dystrophy [15]. A long-term toxic effect of consuming a small dose of methanol was characterized by severe pathologic changes in and poor reserve potential of the cells of the chorioretinal complex, which was reflected in slow repair processes during the period from 1-month to 3-month time points. This likely explains a prolonged serious condition of individuals after consuming surrogate alcohol [16].

Therefore, a single IP injection of the alcohol mixture with a methanol dose of 2.5 g/kg in rats resulted both in signs of hydropic degeneration and slow repair processes in the cells of the chorioretinal complex during the period from 1-month to 3-month time points. A single IP injection of pure methanol (2.5 g/kg) resulted in more uniform and more severe changes in the cells of the chorioretinal complex. Methanol can be attributed a leading role in the development of pathological changes in the examined structures of the chorioretinal complex after a single IP injection of the alcohol mixture.

References

1. Zabrodskiĭ PF. [Immunotoxicological characteristics of methanol. Correction of immune system dysfunction]. Saratov; 2013. 241 p. Russian.
2. Manuchehri AA, Alijanpour E, Daghmechi M, et al. A case of methanol poisoning leading to prolonged respirator dependence with consequent blindness and irreversible brain damage. Caspian J Intern Med. 2015;6 (3):180–3.
3. Rajamani R, Muthuvel A, Senthilvelan M, Sheeladevi R. Oxidative stress induced by methotrexate alone and in the presence of methanol in discrete regions of the rodent brain, retina and optic nerve. Toxicol Lett. 2006 Sep 10;165(3):265-73. doi: 10.1016/j.toxlet.2006.05.005.
4. Sweeting JN, Siu M, McCallum GP, et al. Species differences in methanol and formic acid pharmacokinetics in mice, rabbits and primates. Toxicol Appl Pharmacol. 2010 Aug 15;247(1):28-35. doi: 10.1016/j.taap.2010.05.009.
5. Zhai R, Zheng N, Rizak J, Hu X. Evidence for Conversion of Methanol to Formaldehyde in Nonhuman Primate Brain. Anal Cell Pathol (Amst). 2016;2016:4598454. doi: 10.1155/2016/4598454.
6. Molchaniuk NI. [Light and electron microscopic study of choriocapillaries, pigment epithelium and photoreceptor cells of rat retinas in dynamics after administration of various doses of methanol]. Visnyk Kyivskogo natsional`nogo universitetu imeni Tarasa Shevchenka. Problemy reguliatsii fiziologichnykh funktsii. 2015;1(18):74–8. Ukrainian.
7. Molchaniuk NI. [Ultrastructural changes in the rat choroid and retina after injection of alcohol mixture (40% ethanol and 100% methanol)]. Visnyk Kyivskogo natsional`nogo universitetu imeni Tarasa Shevchenka. Biologiia. 2019;79:16–24. Ukrainian.
8. Zabrodskiĭ PF, Germanchuk VG. [Effect of ethanol on methanol immunotoxicity]. Eksp Klin Farmakol. 2001 Sep-Oct;64(5):40-2. Russian.
9. Pohanka M. Toxicology and the biological role of methanol and ethanol: current view. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2016 Mar;160(1):54-63. doi: 10.5507/bp.2015.023.
10. Akimov PA, Orbidans AG, Terekhin GA, Terekhina NA. The influence of acute alcohol intoxication on glycogen content in the liver and skeletal muscles. Patol Fiziol Eksp Ter. 2010; 2:15-17. Russian.
11. Patra M, Salonen E, Teramaetal E. Under the influence of alcohol: the effect of ethanol and methanol on lipid bilayers. Biophys J. 2006 Feb 15;90(4):1121-35. doi: 10.1529/biophysj.105.062364.
12. Golovenko NIa, Larionov VB, Ovcharenko NV, Borisiuk IIu, Lihota EȊB. [Toxic-kinetic interaction of ethyl and methyl alcohol in white mice]. Sovremennyie problemy toksikologii. 2008;(1):32–6. Ukrainian.
13. Molchaniuk NI. [Effect of alcohol mixture (40% ethanol and 100% methanol) on the ultrastructure of the rat choroid and retina]. Visnyk problem biologii i meditsiny. 2019;4(1):222-7. Ukrainian.
14. Molchaniuk NI. [Ultrastructure of rat hepatocytes in the early stages after intraperitoneal methanol injection]. Bukovynskyi medychnyi visnyk. 2013; (4):100–3. Ukrainian.

15. Ostrovskii MA, Feldman TB. [Chemistry and molecular physiology of vision, light-sensitive protein rhodopsin]. Uspekhi khimii. 2012; 81(11):1071-90. Russian.
16. Paasma R, Hovda KE, Jacobsen D. Methanol poisoning and long term sequelae - a six years follow-up after a large methanol outbreak. BMC Clin Pharmacol. 2009 Mar 27;9:5. doi: 10.1186/1472-6904-9-5.

The authors certify that they have no conflicts of interest in the subject matter or materials discussed in this manuscript.

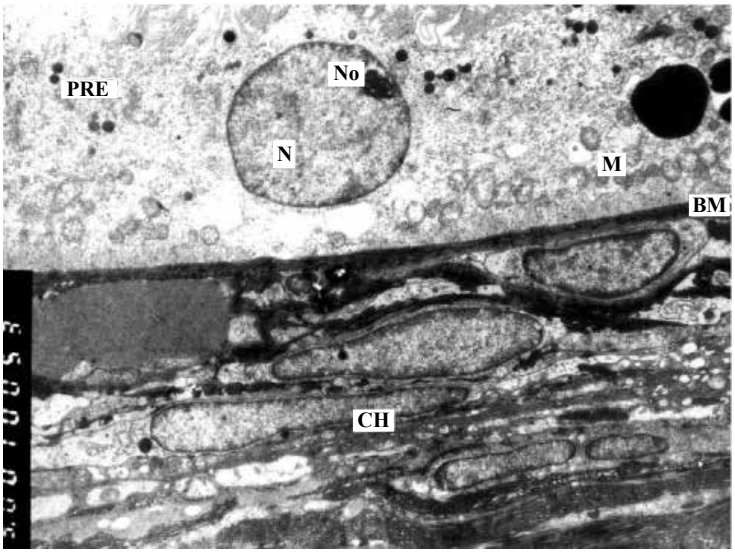

Fig. 1. Electron micrograph showing ultrastructure of cells of the rat chorioretinal complex 3 months after injection of the alcohol mixture with a methanol dose of 2.5 g/kg. The lumen of the choroidal capillaries and of the Bruch's membrane appear osmiophilic. Endothelial cells of the choriocapillaries and retinal pigment epithelium (RPE) cells exhibit hydropic degeneration. Original magnification ×3,000. Notes: CH, choroid; BM, Bruch's membrane; RPE, retinal pigment epithelium; N, nucleus; No, nucleolus; M, mitochondria

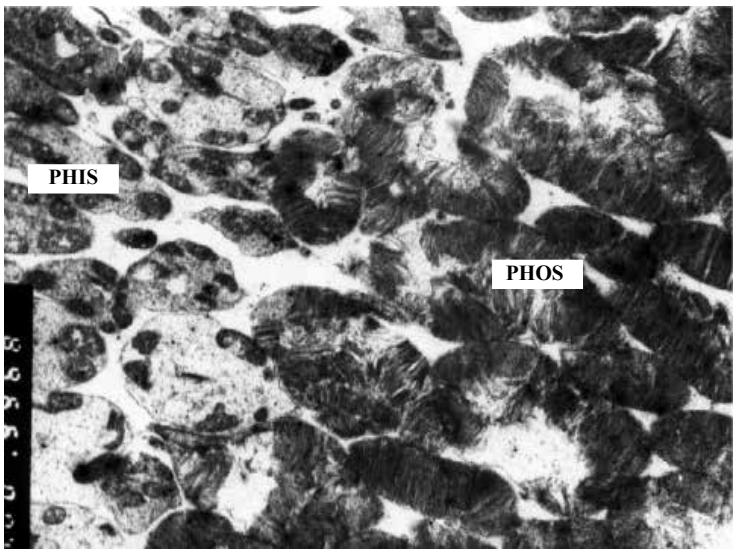

Fig. 2. Electron micrograph showing ultrastructure of cells of the rat chorioretinal complex 3 months after injection of the alcohol mixture with a methanol dose of 2.5 g/kg. Photoreceptor cells show destraction membranes of outer segment discs and signs of intercellular edema of these cells are seen. Original magnification ×6,000. Notes: PHIS, photoreceptor inner segments; PHOS, photoreceptor outer segments

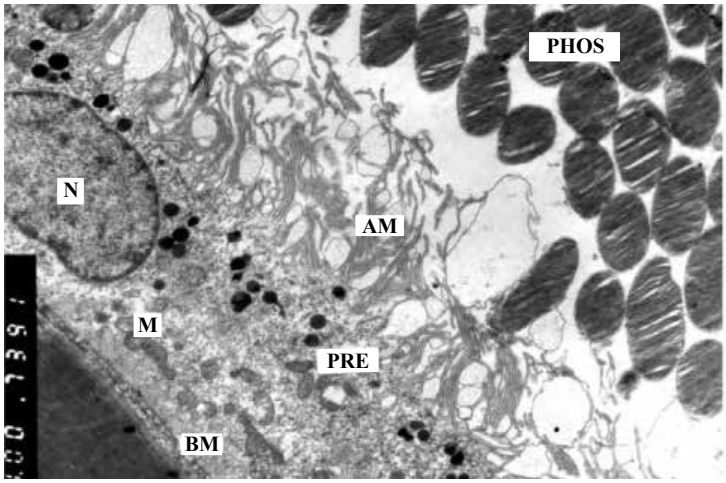

Fig. 3. Electron micrograph showing ultrastructure of cells of the rat chorioretinal complex 3 months after injection of pure methanol at a dose of 2.5 g/kg. Restoration of normal ultrastracture of retinal pigment epithelium cells. Original magnification ×3,000. Notes: BM, Bruch's membrane; RPE, retinal pigment epithelium; AM, apical microvilli; N, nucleus; M, mitochondria; PHOS, photoreceptor outer segments.