Preventive Efficacy of Oxidized Dextran and Pathomorphological Processes in Mouse Lungs in Avian Influenza A/H5N1

O. V. Potapova, V. A. Shkurupiy, T. V. Sharkova, A. V. Troitskiy, N. G. Lusgina, and A. M. Shestopalov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 12, pp. 650-653, December, 2010
Original article submitted January 18, 2010

Oxidized dextran (60 kDa) exerts a pronounced preventive effect in laboratory mice infected with avian influenza subtype H5N1 A/Goose/Krasnoozerskoye/627/05 virus, which manifested in a significant increase in mouse lifetime (by 24.4%) and mortality rate (3.3-fold). This was probably related to significant alleviation of pathological changes in the lungs and severity of hemodynamic and inflammatory complications and early fibrosis.

Key Words: *influenza virus A/H5N1 A/Goose/Krasnoozerskoye/627/05; lungs; oxidized dextran*

Viral diseases (influenza and acute respiratory viral infection) remain in the focus of modern medicine. Zoonotic variants of influenza viruses, *e.g.* viruses transmitted from birds, natural carriers of all known virus subtypes, are especially dangerous [1]. Thus, A/H5N1 virus can be transmitted directly from ill bird to humans and can induce severe damage to various organs, primary to the lungs, associated with high mortality (>60%) [4].

High antigen variability of influenza viruses and improper drug treatment lead to the development of resistance of circulating strains to available antiviral compounds [7,8]. Strains detected in the Russian Federation are not the exception [9]. In light of this, an important problem is creation of new nonspecific preventive anti-influenza drugs not directly affecting the virus, which excludes the possibility of resistance development. Taking into account previous data on the effects of H5N1 virus on cytokine levels and exhaustion of the lymphoid tissue in the central and peripheral immune organs [3], we can assume that immunomodulators may appear the most promising drugs for prophylaxis of highly pathogenic influenza, because they are capable of simultaneously inducing production of endogenous IFN and activate cells of the mononuclear phagocyte system (MPS).

According to previously obtained data [2], oxidized dextran (OD) with a molecular weight of 60 kDa, which is tropic for vacuolar apparatus, particularly in MPS cells, can be regarded as a promising compound [6]. In addition, along with the absence of toxic properties, dextrans were shown to exert detoxification and hepatoprotective properties and intensify plastic properties in cell promoting regeneration in damaged organs [2,6]. Since OD administration has no direct effects on the virus, the risk of the development drug resistance in influenza viruses is excluded [6].

Here we studied pathomorphological features of lung damage in mammals infected with avian influenza virus A/H5N1 (strain A/Goose/Krasnoozerskoye/627/05, AIV) detected in Novosibirsk region and evaluate the efficacy of preventive OD administration.

MATERIALS AND METHODS

The study was carried out on 2-month mongrel white male mice (n=90; laboratory animal nursery, Vector State Research Center of Virology and Biotechno-

Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** potapova@soramn.ru. O. V. Potapova

0007-4888/11/1506-0707

logy, Federal Service for Surveillance on Consumer Rights). The use of mongrel animals as experimental model eliminates the effects of genetic determination on pathological manifestations of inflammation. The animals were kept under standard conditions with free access to food and water in the nursery.

The selected A/H5N1 influenza virus strain was detected in Novosibirsk region in 2005, it is highly pathogenic and replicates in all organs of mammals (mice) [2]. All virological experiments were carried out at Vector State Research Center of Virology and Biotechnology, Federal Service for Surveillance on Consumer Rights (Novosibirsk region, Kol'tsovo). Taking into account the fact that influenza virus A is mainly airborne virus in mammalian population and in humans, the mice were infected with intranasal dose of 10 MLD_{50} (minimum lethal dose) in 50 μl phosphate buffer (pH 7.2).

Three groups (30 animals per group) were formed. Group 1 (intact mice) served as the control. Group 2 mice were infected with AIV A/H5N1 (H5N1); group 3 animals were infected with AIV A/H5N1 after double preventive OD administration 3 and 1 day before the infection in a dose of 1000 mg/kg (H5N1+OD). Twenty animals in each group were used for evaluation of medium lifespan and mortality. The mice were considered to survive if they were alive for 14 days after infection.

The lungs of the experimental animals served as the object for pathomorphological investigation, since they are the main target organ for influenza virus A [5,10]. Lung specimens were taken 1, 3, 6, and 10 days after infection from 10 animals at each time point. The control mice were sacrificed on day 10 by cervical dislocation under ether anesthesia.

For light microscopy, the preparations were fixed in 10% formalin, dehydrated in ascending alcohols, and embedded in paraffin. Deparaffinized 4-5-μ sections were routinely stained with hematoxylin and eosin and van Gieson's picrofuchsin. For immunohistochemical study, the sections were deparaffinized and rehydrated, antigens were unmasked in a microwave oven at 700 W, endogenous peroxidase was blocked, and the sections were incubated in blocking serum and then with specific monoclonal antibodies against $CD4^+$, $CD8^+$, $CD68^+$ (Novocastra).

The preparations were examined under an Axio-Imager A1 microscope and documented using Axio-Cam MRc camera (Carl Zeiss) and AxioVision software (rel. 4.8). Morphometric analysis was performed using a closed test-system consisting of 100 dots with the area of 1.68×10^5 μ^2. Volume density and cellular composition of inflammatory infiltrates, numerical density of the vessels and the relative number of thrombosed vessels, volume density of hemorrhages, and volume density of fibrotic tissue were calculated. Statistical treatment of the data was performed using software Statistica 5.0. Significance of the differences between the mean values was assessed using Student's *t*-test; differences were significant at $p<0.05$.

RESULTS

Mortality in mice infected with AIV A /H5N1 was 73.3%, mean lifetime (MLT) after infection was 9.37 days (Fig. 1), which indicated high pathogenicity of AIV. Preventive OD administration in a dose of 1000 mg/kg increased MLT by 24.2% and 3.3-fold decreased mortality in comparison with the corresponding values in mice receiving no OD (Fig. 1).

Histological examination of lung specimans obtained from experimental animals infected with AIV revealed paretic dilatation of vessels, interstitial and alveolar edema with hyaline membrane formation, multiple hemorrhages, numerous confluent inflammation infiltrates, early peribronchial and perivascular fibrosis. Focuses of acute emphysema were observed at the periphery of sections, which apparently was the compensatory response to functional insufficiency of the organ.

Morphometry of the lung preparations in the dynamics of the infectious process revealed an increase in numerical density of vessels at all time points in comparison with that in intact animals and progression of dystrophic changes in the form of fibrinoid changes in vessel walls with evidences of hypercoagulation. All changes in H5N1+OD mice were significantly lower in comparison with H5N1 group (Table 1). The relative number of thrombosed vessels in H5N1 group on day 3 was 19.54% from the total number of vessels (Table 1) and was 6-fold higher than in H5N1+OD group (Table 1). The decrease in this parameter was

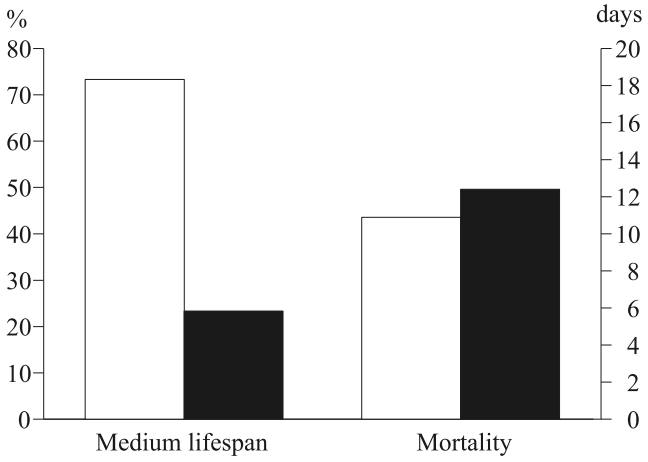

Fig. 1. Preventive efficiency of OD in experimental infection of outbred mice. Light bars: H5N1 group, dark bars: H5N1+OD group.

TABLE 1. Structural Changes in the Lungs of Mice Infected with Influenza Virus A/H5N1 after Preventive OD Administration (*M*±*m*)

Parameter	Days after infection	Experimental groups		
		control (intact animals)	H5N1	H5N1+OD
Nai vessels in 1.68×10^5 μ²	1	—	17.04±0.27	15.92±0.44^{o}
	3	—	19.74±0.30^{+}	18.82±0.49^{+}
	6	—	19.29±0.34	17.75±0.38^{o}
	10	15.13±0.17	18.61±0.26*	15.88±0.55^{+o}
Thrombosed vessels, %	1	—	11.72±0.56	3.07±0.21^{o}
	3	—	19.54±0.58^{+}	3.27±0.24^{o}
	6	—	15.45±0.23^{+}	2.86±0.32^{o}
	10	1.26±0.34	14.97±0.43*	2.27±0.28*^{o}
Vv hemorrhages, %	1	—	1.12±0.27	0.40±0.15^{o}
	3	—	4.02±0.25^{+}	0.71±0.16^{o}
	6	—	4.17±0.22	1.32±0.16^{+o}
	10	0.18±0.11	4.50±0.24*	1.27±0.14*^{o}
Vv infiltrates, %	1	—	21.86±0.60	9.42±0.31^{o}
	3	—	23.26±0.52	11.45±0.66^{+o}
	6	—	26.82±0.61^{+}	10.42±0.72^{o}
	10	0.17±0.09	32.88±0.98*$^{+}$	8.89±0.67*^{o}
Vv fibrosis tissue, %	1	—	0.28±0.16	0.32±0.12
	3	—	2.56±0.45^{+}	0.34±0.15^{o}
	6	—	4.22±0.51^{+}	0.41±0.16^{o}
	10	0.09±0.08	7.91±1.37*$^{+}$	0.61±0.21*^{o}

Notes. Nai: numerical density, Vv: volume density. $p<0.05$ in comparison with: *control, $^{+}$previous term, ^{o}between groups H5N1 and H5N1+OD.

observed in all experimental groups between day 3 and day 10. On day 10 after infection, the relative number of thrombosed vessels in the lung tissue in H5N1 group was 11.9-fold higher than in the control and 6.6-fold higher than in H5N1+OD group (Table 1). Reduction in vessel thrombosis intensity and decrease in the volume density of hemorrhages in lung stroma after preventive OD administration can be associated with better preservation of vessel endothelial lining as a result of indirect OD effect, as well as with dextran ability to improve rheological properties of the blood and to improve reparation processes [6], which also contributes in reduction of thrombosis intensity to microvessels (Table 1).

The dynamics of changes in the volume density of inflammatory infiltrates in the lung interstitium was substantially different in different groups of animals. In H5N1 group, this value permanently increased and on day 10 was 1.5-fold higher than on day 1 postinfection and 3.6-fold higher in comparison with the corresponding value in H5N1+OD group (Table 1).

Cellular composition of inflammatory infiltrates in the lungs was virtually the same in all experimental groups and was characterized by the predominance of macrophages and cytotoxic $CD8^{+}$-lymphocytes involved in lung tissue alteration [5]. The number of $CD68^{+}$-polynuclear cells (appearing in infiltrates, apparently, due to delay in caspase-dependent macrophage apoptosis [3]) in the lung of H5N1 group mice surpassed the corresponding value in H5N1+OD group 2-fold as soon as on day 1 postinfection (4.76 and 2.33%, respectively). In addition, in group H5N1 this parameter increased 1.6-fold (to 7.54%) from day 1 to day 6, and the number of polynuclear cells decreased to 5.27% (by 70%) from day 6 to day 10. Alternatively, in group H5N1+OD, the decrease in the count of polynuclear cells was observed from day 1 to day 10 postinfection (from 2.33 to 1.31%). It possibly at-

tests to indirect antiviral activity of OD reducing the number of viruses capable of penetrating into macrophages and stimulating their fusion with the formation of polynuclear cells. The decrease in this value in the group receiving OD can be also associated with activation of the mechanisms of natural resistance realized through virus elimination by apoptosis of infected macrophages.

Early and rapidly progressing tissue fibrosis is a significant phenomenon in the pathogenesis of growing functional insufficiency of the respiratory system in experimental animals, which can be indicative of fibroblast activation under conditions of ischemia and under the influence of proinflammatory cytokines. Fibrosis was observed primarily in the peribronchial and perivascular areas, at the sites of inflammatory cell infiltrates, where great amounts of mononuclears were concentrated. Volume density of fibrotic tissue in the lungs of mice from H5N1 group during the whole period and on day 10 was 13-fold higher than in group H5N1+OD (Table 1), which probably attests to an increase in the IFN-γ level [4], as well as for antifibrotic effects of OD previously demonstrated in experiments on mice infected with *M. tuberculosis* [2,6].

Thus, it can be concluded that OD in a dose of 1000 mg/kg exerts preventive effect in laboratory mice infected with AIV, when administered two times, 3 and 1 day before the infection.

The work was carried out within the framework of Federal Targeted Scientific-Technical Program "Scientific and Educational Cadres of Innovative Russia at 2009-2013" (state contract No. 02.740.11.0709).

REFERENCES

1. D. K. L'vov, S. S. Yamnikova, I. T. Fedyakina, *et al.*, *Vopr. Virusol.*, No. 3, 17-24 (2004).
2. O. V. Potapova and V. A. Shkurupiy, *Bull. Exper. Biol.*, Suppl. 1, 113-115 (2008).
3. O. V. Potapova, V. A. Shkurupiy, N. G. Luzgina, *et al.*, *Bull. Sib. Otd. Ross. Akad. Med. Nauk*, No. 5, 174-178 (2008).
4. A. M. Shestopalov, K. A. Sharshov, A. V. Zaikovskaya, *et al.*, *Bull. Exp. Biol. Med.*, **145**, No. 9, 317-320 (2008).
5. L. V. Shestopalova, V. A. Shkurupiy, T. V. Sharkova, *et al.*, *Vestn. NGU*, **6**, No. 3, Part 2, 3-10 (2008).
6. V. A. Shkurupiy, *Tuberculous Granulomatosis. Cytophysiology and Targeted Drug Delivery* [in Russian], 109-150, Moscow (2007).
7. C. L. Cheung, J. M. Rayner, G. J. Smith, *et al.*, *J. Infect. Dis.*, **193**, No. 12, 1626-1629 (2006).
8. M. D. de Jong and T. T. Hien, *J. Clin. Virol.*, **35**, No. 1, 2-13 (2006).
9. S. B. Iatsyshina, A. M. Shestopalov, V. A. Evseenko, *et al.*, M*ol. Gen. Mikrobiol. Virusol.*, No. 1, 26-34 (2008).
10. N. Kuiken, G. F. Rimmelzwaan, G. Van Amerongen, A. D. Osterhaus, *Vet. Pathol.*, **40**, No. 3, 304-310 (2003).