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Dynamics of Inhibitors and Proteinases at the First Stage of the Mice Organism Lesion with Experimental Influenza Infection Contamination

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Abstract

The present study examines the impact of an experimental influenza infection when out bred white mice were infested by A flu virus on the changes of proteinase activity in pulmonary tissue and blood serum of animals. These materials study concurrently the activity of these proteinases inhibitor.

Keywords: Flu virus; Trypsin-like Proteinase;Inhibitor of proteinases

Theme Urgency

Interaction of a virus and a cell is a single whole, representing a set of intrinsic relations of two opposite principles. The net result of this interaction is genetical defined both by the owner and the originator and depends on regulation of both the process of virus reproduction and protective forces of an organism. The balance between these processes determines the interaction outcome: the destruction of the owner, its absolute recovery or formation of the chronic form of an infection contamination [1-6].

It is known that in replication of a flu virus; the precursor of the hemagglutinin is synthesized, which splitting on big and small subunit is necessary for acquisition of infectious activity by a virus. The splitting is carried out by cellular Trypsin-like proteinases [7-11]. During influenza infection contamination there is a change of specific cellular proteinases evidencing co-operative interaction of virus proteins with cellular ferments. Most likely, the dynamics of proteinases level change is bound to a reproduction of the virus and its proteolytic activation by the same proteinases. However, this phenomenon is too little studied; there are only the reports [12] about depression of proteinases activity in early periods and its increase in later periods of infection upon infestation of chicken embryos with viruses of flu and Sendai [12]. The purpose of this work was to study the action of experimental influenza infection in white mice and chicken embryos infested with flu viruses A on the dynamics of proteinases and inhibiting activity.

Research Methods

In researches, flu virus A/PR/8/34 (H1N1), adapted for a pulmonary tissue of white mice (the virus is received from laboratory of museum strains of Institute of virology of D.I.Ivanovskiy of the Russian Academy of Medical Science) was used. The infectious titer of virus was 7lg EID50/0.2ml. Besides, we used white BALB/C mice weighing 7-9 and 16-17g. The animals were infested by A/PR/8/34 in intranasal way in volume of 0.05ml under an easy ether narcosis diluted 10-2 that corresponded to 20 LD50 infectious virus dose. Such dose provided 100% destruction of animals in 6 days after infestation. The animals (5 mice in each group) were slaughtered; their lungs and blood were removed in 15, 30 minutes, 1,6h and further in 1-6 days after infestation. The lungs were flushed twice in the cold phosphatic buffer pH 7,5 and triturated in a mortar, suspended in the phosphatic buffer (1ml per 1 lung), homogenized by ultrasound in mode 7 of teh device High Intensity Ultrasonic Procession, Chicago, «Cole Parmel» (USA), centrifuged at 10000 rpm on whizzer RC5c, manufactured by «Sorvall Instruments», rotor SS-34, within 1 hour, at temperature +4 °C. Supernatant and blood serum was used for definition of activity: infectious, proteinases and inhibiting proteinases. Activity of a Trypsin-like proteinases determined by K.M.Veremeenko method [13] modified by S.V.Vovchuk [14]. Identification of activity of proteinases inhibitors in homogenates of lungs and in blood serum made the caseic method offered by A.P.Levitsky [15].

Infectious titer of virus in lungs of the infected mice determined by infestation of 9-10 days chicken embryos and expressed in lg EID50/0,2ml. Hemagglutination reaction was made under the standard method. The received data were processed in Microsoft®Excel.

Results and Discussion

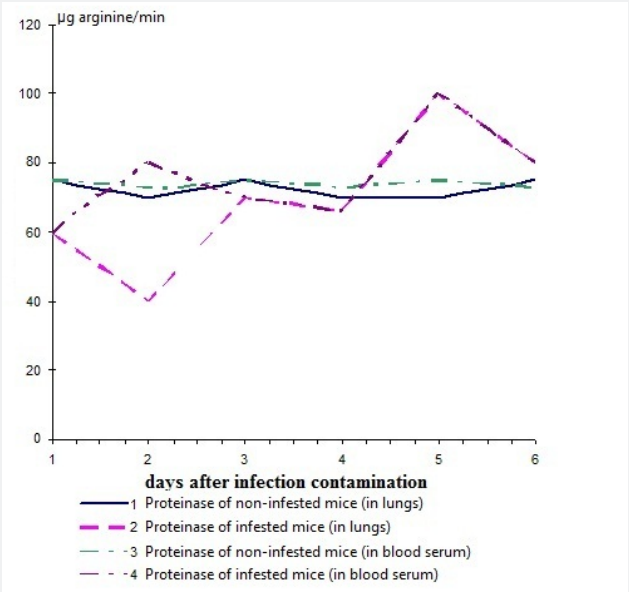

Figure 1: Dynamics of change proteinase activity in lungs and blood serum of the white mice infested with a flu virus A/PR/8/34 in flow of 6 days (the total data of experiences with mice in mass of 6-9g and 16-17 is presented).

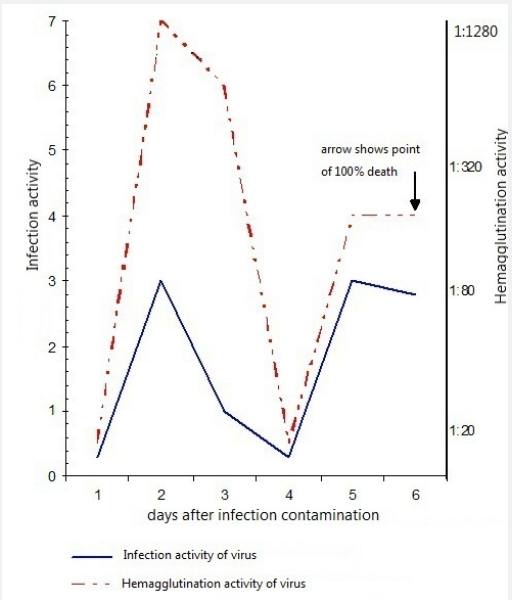

Figure 2: Infectious and hem agglutination activity of a homogenate of the lungs of white mice infested with a flu virus A/PR/8/34 in allantoic fluid of chicken embryos.

In lungs and blood serum of not infested animals, the level of a Trypsin-like proteinases and its inhibitor was not exposed to evaluated changes during the observation period (6 days) and was in balance. During intranasal infestation of mice by flu virus of A/PR/8/34 (H1N1) strain, it was revealed that starting from 15 minutes and up to 6 h after infestation, the proteinases activity in lungs and blood serum decreased, and activity of an inhibitor, on the contrary, raised, especially in lungs (Figure 1). Hem agglutination and infectious activity during the first 6 h after infestation was not determined. During the subsequent period, the proteinases activity increased, and by 3rd day reached a reference level. During this period, hem agglutination and infectious activity sharply increased. By 48 h after infestation, infectious and hem agglutination activity reached its maximum value (Figure 2). By 96 h after infestation, infectious and hem agglutination activity sharply decreased and the mortality of infested animals began. The infested animals who remained alive by 4th day after infestation, the repeated augmentation of proteinases activity by 5-6th day became perceptible, thus both infectious and hem agglutination activity was repeatedly increased. By 6th day, control group of flu virus animals perished.

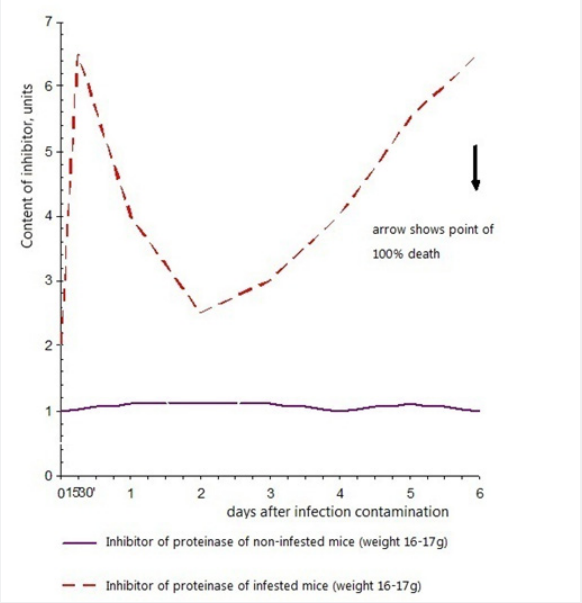

Figure 3: Activity of an inhibitor of Trypsin-like proteinases in lungs of mice infested with flu virus A/PR/8/34.

Thus, the lungs of the infested mice had the correlation between accumulation of infectious virus and depression of proteinases activity by the 2nd day after infestation. At the same time, the augmentation of virus titer by the 5th day in a pulmonary tissue and blood serum of infested animals also coincided with rising proteinases activity. In search of the causes of proteinases activity depression on the 1st day after infestation, we determined inhibiting proteinases activity both in pulmonary tissue and blood serum of white mice. As we can see in Figure 3, in not infested animals, proteinases activity and proteinases inhibiting activity in blood serum were at stable level. In a pulmonary tissue, proteinases inhibiting activity was found in very insignificant quantities. In pulmonary tissue and blood serum of infested animals, proteinases inhibiting activity

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after infestation sharply increased by 6 h and dropped by 2nd day, and then gradually raised by 5th day.

Thus, it is possible to explain the depression of proteinases activity during the first hours after infestation by augmentation of proteinases inhibiting activity (it was clearly observed in pulmonary tissue of infested mice), and it's rising by 2-3rd days of development of infection contamination by depression of inhibiting activity. However, simultaneous increase of proteinases and inhibiting activity by the 5th day after infestation evidence other causes of proteinases activation in this period development of infection. The cited data shows that in blood serum of not infested animals, the level of proteinases activity and proteinases inhibiting activity was in balance which was broken at infestation by a flu virus. It is possible to secure three periods in infectious process which are characterized by different degree of virus reproduction, level of proteinases activity and level of proteinases inhibiting activity. The most critical changes occurred during the first hours after infestation. 6 h after infestation, the quantity of proteinases both in lungs and in serum of the infested animals dropped, and proteinases inhibiting activity increased. Thus, it is possible to assume that reduction of proteolytic activity is related to the respective accumulation of proteinases inhibitor. Apparently, flu virus infested cells induce appearance of inhibitor both in pulmonary tissue, and in blood serum.

Conclusion

During the period of the biggest accumulation of infectious virus (2 days after infestation), proteolytic activity dropped, however, this decrease was accompanied by reduction of inhibitor activity. Probably, one of the causes is use of cellular Trypsin-like proteinases for proteolytic virus activation in the lungs of the infested mice and pool depletion in infested animal's organism. Repeated decrease of proteinases activity by 5-6 days is caused by other causes, apparently, by bacterial flora overlay.

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