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CLINICAL AN D MORPHOLOGIC STUDIES ON THE GUINEA PIG EYE INFECTED WITH HUMAN INFLUENZA VIRUS STRAINS OF DIFFERENT VIRULENCE

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Summary. — Human influenza virus serotypes H3N2 and H2N2 caused iridocyclitis and uveitis when inoculated at doses of $10^{6-6.5}$ EID_{50} into the guinea pig eye anterior chamber. Virulent influenza virus strains and their attenuated variants prepared by passaging in chick embryos (CE) have been compared in this model. These studies showed that virulent viruses cause more severe damage in the eyes than the attenuated strains.

Key words: attenuation; influenza virus; pathogenicity; iridocyclitis; uveitis

Introduction

At present, several animal models for comparative study of virulent and attenuated influenza virus strains have been described. However, none of these models so far has provided an universal tool for differentiation of strains with unequal virulence for humans (Ghendon *et al.*, 1984). In experiments using ferrets, infant rats, mice, hamsters and monkeys, influenza viruses have been basically administered into respiratory organs (Sukhinin *et al.*, 1984; Ghendon, 1984). Recent clinical opthalmological findings allowed to correlate the disease of the vascular system of human eye with influenza virus (Nabieva, Suleimanov, 1972; Belogubko *et al.*, 1979), as well as picornavirus infection (Koroleva *et al.*, 1982, 1984; Khoroshilova-Maslova, Polyn, 1982). Moreover, it has been shown in rabbits (Vinogradova *et al.*, 1977) that the inoculation of influenza virus into the eye anterior chamber is followed by the detection of virus antigens in the eye teguments.

Our aim has been to study the changes occuring in the guinea-pig eye after administration of human influenza virus strains of different virulence into the anterior chamber. It has been found that influenza viruses induced iridocyclitis and uveitis, while attenuated strains caused much less marked changes as compared to the virulent ones.

Materials and Methods

Viruses. Virulent influenza virus strains A/Hong Kong/1/68 (H3N2) (prepared in 1968, underwent one passage) and A/Victoria/35/72 (H2N2) (prepared in 1972, underwent 35 passages), and their variants attenuated for humans, prepared by passages in chick embryos at 36 °C: A/Hong

Kong/1/68/21 (underwent 21 passages) and A/Victoria/35/72/30 (underwent 30 passages). These variants have been kindly provided by Dr. Zhilova (All-Union Research Institute of Influenza Leningrad, U.S.S.R.). In addition, we used the strain A/Ann Arbor/6/60 and its cold-adapted attenuated variant prepared by passaging at low temperature and kindly supplied by Dr. Maassab, U.S.A. All viruses were grown in CE inoculated into allantoic fluid.

Infection of animals. Guinea pigs weighing 300–350 g had been obtained from the Central Nursery of the U.S.S.R. Academy of Medical Sciences. The virus was given into the eye anterior chamber in a volume of 0.1 ml after removal of equal amount of chamber fluid. The animals were examined by means of a stereomicroscope at 16× magnification. Control guinea pigs received the allantoic fluid of uninfected CE in the same manner. Twenty guinea pigs were used for each trial. The infected animals were observed for 10–28 days; on days 7, 14, 21, and 30 some guinea pigs were sacrificed and their eyes were histologically examined.

Pathohistological studies. The guinea pigs were killed by ether sulphate. The enucleated eye was placed into 10% formalin solution. After treatment with a series of alcohols the eyes were embedded into paraffin; 10μm thick sections were stained with haematoxylin-eosin.

Results

Examination of virulent influenza virus strains

Clinical observations have shown (Fig. 1) that on day 2 after infection of guinea pigs with virulent influenza virus strains the anterior chamber humor was significantly opacified, the colour of iris was changed and a pericorneal vascularization appeared around the limbus. Then, on days 3 or 4 the vascular

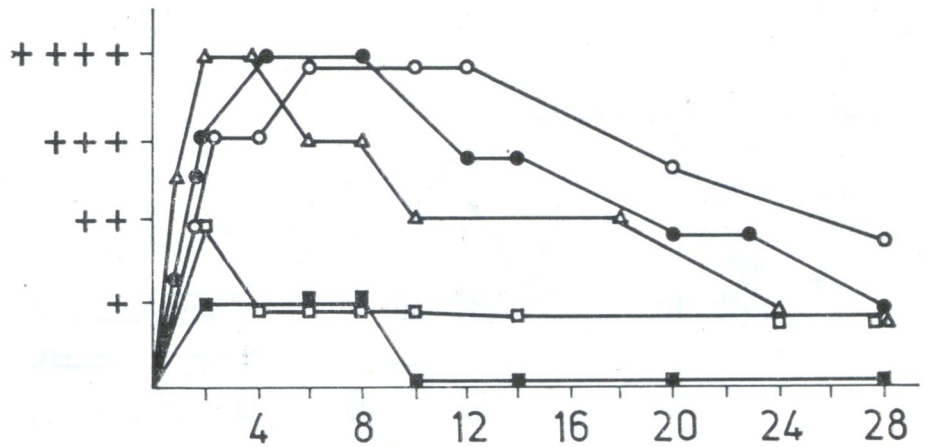

Fig. 1.

Intensity of clinical changes after administration of virulent influenza virus strains into the guinea pig eye anterior chamber

Ann Arbor/6/60 (○), A/Victoria/35/72 (●), A/Hong Kong/1/68 (△), allantoic fluid of uninfected chick embryos (□, ■)

Abscissa: days post-infection; ordinate: intensity of clinical changes

(+) – macula corneae, opalescence of chamber humor, (+ +) – changes of iris colour, pupil shape, pericorneal injection, delayed response to light, (+ + +) – corneal leukoma, changes of iris colour, vascularization around limbus, delay and absence of pupil response to light, (+ + + +) – changes of the iris colour, corneal oedema and caligo corneae, ingrowth of vascular network into cornea, absence of pupilar response to light.

injection surrouding the limbus was enhanced, and an expanded network of dilated vessels appeared in the limbus area. The iris usually changed its colour, the pupil was myotic and in some cases failed to respond to light. On week 2 post-infection (p.i.) a small macula or leukoma was often found in the cornea. Corneal changes were local and observed in the injection site. All 3 virulent human influenza virus strains tested caused similar changes.

At microscopic examination the earliest changes were observed on day 2 p.i., they reached their maximum on days 4 or 5; by the end of second week residual changes in the ciliary body and iris could be seen only. Within the first week pathologic changes consisted of fibrinous exudation of the iris and ciliary body with lymphocytic and mononuclear infiltration in the stroma. The infiltration was mostly diffuse, but in some cases it was predominating near the iris root and Schlemm's chanel (Fig. 2). Iris chromatophores were partially destroyed, the pigment was seen outside cells and fibrinous exudate was located on the surface, the vessels of the iris stroma, choroid and ciliary bodies were dilated with an inflammatory infiltrate surrounding them (Fig. 3). By the second week, the inflammatory infiltration and exudation of the iris stroma and choroid were decreased, as was the destruction of pigment layer

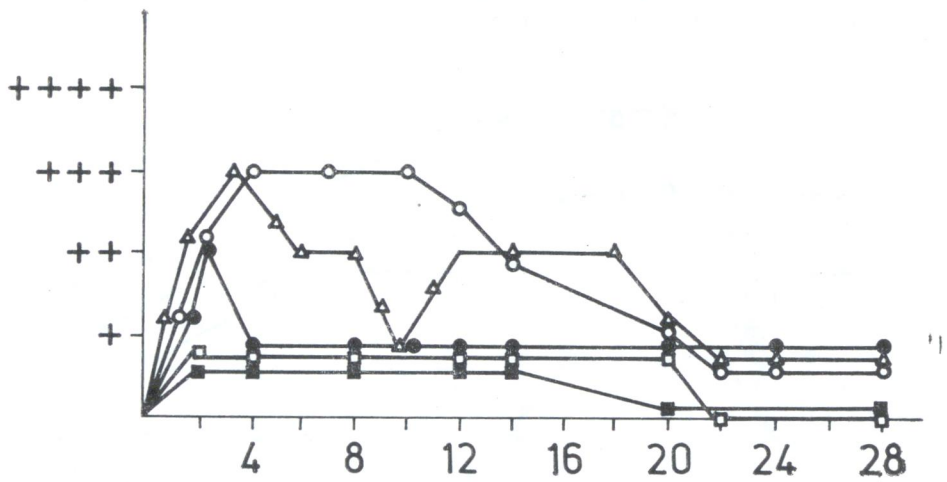

Fig. 6.

Intensity of clinical changes after infection of the guinea pigs into the eye anterior chamber by attenuated influenza virus strain

Abscissa: days post-infection; ordinate: intensity of clinical changes

Cold-adapted strain Ann Arbor/6/60 (○), attenuated strain A/Victoria/35/72/30 (●), attenuated strain A/Hong Kong/1/68/21 (△), allantoic fluid of uninfected chick embryos (□, ■). (+) — macula corneae, opalescence of chamber humor, (++) — changes of iris colour, pupilar shape, pericorneal injection, delayed response to light, (+++) — corneal leukoma, changes of iris colour, vascular corona around the limbus, delayed response to light, (++++) — changes of iris colour, corneal oedema and caligo corneae, ingrowth of vascular network into cornea, no pupilar response to light.

chromatophores. Vascular inflammatory infiltration was most prolonged in the ciliary body and in choroid.

Corneal changes were found near the injection scar where local destruction of epithelial and stromal elements was noted. At the third week p.i. only a narrow fibroblast strip was seen along the injection track. In two cases, in addition to above-mentioned changes a destruction of the retinal pigment layer (chorioretinitis) was observed in the guinea pig eye uveal tract (Figs. 4 and 5).

Examination of attenuated influenza virus strains

In the 2nd trial 3 attenuated influenza virus strains have been tested. The first changes have been clinically registered on days 2 or 3 of the experiment (Fig. 6). However, they were not as intensive as after infection with virulent strains and were much less marked. Local corneal opacification of macular type, pericorneal injection, and changes of the iris colour were observed. These signs persisted for 3 or 4 days and then gradually disappeared. After 2 days, in general, no clinical changes could be detected.

Microscopic examination has detected granular fibroblasts which replaced the destroyed corneal tissue at the injection site, as well as limited inflammatory infiltration in the stroma of ciliary body and iris (Fig. 7). Changes of the pigment layer were observed in few chromatophores only, and no perivascular infiltration could be seen. By the end of week 2, residual restorative signs were observed in the ciliary body and in the iris.

Twenty control animals inoculated with allantoic fluid from uninfected CE showed changes related to traumatic injury at the inoculation site. Corneal macula and local injection of conjunctival vessels were observed. No changes of either the iris color or the pupil size were found; pupil response to light was unimpaired. Microscopic findings indicated no inflammatory changes in the eye uveal tract. Scarring replacement of the destroyed tissue in the inoculation site was seen in the cornea (Fig. 8).

Discussion

It has been shown that administration of different influenza virus strains serotypes H3N2 and H2N2 at a dose of $10^{6-6.5}$ EID_{50} into the guinea pig eye anterior chamber causes local inflammation in the uveal tract. The changes observed — iridocyclitis, uveitis, and chorioretinitis — were specific as was evident from the absence of typical changes after injection of uninfected chick embryo allantoic fluid into the eye anterior chamber, as well as from the possibility to isolate the virus, although rarely, from the anterior chamber on days 2—5 p.i. (in preparation).

It is known that guinea pigs are resistant to human influenza virus administered by intranasal, intravenous and intramuscular routes, since they do not replicate infectious virus (Sukhinin *et al.*, 1984). Probably, administration of the virus directly into the eye chamber allows it to penetrate through blood tissue barriers resulting in a short-lasting virus reproduction

in the epithelial lining of anterior chamber wall. Infection of guinea pigs with virulent influenza virus strains was followed by the development of focal keratitis and uveitis, affecting all regions of the uveal tract. In especially severe cases damage of the uveal tract was associated with chorioretinitis. Meanwhile, the administration of attenuated variants of the same influenza virus strains caused slight iridocyclitis in anterior region of the uveal tract and local posttraumatic corneal changes.

It cannot be ruled out that in some cases of virulent influenza viruses inoculation at early intervals (1st week) we found lesions due to the toxic effect of virus on the anterior chamber wall lining cells. However, the duration of pathologic process in different regions of the eye uveal tract, the character of the observed changes, as well as the reproduction of the virus in these structures, indicate that the specific pathogenic effect of the viruses tested predominated.

Localization of histologic changes, the course and severity of clinical signs allowed to differentiate influenza virus strains of unequal virulence for humans. It is important that the correlation between the virulence of influenza virus and its ability to cause clinical and pathological changes of the eye of different degree was observed with all three pairs of virulent and attenuated influenza virus strains tested. Further studies of virulent and attenuated influenza virus strains and their recombinants with known genome composition that are presently underway to show whether this model can be used for the estimation of influenza virus attenuation in humans.

The induction of pathologic lesions in the guinea pig eye uveal tract by human influenza virus may probably be used for screening of chemotherapeutic agents in the treatment of uveitis during human influenza infection.

References

Belogubko, E. N., Georgiladze, T. U., and Kuksa, V. D. (1979): Study of the role of some viruses in eye diseases, pp. 95—96 (in Russian). *All-Union Congress of Ophthalmologists* **4**, Moscow.

Ghendon, Y. Z. (1984): Molecular characteristics and biological properties (genetic markers) of candidate strains for preparation of live influenza virus vaccines. *Bull. Wrld. Hlth Org.* **62**, 493—499.

Ghendon Yu. Z. Klimov A. I. Lisovskaya K. V. Aleksandrova G. I. and Medvedeva T. I. (1984): Molecular genetic analysis of cold-adapted strain — an attenuation donor for live influenza virus vaccine for use in children recombinants prepared on its basis and isolates isolated from vaccinated children (in Russian). *Vop. Virus.* **29**, 22—28.

Khoroshilova-Maslova I. P. and Polyn V. D. (1982): Pathohistology of ECHO-9 virus uveitis in children pp. 59—61 (in Russian). In: *Viral Eye Diseases*, Moscow.

Koroleva G. A. Lakshevich V. A. and Savinov A. P. (1984): Damage of the eye uveal tract (uveitis) caused by enteroviruses in humans and monkeys (in Russian). *Vop. Virus.* **29**, 447—453.

Koroleva, G. A., Savinov, A. P., Krichevskaya, G. I., Vinogradova, L. A., Zayecevy, N. S., and Sobolev, S. G. (1982): Specific features of virus ECHO-19 strain Rad/40 causing experimental damage of the eye teguments (uveitis) in Javanese monkeys (in Russian). *Vop. Virus.* **27**, 81—87.

Nabieva, Z. A., Suleimanov, A. G. (1972): Eye damages during influenza (in Russian). *Transact. Azerbaijanian Res. Inst. Ophthalm.* **6**, 107—110.

Sukhinin, V. D., Ovdeichuk, F. A., Osipova, M. V., Belyavskaya, S. V., Bichurina, M. A., Shvarsman, Y. S. and Fridman, E. A. (1984): Immunity and nonspecific resistence to influenza and