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INFLUENCE OF TICK-BORNE ENCEPHALITIS AND WEST NILE VIRUSES ON THE CHROMOSOMES OF PIG KIDNEY CELLS

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Summary. — Changes of the mitotic index (MI) were studied in pig kidney (PS) cells infected either with tick-borne encephalitis (TBE) virus strains Hypr and Ir-32, or with West Nile (WN) virus prototype strain, strains K-99 and E-13. The cell division was arrested by the infection (metaphase barrier). The effect of TBE virus strains was manifested by the appearance of lagging chromosomes while the action of WN virus strains by colchicine-like metaphases. The prototype TBE virus strain Hypr affected the chromosomes and mitotic apparatus of PS cells less markedly than did the freshly isolated Ir-32 strain.

Key words: *Flaviviruses*; *tick-borne encephalitis virus*; *West Nile virus*; *mitotic index*; *mitotic apparatus*; *metaphase barrier, chromosomes*

Introduction

As shown in our previous study, TBE virus infection of chick embryo cells (CEC) was accompanied by structural chromosome changes (Varadinova and Grešíková, 1980). In explants of human embryonal cerebellar and retinal tissues inoculated with TBE virus, a decrease in the MI was observed at late intervals after infection (Mayer and Mitrová—Bellová, 1969). In contrast, an increase in the MI has been reported at early intervals of infection with TBE virus strains (Zalkind and Zaslavskii, 1962; Monastireva and Blumkin, 1969; Gordeeva and Chekova, 1979; Varadinova and Grešíková, 1980). Therefore, the present study was aimed to compare the changes of MI in PS cells caused by TBE and WN virus infections.

Materials and Methods

Viruses. Two strains of TBE virus were used; the prototype strain Hypr (Pospíšil *et al.*, 1954) in its 60th passage and the Ir-32 strain (Grešíková and Sekeyová, 1980) in its 2nd passage. Following WN virus strains were employed: the prototype WN virus strain (Taylor *et al.*, 1956) kindly supplied by Dr. Casals (The Rockefeller Foundation Virus Laboratory, New York, U.S.A.) has undergone 12 mouse passages since its 7th passage in 1963 at the WHO Collaborating Centre for Arbovirus Reference and Research; the K-99 strain isolated from *Aedes cantans* mosquitoes in West Slovakia (Labuda *et al.*, 1974) was in its 8th mouse passage and the E-13 strain isolated from a blood of *Tringa ochropus* (Grešíková *et al.*, 1975) was in its 8th passage.

Virus titration was carried out on intracerebrally (i.c.) inoculated suckling white mice which were killed after the typical signs of encephalitis had developed. The 10% mouse brain suspensions were prepared in saline enriched with 10% inactivated calf serum. The mouse brain suspensions were clarified by low speed centrifugation and the supernatant fluids were collected. The viruses weretitrated in tube cultures on two-day-old PS cells. The cells were seeded at a concentration of 6×10^4 cells/ml.

Mitotic index (MI). Changes of the MI were examined 4, 8, 12, 48, 72 and 96 hr post infection (p.i.). Cells infected with 0.6 LD_{50}/cell were fixed at given intervals and stained with hematoxyline-

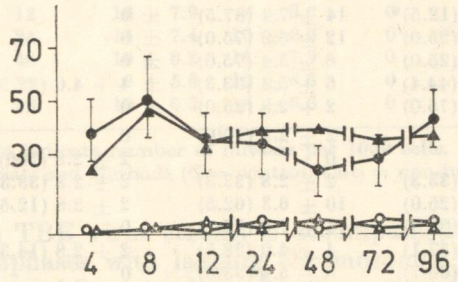

Fig. 1.
Changes of MI in non-infected PS cells MI (●) and abnormal forms (○) in control PS cells; MI (▲) and abnormal forms (△) in cells treated with the non-infected mouse brain suspension
Abscissa: hr p.i.; ordinate: MI and No. of abnormalities in $^0/_{00}$

eosine (H.E.). The MI was calculated by counting the dividing cells per 1 000 cells of the monolayer and expressed in promiles ($^0/_{00}$). The data were compared with those obtained from following controls; a) non-infected cells (C): b) cells exposed to normal mouse brain (Cnb). The experimental results were processed statistically with guaranteed probability of $P(t) = 0.95$.

Results

No significant differences in the dynamics of the MI of PS culture after treatment with non-infected mouse brain (Cnb) were obtained in comparison with the control (C) (Fig. 1). The number of abnormal forms — metaphases with sticking and with lagging chromosomes did not exceed 4% (Table 1). The results showed that 4 hr after virus infection the MI of PS cells was significantly increased (Figs 2 and 3) and that the virus titre began to decrease. After 8 and/or 12 hr p.i. the MI sharply decreased. The analysis of the number of dividing cells showed that the prophases were reduced 8 hr p.i. with TBE virus strains and 12 hr p.i. with WN virus strains (Table 2).

The number of metaphases increased 4 hr after TBE virus infection (Table 3). By 8 hr p.i., the number of metaphases sharply decreased and 24 hr p.i. almost no metaphases were found. The action of WN virus strains was characterized by an increased number of metaphases by 4, 8, and/or 12 hr p.i. Subsequently, in accordance with the time of virus replication, the number of metaphases decreased, while at 48 and 72 hr p.i. almost no metaphases were found. It is of interest that the action of K-99 strain was characterized by a significant reduction of metaphases at 12 hr p.i. There were no metaphases at later intervals.

The number of anaphases (Table 4) and telophases (Table 5) decreased sharply after infection with the viruses employed. Approved differences in the type of abnormal forms after infection of PS culture with TBE and WN viruses were obtained. The data showed that the infection of PS culture

Table 1. Number of abnormal metaphases in PS cells infected with flaviviruses

Duration of infection (hr)	Virus strain	Number of abnormal metaphases		
		Colchicine-like	Lagging	Sticking
4	C	0	0	0
	Cnb	0	0	0
	Hypr	2 ± 2.8 (12.5)*	14 ± 7.4 (87.5)	0
	Ir-32	4 ± 4.0 (25.0)	12 ± 6.9 (75.0)	0
	WN	2 ± 2.8 (25.0)	6 ± 5.4 (75.0)	0
	K-99	8 ± 5.6 (44.4)	6 ± 5.4 (33.3)	4 + 4.0 (22.2)
	E-13	6 ± 5.4 (75.0)	2 + 2.8 (25.0)	0
8	C	0	2 ± 2.8 (100)	0
	Cnb	0	0	2 ± 2.8 (100)
	Hypr	2 ± 2.8 (33.3)	2 ± 2.8 (33.3)	2 ± 2.8 (33.3)
	Ir-32	4 ± 4.0 (25.0)	10 ± 6.3 (62.5)	2 ± 2.8 (12.5)
	WN	4 ± 4.0 (22.3)	14 ± 7.4 (77.7)	0
	K-99	8 ± 5.6 (57.1)	4 ± 4.0 (28.6)	2 + 2.8 (14.3)
	E-13	12 ± 6.9 (66.7)	6 ± 5.4 (33.3)	0
12	C	0	0	0
	Cnb	0	2 ± 2.8 (100)	0
	Hypr	4 ± 4.0 (50.0)	4 ± 4.0 (50.0)	0
	Ir-32	6 ± 5.4 (33.3)	12 ± 6.8 (66.7)	0
	WN	4 ± 4.0 (33.3)	6 ± 5.4 (50.0)	2 ± 2.8 (16.7)
	K-99	14 ± 7.4 (77.7)	4 ± 4.0 (22.2)	0
	E-13	10 + 6.3 (100)	0	0
24	C	0	2 ± 2.8 (50.0)	2 ± 2.8 (50.0)
	Cnb	0	2 ± 2.8 (50.0)	2 ± 2.8 (50.0)
	Hypr	2 ± 2.8 (50.0)	2 ± 2.8 (50.0)	0
	Ir-32	4 ± 4.0 (28.6)	10 ± 6.3 (71.4)	0
	WN	4 ± 4.0 (100)	0	0
	K-99	8 ± 5.6 (100)	0	0
	E-13	8 + 5.6 (100)	0	0
48	C	2 ± 2.8 (100)	0	0
	Cnb	0	2 ± 2.8 (50.0)	2 ± 2.8 (50.0)
	Hypr	2 ± 2.8 (33.3)	4 ± 4.0 (66.6)	0
	Ir-32	6 ± 5.4 (33.3)	10 ± 6.3 (55.5)	2 ± 2.8 (11.1)
	WN	8 ± 5.6 (80.0)	0	2 ± 2.8 (20.0)
	K-99	4 ± 4.0 (100)	0	0
	E-13	8 ± 5.6 (80.0)	2 ± 2.8 (20.0)	0
72	C	0	2 ± 2.8 (50.0)	2 ± 2.8 (50.0)
	Cnb	0	2 ± 2.8 (100)	0
	Hypr	2 ± 3.8 (50.0)	2 ± 2.8 (50.0)	0
	Ir-32	2 ± 2.8 (20.0)	8 ± 5.6 (80.0)	0
	WN	6 ± 5.4 (100)	0	0
	K-99	2 ± 2.8 (100)	0	0
	E-13	2 + 2.8 (100)	0	0
96	C	0	2 ± 2.8 (100)	0
	Cnb	0	2 ± 2.8 (100)	0
	Hypr	2 ± 2.8 (50.0)	2 ± 2.8 (50.0)	0
	Ir-32	2 ± 2.8 (25.0)	6 ± 5.4 (75.0)	0
	WN	2 ± 2.8 (100)	0	0
	K-99	0	0	0
	E-13	0	0	0

Table 2. Changes in the number of prophases in flavivirus-infected PS cells

Duration of infection (in hr)	Virus strain						
	C	Cnb	Hypr	Ir-32	WN	K-99	E-13
4	10 ± 6.3*	12 ± 6.9	16 ± 7.0	12 ± 6.9	12 ± 6.9	12 ± 6.9	14 ± 7.4
8	16 ± 7.9	10 ± 6.3	2 ± 2.8	2 ± 2.8	8 ± 5.6	8 ± 5.6	4 ± 4.0
12	16 ± 7.9	8 ± 5.6	0	0	4 ± 4.0	4 ± 4.0	2 ± 2.8
24	14 ± 7.4	10 ± 6.3	0	0	2 ± 2.8	0	2 ± 2.8
48	10 ± 6.3	16 ± 7.9	0	0	0	0	0
72	6 ± 5.6	10 ± 6.3	0	0	0	0	0
96	10 ± 6.3	8 ± 5.6	0	0	0	0	0

* Data indicate number of mitoses per 1000 cells. (means ± SD). For further explanations see Materials and Methods (C = control; Cnb = non-infected mouse brain suspension).

with TBE virus strains is accompanied by a sharply increased number of metaphases with lagging chromosomes (over 50% of the total number of abnormal forms), while after the action with WN virus strains the colchicine--like metaphases were more frequently observed (over 40% of the total number of abnormal forms).

Discussion

In the present study a sharply increased MI of PS culture 4 hr p.i. with some flaviviruses (except strain K-99) was obtained. We supposed that the increased number of metaphases is a result of the appearance of metaphase barrier, the duration of which depends on the specific properties of the virus strain. The metaphase barrier lasted from the 4th till the 12th hr p.i. with WN virus (strain Egypt) and till the 4th hr only under influence of TBE

Table 3. Changes in the number of metaphases in flavivirus-infected PS cells

Duration of infection (in hr)	Virus strains						
	C	Cnb	Hypr	Ir-32	WN	K-99	E-13
4	16 ± 7.9	12 ± 6.9	48 ± 13.6	46 ± 13.6	42 ± 12.7	18 ± 8.4	40 ± 12.4
8	20 ± 8.8	30 ± 10.6	6 ± 5.4	20 ± 8.8	34 ± 11.4	20 ± 8.8	30 ± 10.6
12	16 ± 7.9	12 ± 6.9	8 ± 5.6	4 ± 4.0	40 ± 12.4	8 ± 5.6	6 ± 5.4
24	10 ± 6.3	18 ± 8.4	0	0	2 ± 2.8	2 ± 2.8	2 ± 2.8
48	6 ± 5.4	10 ± 6.3	0	0	2 ± 2.8	2 ± 2.8	0
72	8 ± 5.6	12 ± 6.9	0	0	0	0	0
96	16 ± 7.9	18 ± 8.4	0	0	0	0	0

For explanations see Table 2.

Legend to Table 1.
* Mean number ± SD (per cent in brackets)
For further explanations see Materials and Methods (C = control; Cnb = non-infected brain suspension).

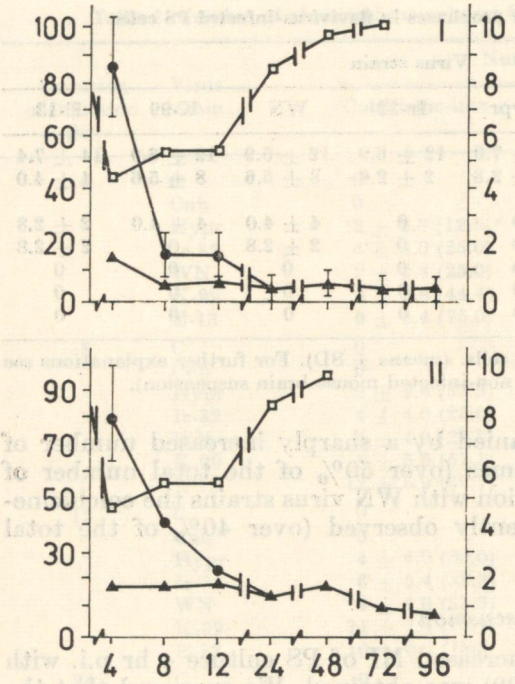

Fig. 2.

Changes of MI in cells infected with TBE virus

MI (●) and abnormal forms (▲) in cells infected with strain Hypr (I) and strain Ir-32 (II); □——□ virus growth curve. Abscissae: hr p.i.; left ordinates: MI and abnormal forms in $^0/_{00}$; right ordinates: viral titre in $\log_{10}$ $TCID_{50}/0.1$ ml.

virus strains Hypr and Ir-32 and WN virus strain E-13, respectively. No metaphase barrier was observed upon action of the K-99 strain.

The presented results showed that at early intervals of infection, some flaviviruses were capable blocking the normal course of mitotic process. A delayed division of PS and chick embryo cells manifested by increased number of cells in different mitotic phases was reported (Gordeeva and Chekova, 1979; Varadinova and Grešíková, 1980). The appearance of a barrier at the definite mitotic stage depends on specificities of cells as well as on the virus strains used.

Table 4. Changes in the number of anaphases in flavivirus-infected PS cells

Duration of infection (hr)	Virus strains						
	C	Cnb	Hypr	Ir-32	WN	K-99	E-13
4	6 ± 5.4	0	2 ± 2.8	4 ± 4.0	2 ± 2.8	2 ± 2.8	2 ± 2.8
8	4 ± 4.0	2 ± 2.8	2 ± 2.8	2 ± 2.8	2 ± 2.8	0	2 ± 2.8
12	0	2 ± 2.8	0	0	4 ± 4.0	0	0
24	2 ± 2.8	4 ± 4.0	0	0	0	0	0
48	0	2 ± 2.8	0	0	0	0	0
72	4 ± 4.0	2 ± 2.8	0	0	0	0	0
96	6 ± 5.4	2 ± 2.8	0	0	0	0	0

For explanations see Table 2.

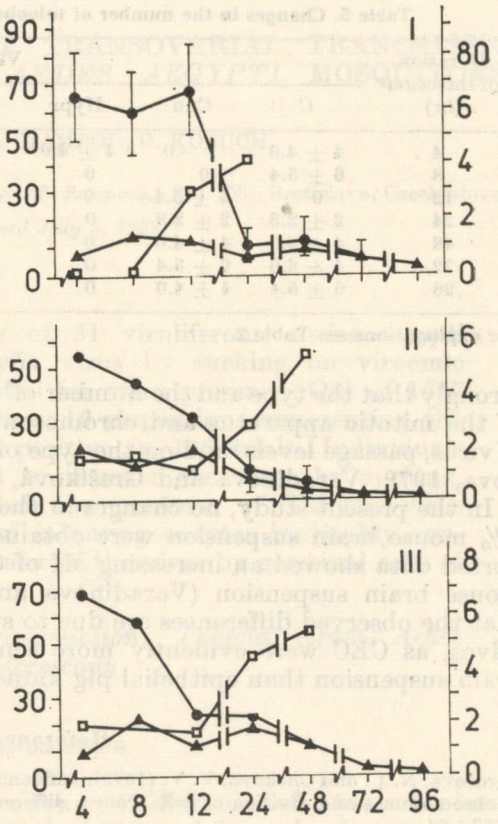

Fig. 3.
Changes of the MI cells infected with WN virus
MI (●) and abnormal forms (▲) in cells infected with prototype WN strain (I), strain K 99 (II) and E 13 (III): □——□ virus growth curve.
Abscissae: hr p.i.; left ordinates: MI and abnormal forms in $^0/_{00}$; right ordinates: viral titre in $\log_{10}$ $TCID_{50}/0.1$ ml.

In accordance with the delayed mitotic process at the early intervals of flavivirus infection, no mitoses accompanied by a thinner monolayer occurred at the late intervals (72—96 hr) as compared to the C group. These results were in accordance with the data previously reported (Mayer and Mitrová——Bellová, 1969). Differences were also found in the action of TBE virus strains on the structures of chromosomes and mitotic apparatus. The results showed that the prototype Hypr strain affected the mitotic apparatus and chromosomes milder than the freshly isolated Ir-32 strain. These data also were in accordance with previously reported ones (Varadinova and Grešíková, 1980) confirming differential action of Hypr and Ir-32 strains on mitotic structures of CEC.

The differences in qualitative characteristics of the MI (type of the abnormal forms) of PS culture after infection with TBE and WN virus strains were manifested by sharply increased number of metaphases with lagging chromosomes after TBE virus infection, in contrast to the action of WN virus strains characterized by colchicine-like mitoses. These results suggest

Table 5. Changes in the number of telophases in flavivirus-infected PS cells

Duration of infection (hr)	Virus strain						
	C	Cnb	Hypr	Ir-32	WN	K-99	E-13
4	4 ± 4.0	−0	4 ± 4.0	2 ± 2.8	0	2 ± 2.8	0
8	6 ± 5.4	0	0	0	0	0	0
12	0	6 ± 5.4	0	0	6 ± 5.4	0	2 ± 2.8
24	2 ± 2.8	2 ± 2.8	0	0	0	0	0
48	4 ± 4.0	4 ± 4.0	0	0	0	0	0
72	4 ± 4.0	6 ± 5.4	0	0	0	0	0
96	6 ± 5.4	4 ± 4.0	0	0	0	0	0

For explanations see Table 2.

strongly that the type and the number of virus induced changes in structures of the mitotic apparatus and chromosomes depended mainly on properties of virus, passage levels, and on the type of the cells used (Gordeeva and Chekova, 1979; Varadinova and Grešíková, 1980).

In the present study, no changes in the MI of PS cells after influence of 1% mouse brain suspension were obtained. In contrast, our previously reported data showed an increasing MI of CEC 4 hr after treatment with 1% mouse brain suspension (Varadinova and Grešíková, 1980). We suppose, that the observed differences are due to specific properties of the cells themselves, as CEC were evidently more sensitive to the action of the mouse brain suspension than epithelial pig kidney (PS) cells.

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