Acta virol. **30** : 7—12, 1986

THE INFLUENCE OF HIGH TEMPERATURE ON HUMAN CELLS IN CULTURE, THEIR SENSITIVITY TO VIRUSES AND THEIR INTERFERON-PRODUCING CAPACITY

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Received October 10, 1985

Summary. — The influence of extremely high temperatures on the course of virus infection in vitro was studied. It has been shown that exposure of human cells (continuous cell line of embryonal skin-muscle tissue, donor blood leukocytes) to 43 °C leads to a significant increase in their sensitivity to cytopathic action of the viruses tested. Noteworthy, the reproduction of these viruses under tested conditions did not differ from their reproduction in control cells. A dramatic inhibitory effect of high temperatures on the interferon-producing capacity of cells was observed.

Key words: interferon; induction; heatshock; human cells

Introduction

The influence of non-physiologic temperatures on the animal and plant cells has not been resolved so far. The studies of gene organization of cells exposed to hyperthermia, their chromatin structure, proteins produced during heatshock, chemical inducers of this shock and physiologic response of the cells are in progress. Temperatures ranging from 41 °C to 43 °C have been most often tested (Anderson *et al.*, 1983; Bromley and Voellmy, 1983; Morimoto and Meselson, 1983), that of 45 °C has been used in some experiments (Opperman *et al.*, 1981; Schlesinger *et al.*, 1983).

It was demonstrated that cells exposed to high temperatures produce so-called heatshock proteins with molecular mass ranging from 22 to 98 kD (Morimoto and Meselson, 1983; Opperman *et al.*, 1981; Rubin and Swift, 1983). It is important that these proteins isolated from cells of different origin (chick fibroblasts, human lymphoblastoid cells, drosophila cells) had not only a similar molecular mass (ranging from 70 to 84—85 kD), but also showed a similar polypeptide structure (Voellmy *et al.*, 1983). These proteins seem to act as protectors of cells against damaging effect of high temperatures (Field and Anderson, 1982; Henle and Dethlefsen, 1978). In the course of and directly after heatshock dramatic changes in the synthesis of cellular proteins and of virus polypeptides have been observed. It

has been shown that 3 to 5 hr exposure of Rous-sarcoma-infected chick fibroblasts to 44 °C leads to a decrease of the yield of proteins, whereas the exposure of chick fibroblasts or hamster kidney cells infected by vesicular stomatitis virus to 42 °C for 3 hr caused enhancement of the virus protein yield 8 hr post-infection (Bromley and Voellmy, 1983). The analysis of the effects of temperature on interferon (IFN) production is also controversial. Most of papers report a decrease of IFN production in the animal body and/or tissue cultures during hypothermia (Postic *et al.*, 1966; Ruiz-Gomez and Martinez, 1965). However, some papers do not confirm this finding (Henle and Dethlefsen, 1978).

The aim of present paper has been to elucidate the effect of temperatures, extremely high for vital activity of human cells, on the sensitivity of these cells to viruses and on IFN-producing capacity. In these studies, the cells were infected after preliminary exposure to the temperatures tested, i.e. when the temperature was lowered to optimal (37 °C).

Materials and Methods

Viruses. Allantoic cultures of vesicular stomatitis virus (VSV, strain Indiana) and influenza virus (strain A/WSN) prepared in 9—10-day-old chick embryos were used. Infectious titres of the viruses in human diploid cells were 10^9 and 10^6 TCD_{50}/ml, respectively. Mouse encephalomyocarditis virus (EMC) with infectious titre 10^7 TCD_{50}/ml was prepared by conventional technique in human diploid cells. Newcastle disease virus (NDV), strain H, with a titre of 10^{10} ID_{50}/ml was prepared and titrated in chick embryos.

Cell cultures. Continuous cell line of skin-muscle human embryo tissue (M-19) obtained from the Institute of Poliomyelitis and Viral Encephalitides and cells FS-4 (foreskin diploid cell line) supplied by Dr. Vilček (U.S.A.) were passaged in Eagle's medium supplemented with double concentration of amino acids and 10% foetal calf serum. Human white blood cells were prepared by centrifugation of fresh donor blood at 1000 rev/min followed by double treatment of cellular mass with five-fold volume of cold 0.83% solution of NH_4Cl during 10 min at 4 °C for red blood cell lysis. After centrifugation at 1000 rev/min the leukocytes were diluted with Eagle's medium containing 5% foetal calf serum to a concentration of 4.5×10^7—6.0×10^7 cells per sample.

Interferon production. IFN was induced with NDV inoculated at a multiplicity of 10—50 ID_{50} per cell and incubated for 24 hr at 37 °C. After inactivation at pH 2.2 for 6 or 7 days, IFN was titrated in homologous diploid cell cultures on microplates against 100 TCD_{50} of EMC virus. The last IFN dilution protecting 50% of cells was considered for the titre.

Experimental design. Two-day monolayer cultures of M-19 and FS-4 cells grown at 37 °C and human leukocytes were exposed to high temperatures (38 °C and 43 °C) during various time intervals. The cultures were then transferred to optimal temperature (37 °C) and the cells were used either for assessment of their sensitivity to viruses and virus reproduction or for estimation of their IFN-producing capacity, depending on the purpose of the test.

Viability of human diploid cells was determined by the treatment of the cells with 0.1% neutral red solution for 20 min at 37 °C. The count of stained (live) cells in Goryaev chamber was carried out directly after heatshock and 24 hr after cultivation of cells at 37 °C in Eagle's medium with 2% of bovine serum.

The sensitivity of M-19 cells to viruses after exposure of the cells to various temperatures was determined by titration of EMC virus, VSV and WSN virus in these cells. For the estimation of reproduction of EMC, VSV and WSN viruses under heatshock conditions, M-19 cells grown under normal temperature were trasferred to 43 °C for 4 hr. The medium was removed, and after 1 hr adsorption of viruses at 37 °C the monolayer was twice washed; then fresh medium with 2% serum was added into the flasks and the cultivation was confirmed at 37 °C. Zero and 16 hr samples were frozen for further determination of virus content. For the assessment of IFN-producing capacity human leukocytes (4.5×10^7—6.0×10^7 cells per sample) and diploid cells M-19 and FS-4 were used.

Table 1. Cytopathic effect of different viruses in M-19 cells exposed to hyperthermia

Temperature and incubation time before introduction of virus	Virus titre, TCD_{50}/0.1 ml*		
	EMC	VSV	WSN
37 °C (control)	$10^{5.1}$	$10^{5.5}$	$10^{3.5}$
38 °C, 1 hr	$10^{5.3}$	$10^{5.5}$	$10^{3.5}$
38 °C, 4 hr	$10^{5.3}$	$10^{5.3}$	$10^{3.5}$
43 °C, 1 hr	$10^{5.6}$	$10^{5.2}$	$10^{4.0}$
43 °C, 4 hr	$10^{7.5}$	$10^{8.0}$	$10^{6.5}$
43 °C, 4 hr without virus	unchanged tissue	unchanged tissue	unchanged tissue

* Mean value of 4 tests

Results

Sensitivity of cells to EMC, VSV and WSN viruses

The results of titration of the viruses in M-19 cells exposed to high temperatures are represented in Table 1. Exposure of cells to 38 °C (1 hr and 4 hr), or short-term incubation at 43 °C (1 hr) appeared not to alter the sensitivity of M-19 cells to the viruses tested. After prolonged exposure to 43 °C (4 hr), the situation was different. Cytopathic effect was increased for EMC, VSV and influenza viruses by 2.4, 2.5 and 3 log, respectively. Therefore, further tests were carried out at 43 °C only.

It should be noted that studies on cell viability based on incorporation of neutral red which had been made directly after a 4-hr heatshock and after 24 hr cultivation of these cells at 37 °C, showed no increase in the number of dead cells. Microscopy of unstained cell cultures (during 3 or 4 days) also failed to reveal any increase of cytopathologic changes as compared to control cultures.

The findings obtained allowed us to expect that under the tested conditions reproducibility of the viruses investigated will be enhanced. A special experimental series was devoted to this suggestion.

Table 2. Reproduction of EMC, VSV and WSN viruses in M-19 cells exposed to heatshock

Temperature and incubation time before introduction of virus	Time of virus reproduction	Virus titre in 0.1 ml*		
		EMC	VSV	WSN
37 °C, 4 hr	0	$< 10^1$	$< 10^1$	$< 10^1$
37 °C, 4 hr	16	$10^{5.8}$	$10^{5.9}$	$10^{5.7}$
43 °C, 4 hr	0	$< 10^1$	$< 10^1$	$< 10^1$
43 °C, 4 hr	16	$10^{5.5}$	$10^{5.8}$	$10^{5.5}$

* Mean value of 7 tests

Table 3. Effect of heatshock on IFN-producing capacity of human cells

Temperature and incubation time before introduction of virus	IFN titre units/ml*		
	leukocytes	M-19	FS-4
37 °C, 4 hr — NDV	1280	128	64
43 °C, 2 hr — NDV	428	< 16	< 16
43 °C, 4 hr — NDV	< 40	< 16	< 16
37 °C, 4 hr without NDV	< 40	< 16	< 16
43 °C, 4 hr without NDV	< 40	< 16	< 16

* Mean value of 3 tests

Reproduction of EMC, VSV and WSN viruses under heatshock conditions

As seen from Table 2 the reproduction of different viruses in M-19 cells incubated at 43 °C for 4 hr did not differ from the reproduction in cells incubated at optimal temperature (infectious virus titre after 16 hr of cultivation was the same in test and control cells). IFN-producing ability of human cells under heatshock is presented in Table 3 showing IFN titres 48 hr after addition of the inducer. Exposure of leukocytes to 43 °C for 2 hr reduced IFN production 3-fold as compared with the control. Four-hour incubation of leukocytes at 43 °C completely abolished the IFN-producing capacity of cells. Complete absence of its production was observed in the cells M-19 and FS-4 exposed to heatshock for 2 and 4 hr.

Discussion

The data reported in the present paper indicate drastic changes associated with the effect of heatshock (exposure of cells to 43 °C for 4 hr followed by transfer to standard cultivation conditions at 37 °C) in different human cells. Thus, for instance in M-19 cells exposed to such treatment, upon infection with VSV, EMC and WSN viruses, the infectious titre as determined by cytopathic effect titration was higher by an average of 2 to 3 log, than the titre registered in control cells.

This increase in sensitivity to cytopathic effect of viruses appeared to be unrelated to the changes of virus reproduction, since the plots of infectious activity curves were the same in experimental and control cells. Several reports about the effects of hyperthermia on the viability of animal cells and the character of reproduction in these cells can be found in available literature. Thus, for instance, Bromley and Voellmy (1983) have found that the exposure of chick fibroblasts or hamster kidney cells, preinfected with VSV, to high temperature (42 °C) for 3 hr caused an enhanced production of virus proteins 8 hr post-infection. On the other hand, heat treatment of

cells (44 °C, 3 to 5 hr) infected with Rous sarcoma virus caused a decrease of virus protein yield. These data point not only to the changes developing in the cells under heatshock, but probably also to differences in the genetic properties of viruses related to the ability of the genome to replicate at high temperatures.

Experimental conditions designed in this paper ruled out any effects of hyperthermia on reproducibility of viruses, since the cells were infected after the exposure to heatshock. Therefore, the increase in the fluidity of cell membranes during heatshock seems a likely explanation of enhanced sensitivity to the cytopathic effect of viruses (Li *et al.*, 1980). This suggestion is also supported by the fact that virus reproduction in M-19 cells was unchanged in spite of dramatic decrease of their IFN-producing capacity after prolonged heatshock (from 128 to less than 16 units/ml).

Thus, heatshock of diploid M-19 cells causes a rise in their sensitivity to cytopathic effect of viruses and a dramatic reduction of IFN synthesis, which, however, does not result in the enhancement of virus reproduction. We were the first who observed a striking inhibition of IFN production following heatshock not only in human diploid cells (M-19 and FS-4), but also in donor blood leukocytes. This observation seems important not only for the studies of interaction of infectious viruses with the cell, but also for tackling the problems of experimental cancerogenesis. It is known that the loss of IFN protection in the cells makes them more sensitive to the effects of oncogenes (Balitsky and Vorontsova, 1973). Therefore, the absence of IFN may promote the appearance of malignant transformation. Moreover, it has been demonstrated that specific "heatshock" proteins produced as a result of heatshock can interact with transforming protein of Rous sarcoma virus (Opperman *et al.*, 1981; Yonemoto *et al.*, 1983). Dramatic inhibition of IFN production by immunocompetent cells should be regarded as a factor reducing immunological protection in general.

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