Virus-Induced Morphological Changes in Arteries of Rabbits with Herpetic Keratoconjunctivitisand of Patients with Generalized Herpes Infection

T. V. Amvros'eva, M. K. Nedz'ved', V. I. Votyakov, and O. T. Andreeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 6, pp. 702-706, June, 1996
Original article submitted April 5, 1995

It is found that herpetic keratoconjunctivitis in rabbits and generalized herpes infection in humans is associated with virus-specific arterial damage. Experiments on rabbits show that virus-induced morphological changes in the aorta can be corrected with furavir. The role of herpes infection as a possible risk factor in atherogenesis is considered.

Key Words: *herpes infection; arteries; morphological changes*

Recent findings indicate that viruses may contribute to the pathogenesis of atherosclerosis. Convincing evidence that herpes viruses are involved in atherogenesis has been reported [4,7,8,10-12,14]. For example, Fabricant *et al.* succeeded in identifying atherosclerotic lesions in chickens infected by the causative agent of Marek's disease and showed that this virus changes lipid metabolism and stimulates lipid accumulation in arterial cells and tissues [8,11]. Concerning human herpes viruses, atherogenic activity has been demonstrated for cytomegalovirus [14], Epstein-Barr [4], and herpes simplex [10,12]. While studying the atherogenic potential of type I herpes simplex (HS-I), we found that it causes dyslipidemia, which was observed in rabbits with herpetic keratoconjunctivitis [1] and in patients with ophthalmoherpes [2]. We have shown that HS stimulates intracellular lipid accumulation in cultured human aortic smooth muscle cells (SMC) [1], which is consistent with previously reported data [12].

Lipid metabolism disorders, even if they are atherogenic, are not an absolute condition for the development of atherosclerosis. Damage (alteration) to the arterial wall is another important factor that initiates and/or accelerates atherogenesis [1].

In studying the damaging properties of HS-1 *in vitro*, we found that human embryonic SMC are a permissive system for the reproduction of HS-1, which is accompanied by a more or less pronounced (depending on the dose of HS-1) cytopathogenic response [1].

Bearing in mind that herpes infection generally proceeds with viremia and the fact that mRNA [5] and HS virions [9] are present in atherosclerotic arteries [9], we studied the arterial wall under conditions of herpes infection at the organismic level. Histopathological investigation of blood vessels of rabbits with herpetic keratoconjunctivitis (HKC) and of patients with generalized herpes infection (GHI) was performed.

MATERIALS AND METHODS

Herpetic keratoconjunctivitis was modeled in Chinchilla rabbits weighing 2-2.5 kg, using the HS-1 strain Koptev in a dose equal to 100 LD_{50}. The virus was isolated and titrated on outbred albino mice. The rabbits were treated with furavir: intravenous injections (20 mg/kg) were combined with five instillations of 3% solution into the conjunctival sac during a 7-day period [1]. The rabbits were sacrificed on day 14 of infection and the aorta was studied histologically. The aorta and cerebral and coronary arteries of patients who had died of GHI were studied histopathologically. In 9 patients (7 men and 2 women) GHI was accompanied by atherosclerosis of the aorta (patients Nos. 1-9) and of the cerebral (patients 3-5, 7, and 8) and coronary (patients 2, 3, 6, and 8) arteries. Specific HS antigens in the aorta, arteries,

Department of Viral Activity Inhibitors, Belarus Institute of Epidemiology and Microbiology, Minsk

0007-4888/96/0006-0635$15.00

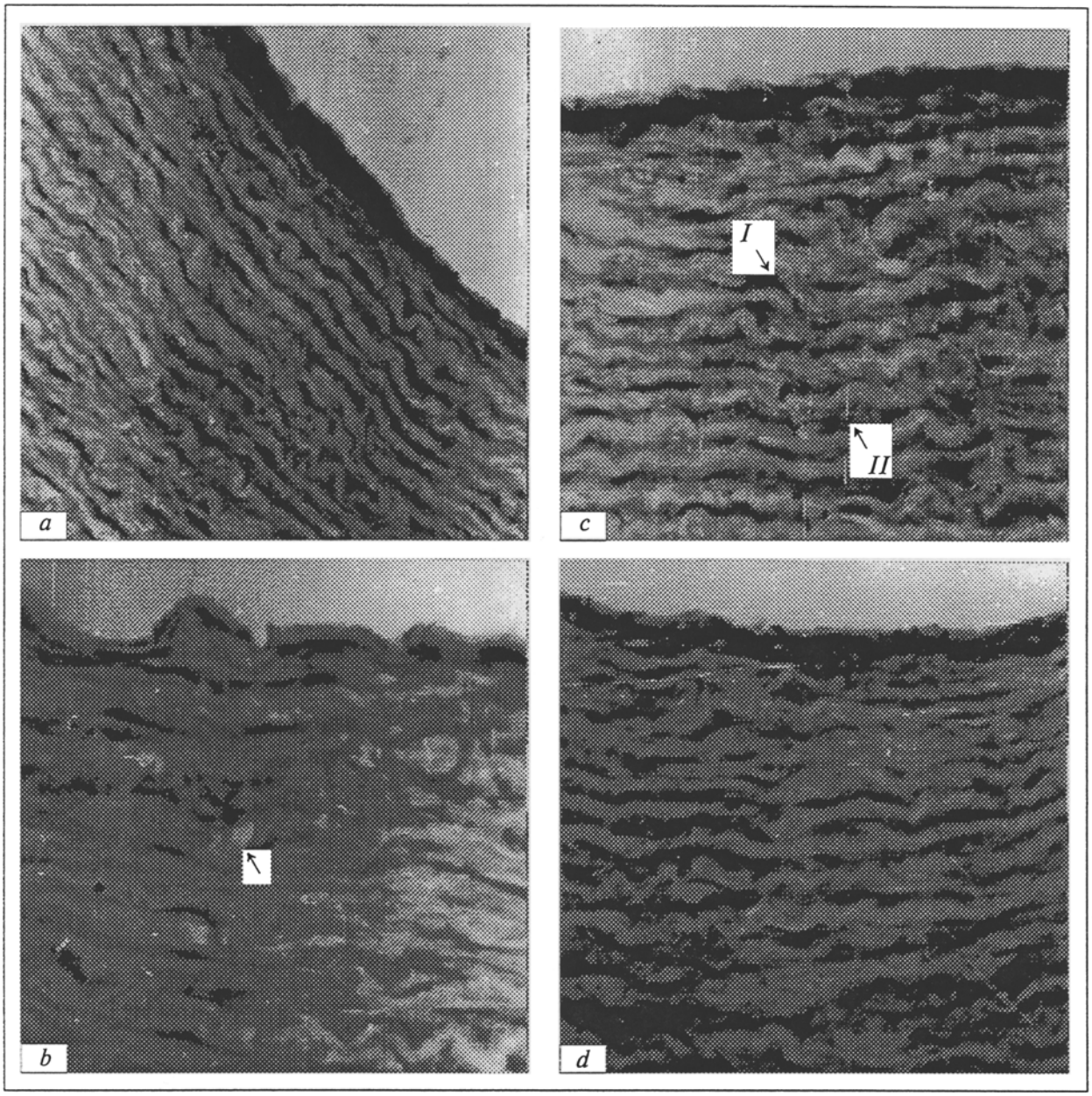

Fig. 1. Morphological changes in the aorta of rabbits with HKC. *a*) proliferation of endothelial cells with enlarged hyperchromatous nuclei. Cell borders are not pronounced. Hematoxylin and eosin staining, × 200; *b*) desquamation of endothelial cells, convoluted basal membrane, focal destruction of collagen and elastic fibers in the upper layer of the tunica media (arrow). Van Gieson staining, × 400; *c*) enlarged hyperchromatous nuclei of SMC (type I inclusions) and vacuolization of enlarged nuclei in these cells (type II inclusions), hematoxylin and eosin staining, × 400; *d*) homogenization of the ground substance of the tunica media; hematoxylin and eosin staining, × 200.

and adjacent tissues were revealed by immunofluorescence on deparaffined sections. For histological investigations preparations were stained with hematoxylin and eosin and picrofuchsin after Van Gieson.

RESULTS

Experimental HKC in rabbits ran a classical course with typical clinical manifestations. The virus was isolated from the blood (the 7th-day titer was 5.0 lg LD_{50}), brain, and liver (the 14th-day titers were >5.5 and 3.5 lg LD_{50}, respectively), indicating that the infection tended to spread to other organs and tissues [3].

Microscopy of rabbit aortas revealed considerable morphological changes. Proliferation of intimal endothelial cells with the formation of several layers was observed in some aortic areas. The nuclei of these cells were hyperchromatous, and cell borders were not seen (Fig. 1, *a*). Desquamation of endothelial cells occurred in other areas of the aorta.

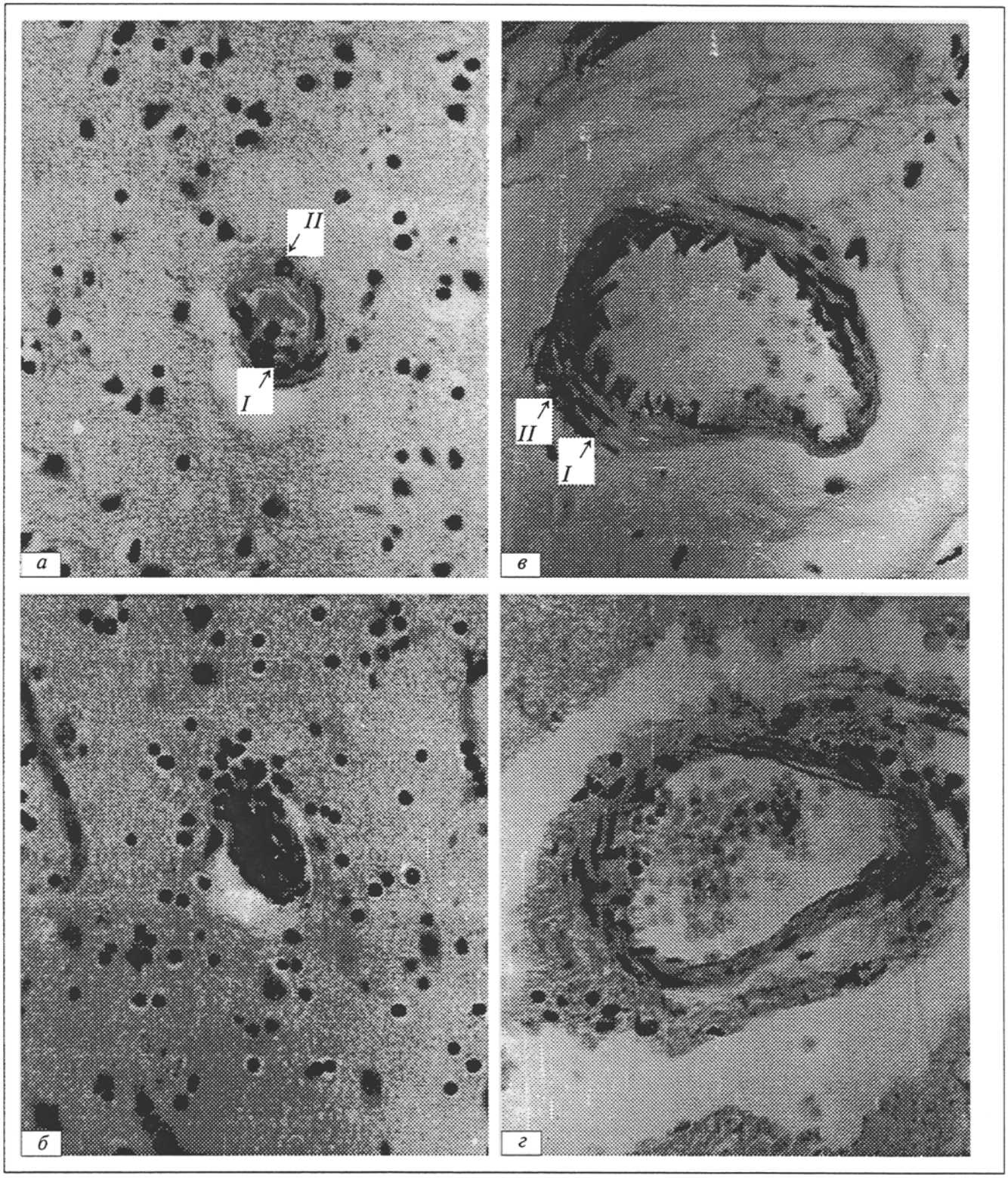

Fig. 2. Morphological changes in the walls of cerebral and coronary arteries of patients with GHI.

a) brain cortex (patient No. 7). Proliferation of aortic endothelium. Types I and II inclusions in endotheliocyte nuclei;

b) brain cortex (patient No. 7). Pronounced proliferation of the endothelium with obliteration of the vessel lumen;

c) heart (patient No. 4). Focal infiltration of SMC nuclei with types I and II inclusions;

d) brain, white matter (patient No. 3). Perivascular infiltration and signs of pronounced encephalomalacia; hematoxylin and eosin staining, × 400.

The inner surface of the intima in these areas was rough, the basal membrane was not pronounced, and occasional foci of metachromasia and "melting" of elastic fibers and ground substance with small foci of disorganization were seen in the intima (Fig. 1, *b*). The elastic fibers of the tunica media were involved in this process. These areas looked homogeneous, eosinophilic-basophilic with a noticeably increased number of cells. In some SMC the nuclei were enlarged and the perinuclear space was transparent. The elastic fibers of the tunica media were thickened, vacuolized, and convoluted. Type I viral inclusions were seen in the nuclei of SMC and of some fibroblasts. The nuclei were enlarged and hyperchromatous. In addition to having type I inclusions, the nuclei of adjacent cells were vacuolized, and each vacuole contained small punctate inclusions consistent with the type II nuclear inclusions typical of herpes infection (Fig. 1, *c*). Sometimes the vacuolized nuclei looked like mulberries. In addition to the nuclear transformations due to viral inclusions, foci of destruction and homogenization of elastic fibers with signs of metachromasia of the ground substance were found. Occasional round cells infiltrated the adventitia, in which early sclerotic manifestations were seen.

Microscopy of the aortas of furavir-treated rabbits also revealed typical manifestations of viral infection, but they were much less pronounced than in untreated animals. The elastic fibers of the tunica intima and tunica media were convoluted but preserved their orientation. Despite some changes in nuclear shape, most SMC and fibroblasts remained elongated and appropriately oriented. Only occasional foci of metachromasia and homogenization of the ground substance were seen (Fig. 1, *d*).

Thus, in the aorta of HS-infected rabbits with typical clinical manifestations of HKC morphological markers typical of herpes infection and previously described for other organs [3] were revealed.

Histopathological investigation of small and large blood vessels of GHI patients showed that the virus caused considerable changes in vascular cells and tissues. It should be emphasized that all the studied vessels were analyzed for the presence of HS-specific antigens in the vascular wall and adjacent tissues. Types I and II viral inclusions were detected in endothelial cells (Fig. 2, *a*). These morphological changes were often accompanied by perivascular hemorrhages. Proliferation of endothelial cells and transformation of their nuclei due to viral inclusions led to marked occlusion and in some cases to obliteration of the lumen (Fig. 2, *b*). Scarce lymphocyte and plasma cell infiltrates and outgrowth of the connective tissue were seen around the vessels. Proliferation of SMC often occurred in the vascular wall (Fig. 2, *c*). Their nuclei were large, hyperchromatous, and had an irregular shape. Generally, this proliferation was focal. In four cases, these changes went along with pronounced circulatory disorders and the formation of thrombotic alterations with the development of necrotic foci around them (Fig. 2, *d*). It should be noted that these morphological changes were observed to various degrees in major and organ blood vessels.

Taken together with the published data [1,2, 4,10,12,14], our results provide new information regarding the role of herpes viruses in human pathology. It is obvious that HS-I not only induces the known nosological forms of herpes infection but (under certain conditions) also acts as an additional risk factor in atherogenesis due to its ability to induce lipid metabolism disorders [1,2,12]. The virus can also cause morphological changes in tissue and cell components of blood vessels. It can be assumed that the vascular changes pose an atherogenic risk not only during the intensive development of infection but also during its decline. This is confirmed by the results of histopathological studies of the aortas of rabbits practically recovered (according to clinical criteria) from HKC.

The experimental data on the correction of HS-induced vascular alterations by the antiviral agent furavir and on its ability to correct lipid disorders [1] are of dual interest. First, they indicate that morphological changes occurring in blood vessels during herpes infections and dyslipidemia are virus-specific and, second, they pose a new problem: the feasibility of using antiviral chemotherapy in somatic pathology accompanied by virus-induced atherogenic alterations.

REFERENCES

1. T. V. Amvros'eva, V. I. Votyakov, O. T. Andreeva, *et al.*, *Vopr. Virusol.*, No. 1, 61-64 (1992).
2. T. V. Amvros'eva, V. I. Votyakov, A. Yu. Chekina, *et al.*, *Ter. Arkh.*, No. 11, 48-50 (1994).
3. A. G. Kolomiets *et al.* (Eds.), *Generalized Herpes Infection: Facts and Concepts* [in Russian], Minsk (1992).
4. R. A. Kupchinskii, L. I. Matskin, T. N. Azarovskaya, *et al.*, *Vopr. Virusol.*, No. 4, 479-481 (1988).
5. E. P. Benditt, T. Barrett, J. K. McDougall, *Proc. Natl. Acad. Sci. USA*, **80**, 6386-6389 (1983).
6. R. S. Cottrell, *Lancet*, **1**, 520-524 (1984).
7. C. G. Fabricant, J. Fabricant, M. M. Litrenta, *et al.*, *J. Exp. Med.*, **148**, 335-340 (9178).
8. C. G. Fabricant, D. P. Hajjar, C. R. Minick, *et al.*, *Fed. Proc.*, **39**, 1110-1118 (1980).
9. F. Gyorkey, J. L. Melnick, G. A. Guinn, *et al.*, *Exp. Mol. Pathol.*, **40**, 328-329 (1984).
10. D. P. Hajjar, *Am. J. Pathol.*, **139**, 1195-1211 (1991).
11. D. P. Hajjar, C. G. Fabricant, C. R. Minick, *et al.*, *Am. J. Pathol.*, **122**, 62-70 (1986).
12. D. P. Hajjar, K. B. Pomerantz, D. J. Falcone, *et al.*, *J. Clin. Invest.*, **80**, 1317-1321 (1987).
13. W. B. Kannell, *Med. Clin. North Am.*, **58**, 363-369 (1974).
14. J. L. Melnick, E. Adam, and M. E. DeBakey, *J. Am. Med. Assoc.*, **263**, 2204-2207 (1990).