RIDOSTIN INHIBITS TRANSFER OF HIV-1 FROM ABORTIVELY INFECTED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS TO T LYMPHOBLASTOID CELLS

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Summary. – The exposure of human umbilical vein endothelial (HUVE) cells to Ridostin resulted in reduced extent of virus rescue after the addition of indicator T cells. The inhibitory effect was accompanied by decreased synthesis of tumor necrosis factor (TNF) alpha by HUVE cells. The reduced TNF alpha synthesis caused by Ridostin treatment could be responsible, at least in part, for the inhibition of human immunodeficiency virus 1 (HIV-1) infection in our experimental system, at multiple steps of this process, such as: infection of HUVE cells, transfer to and subsequent replication in rescuing cells.

Key words: Ridostin; human umbilical vein endothelial cells; C8166 cells; HIV-1 replication; TNF alpha

Introduction

Endothelial cells represent one of the most important blood tissue barriers (Beilke, 1989; Pober and Ramzi, 1990; Pober *et al.*, 1990). In response to inflammatory stimuli these cells recruit leukocytes that rapidly cross the endothelial barrier by an active process. Resent findings suggest a participation of endothelial cells in the immune network (Hirschberg, 1981; Miossec and Ziff, 1986; Teitel *et al.*, 1989). The infection of endothelial cells can be involved in the pathogenesis of some viral diseases and the vasculitis usually accompanies HIV-1 diseases (Pantaleo *et al.*, 1993). Furthermore, Kaposi's sarcoma is possibly the result of malignancy of endothelial cells after HIV infection (Pandolfi, 1993).

The possibility that HIV infects endothelial cells derived from different vessels of the organism has been reported (Lafon *et al.*, 1992; Re *et al.*, 1991; Steffan *et al.*, 1992). We have reported earlier that after HIV-1 infection of HUVE cells the reverse transcription of input viral RNA does occur, but virus maturation and release are undetectable (Scheglovitova *et al.*, 1993). However, productive HIV infection can be rescued by the addition of either T lymphoblastoid cells or activated peripheral blood mononuclear (PBM) cells. Prevention of cell-to-cell transmission of HIV-1 infection is an attractive target of antiviral therapy, since, although unable to irradicate the virus, it could limit the spread of the infection through the organism. This may be particularly relevant in the mother-to-child transmission during pregnancy. The transmission of HIV infection has been shown to be not influenced by antibodies to HIV (Gupta *et al.*, 1989), but inhibited by some compounds such as interferon (IFN) alpha (Pitha, 1994; Poli, 1994), and alpha glucosidase 1 inhibitor (Bridges *et al.*, 1994). We have recently shown that Ridostin is able to inhibit HIV-1 replication in acutely infected T lymphoblastoid C8166 cells (Scheglovitova *et al.*, 1995). In the present paper we investigated the question whether Ridostin could be able to prevent HIV infection in a original experimental system of HUVE cells abortively infected with HIV-1 and co-cultivated with C8166 cells.

Materials and Methods

Cells. Primary cultures of HUVE cells were obtained as described by Jaffe (1980). Briefly, fresh umbilical vein was cannulated and filled with 10% dispase solution (Collaborative Research, Bedford, MA), and incubated at 37 °C for 30 mins. Then the veins were perfused with PBS without Ca^{++} and Mg^{++} cations. Cells were collected from the perfusate by centrifugation at 800 x g for 5 mins,

Abbreviations: EDTA = ethylendiamine tetraacetate; FCS = foetal calf serum; HIV-1 = human immunodeficiency virus 1; HUVE = human umbilical vein endothelial; ICAM-1 = intercellular adhesion molecule 1; IFN = interferon; MOI = multiplicity of infection; PBS = phosphate buffered saline; TNF = tumor necrosis factor

resuspended in RPMI-1640 medium supplemented with 20% heat-inactivated foetal calf serum (FCS), heparin (100 μg/ml), endothelial growth factor (100 μg/ml) and gentamycin (50 μg/ml), and seeded into culture flasks. The confluent monolayers were washed and trypsinised (0.05% trypsin, 0.02% EDTA). The cells were resuspended in complete medium and seeded into the wells of plastic microtiter plates at a density of $10^5/cm^2$. Only cells from the first subculture were used for the experiments. Cells were identified as HUVE by staining with antibodies to factor VIII (von Willebrand factor) and to CD31 (PECAM-ENDOCAM) (Shimizu *et al.*, 1992). A contamination by monocytes was excluded by staining with the monocyte-specific anti-CD14 antibodies. These cultures were found not to contain CD4-positive cells by FACS analysis with anti-Leu3A antibodies.

CD4-positive T lymphoblastoid C8166 cells were grown in suspension in RPMI-1640 medium supplemented with 10% FCS and 50 μg/ml gentamycin. For co-cultivation, C8166 cells were added 24 hrs later to the endothelial cells obtained by trypsinisation of the infected HUVE cell monolayers, at a concentration of 8 x 10^5/ml in complete medium. Supernatants were assayed for both the virus antigen and infectivity at various time intervals as described by Dianzani *et al.* (1989).

Virus. HIV strain P1 was obtained from a clinical isolate. It was grown in C8166 cells, and stock virus was obtained from clarified lysates of virus-infected C8166 cells by repeated freezing and thawing of the cell pellets. Confluent HUVE cell monolayers were infected at a different multiplicity of infection (MOI). After 1 hr incubation at 37 °C with the virus inoculum, the cells were washed twice to remove residual virus inoculum and uninternalized virus (Tang and Levy, 1991), trypsinized and seeded into replicate wells.

Titration of HIV-1 infectivity was performed by the standard method in C8166 cells in 96-well microtiter plates. The titer was determined after 4 days of cultivation by scoring syncytia under the microscope and calculated according to Reed and Muench (Dianzani *et al.*, 1989).

Viral antigens were estimated by the Abbott antigen capture assay (Abbott Laboratories, Chicago, IL), detecting mainly p24 antigen.

Ridostin, a dsRNA preparation derived from *S. cervisiae* (Scheglovitova *et al.*, 1995), was a gift of Dr. V. Masycheva, The Biologically Active Substances Research and Technoloogy Institute, Berdsk, Russia. A stock solution of Ridostin was made in distilled water.

Results

In the first series of experiments the effect of Ridostin on HIV-1-infected HUVE cells was investigated. An evidence of the infection was obtained through rescue by T lymphoblastoid C8166 cells co-cultivated with HUVE cells. Ridostin was added to HUVE cell cultures in 3 different ways: before, after, and both before and after HIV-1. The extent of HIV-1 rescue by co-cultivation with C8166 cells is shown in Table 1. Since the interexperimental variation was usually rather high due to the fact that HUVE cells from different donors were used for different experiments, just one representative experiment is shown. The amounts of infectious virus and viral antigens were reduced in HUVE cells treated with Ridostin, the effect being more evident when the inhibitor was added to them after infection: infectious titer in control cultures was 10^5 $TCID_{50}$/ml and that in cells treated after infection was $10^{3.75}$ $TCID_{50}$/ml, while virus antigens were above 4,000 pg/ml in the control culture and 2,100 pg/ml in the Ridostin-treated culture. Similar results were obtained in the case of Ridostin added both before and after HIV-1 infection of HUVE cells.

Table 1. Effect of Ridostin on HIV-1 infection of HUVE cells

Ridostin added	HIV-1 yield	
	log $TCID_{50}$/ml	p24 (pg/ml)
Before infection	4.5	3400
After infection	3.75	2100
Before and after infection	3.75	2300
Control (no Ridostin)	5.0	>4000

Ridostin (400 μg/ml) was administered to HUVE cells before infection (24 hrs), after infection (24 hrs), or both (48 hrs). Then the cell monolayers were washed and C8166 cells were added to rescue HIV-1 infection. Data on HIV-1 yield refer to day 4 after infection.

In the next series of experiments Ridostin was added to HUVE cells after infection and either removed before the addition of C8166 cells or left also during the co-cultivation. In parallel cultures Ridostin was added only after the addition of rescuing cells (Table 2). The results indicate that the effect of Ridostin was invariably inhibitory, although the most impressive inhibition was obtained when Ridostin was present during the entire experiment. In this case infectious virus and viral antigens were undetectable. These re-

Table 2. Effect of Ridostin on rescue of HIV-1 infection by C8166 cells

Ridostin added to		HIV-1 yield	
HUVE cells	C8166 cells	log $TCID_{50}$/ml	p24 (pg/ml)
+	–	3.1	670
–	+	2.15	230
+	+	<1.5	<20
–	–	3.35	6200

Ridostin treatment (400 μg/ml) was performed according to the following schedule: before addition of C8166 cells (24 hrs), after addition of C8166 cells (24 hrs), or both (48 hrs). Data on HIV-1 yield refer to day 4 after infection.

sults indicate that Ridostin could act both in the infected HUVE cells and in the subsequent rescue of HIV infection by C8166 cells, giving rise to a synergistic effect when present in both phases of the experiment.

Table 3 describes the dependence of the Ridostin effects on different MOI with HIV (0.1, 1 and 10 $TCID_{50}$/cell). The effect of Ridostin was invariably inhibitory at all the tested MOI.

Table 3. Effect of Ridostin on rescue of infection by C8166 cells from HUVE cells infected with HIV-1 at different MOI

MOI	Ridostin	HIV-1 yield	
		log $TCID_{50}$/ml	p24 (pg/ml)
0.1	–	1.5	250
	+	<1.0	<20
1.0	–	2.5	660
	+	1.25	<20
10	–	2.75	>7000
	+	1.75	1900

HUVE cells were infected with HIV-1 at different MOI and Ridostin (400 μg/ml) was present (+) or absent (–) during the whole experiment. The experimental conditions were as described in Table 2.

Since the cellular expression of ICAM-1 is under the control of several cytokines, including TNF alpha (Mantovani *et al.,* 1993), we explored the possibility that Ridostin could affect the amount of TNF alpha released by HUVE cells. To test this hypothesis, we investigated the effect of Ridostin on TNF alpha release by HUVE cells.

The results of such experiments showed that HUVE cells treated for 24 hrs with Ridostin (400 μg/ml) released a lower amount of TNF alpha as compared to control cells (2.8 pg/ml versus 9.7 pg/ml).

Discussion

In this study the influence of Ridostin on HIV-1 infection of HUVE cells was investigated. The results indicate that Ridostin exerted an inhibitory effect, most evident when it was present during both the infection of HUVE cells and the subsequent co-cultivation with rescuing C8166 cells. Furthermore, the effect was not dependent on MOI, since it was of the same order of magnitude at all the tested MOI. The experiments aimed to clarify the mechanism of action of Ridostin were based on several points. HIV-1 infection of HUVE cells is abortive since only the reverse transciption of the viral genome occurs and the virus maturation is not detectable (Scheglovitova *et al.,* 1993). Similarly, the rescue of HIV infection by C8166 cells does not appear to involve transfer of mature virions. HUVE cells spontaneously produce IL-1 beta, IL-6 (Montovani *et al.,* 1993) and TNF alpha (our own data). Ridostin has been shown in this work to inhibit TNF alpha release by HUVE cells. Since it is well established that TNF alpha is an HIV-1 enhancing cytokine, it is possible that the reduction of endogenous TNF alpha released by HUVE cells treated with Ridostin could be, at least in part, responsible for the inhibition of HIV-1 infection in our experimental system. The data obtained with the different time schedule of treatment suggest that both the pre-rescue and rescue events *per se* could be affected by Ridostin. However, we do not have at present any evidence on the influence of Ridostin on any particular infection event. Further experiments are necessary to clarify these points that appear to be important in view of the possibility that HUVE cell infection can be involved in the HIV-1 transmission from mother to child. However, our present results suggest that Ridostin can be an attractive candidate for the prevention of HIV-1 spread from endothelial to other cells, and thus for limiting of spread of HIV-1 through the organism.

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