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Sulfated colominic acid: an antiviral agent that inhibits the human immunodeficiency virus type 1 in vitro

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Abstract

Colominic acid is a homopolymer of *N*-acetylneuraminic acid (NANA), which has an α-2,8 ketosidic linkage between its polymer units. In this study, colominic acids were sulfated under different conditions and their antiviral activities against human immunodeficiency virus type 1 (HIV-1) were examined. Sulfated colominic acids, containing 6–12% sulfur, blocked the expression of HIV-1 antigen in MT-4 cells or C8166 cells following exposure to MOLT-4/HTLV-IIIB or HIV-1[GUN-1]. The compounds inhibited syncytium formation upon co-cultivation of MOLT-4 cells (clone 8) with MOLT-4/HTLV-IIIB cells and abolished the production of HIV-1 p24 antigen in culture medium of peripheral blood lymphocytes (PBLs). HIV-1 reverse transcriptase (RT) activity was not directly affected by the drugs. The compounds did not prolong activated partial thromboplastin time (APTT) at 10 and 1.0 μg/ml, suggesting that they may not have appreciable side effects in vivo. These agents were still able to block the expression of HIV-1 antigen even when the cells were infected with HIV-1 in RPMI-1640 medium containing high percentages of fetal calf serum (FCS). These properties may be therapeutically advantageous if these compounds were considered for possible clinical use.

Keywords: Acquired immunodeficiency syndrome; Enzyme-linked immunosorbent assay; Human immunodeficiency virus type 1; Indirect immunofluorescence assay; Sulfated colominic acid; Reverse transcriptase

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983; Gallo et al., 1984; Levy et al., 1984). In addition to searching for vaccines against HIV-1,

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it is important to look for other approaches to block viral replication as a means of chemotherapeutic control of the disease in patients suffering from AIDS and AIDS-related complex (ARC). Zidovudine (AZT), Didanosine (DDI) and Zalcitabine (DDC) (Mitsuya et al., 1985; Mitsuya and Broder, 1986) are available for the treatment of these patients. Treatment with these drugs has resulted in improved immunologic functions and reduction of other clinical abnormalities (Fischl et al., 1987; Yarchoan et al., 1986, 1988, 1989). However, these drugs used in clinical treatment have shown substantial side effects (Lang et al., 1993; Richman et al., 1987). Moreover, the appearance of severe dose-dependent toxicity and the emergence of drug-resistant mutant viruses have been reported after long-term administration of these drugs (Fitzgibbon et al., 1992; Larder and Kemp, 1989; St. Clair et al., 1991). Thus, development of new anti-HIV-1 agents is urgently needed for the treatment of AIDS patients.

It has been reported that CD4 is a major receptor for HIV-1 (Dalgleish et al., 1984; Klatzmann et al., 1984) and CD26 acts as a co-factor for entry of HIV-1 into CD4-positive cells (Callebaut et al., 1993). Dextran sulfate was shown to block the binding of HIV virions to CD4-positive target cells, inhibit virally induced syncytium formation, and exert a potent inhibitory effect against HIV-1 in vitro (Baba et al., 1988; Mitsuya et al., 1988; Nakashima et al., 1987; Ueno and Kuno, 1987; Ito et al., 1987). The gangliosides, the most complex of the glycolipids, contain one or more sialic acid residues (also known as *N*-acetylneuraminic acid, or NANA). It was shown that sulfated gangliosides bind to the CD4 antigen on the surface of T-cells, down-regulating its expression and inhibiting the HIV-1 infection (Handa et al., 1991; Kawaguchi et al., 1989). Colominic acid is a homopolymer of *N*-acetylneuraminic acid (NANA), which has an α-2,8 ketosidic linkage between the polymer units (McGuire and Binkley, 1964). We examined the effects of several preparations of sulfated colominic acids for anti-HIV-1 activity and noticed that they had a potent antiviral activity against HIV-1. We therefore further investigated the mode of action of these compounds in vitro.

2. Materials and methods

2.1. Cells

MT-4 cells are a human T-cell line harboring human T-cell leukemia virus type 1 and susceptible to HIV-1 (Harada et al., 1985). Cells of the human CD4-positive lymphocyte line, C8166, were chosen as the target cells for the assay because this cell line expresses markers typical of activated T-cells in addition to its sensitivity to HTLV-III infection as well as its cytopathicity (Salahuddin et al., 1983). MOLT-4 cells (clone 8) (Kikukawa et al., 1986) were also a human T-cell line, and were used for the syncytium formation assay. MOLT-4/HTLV-IIIB cells were MOLT-4 cells persistently infected with the HTLV-IIIB strain of HIV-1. These cells were maintained in RPMI 1640 medium containing 10% fetal calf serum (FCS). The fetal calf serum (Sigma, St. Louis, MO) was heat-inactivated (56°C, 30 min) before use.

Primary human peripheral blood lymphocytes (PBLs) were collected, stimulated for 24–48 h with phytohemagglutinin (PHA) and then cultured in RPMI 1640 medium containing 15% FCS and 100 U/ml of interleukin-2.

2.2. Viruses

HTLV-IIIB strain of human immunodeficiency virus type I (HIV-1) (Popovic et al., 1984) was propagated in MOLT-4 (clone 8) cells and titrated in MT-4 cells. HIV-1[GUN-1] was isolated from the PBLs of a Japanese hemophilia B patient infected with AIDS (Takeuchi et al., 1987). Then, PBLs were co-cultivated with MT-4 cells and expression of HIV-1 antigen was examined during incubation by indirect immunofluorescence assay (IFA). In this study, C8166 cells were infected with HIV-1[GUN-1] and the culture supernatant was titrated in C8166 cells. This strain differs from HTLV-IIIB strain in the amino acid sequence of its envelope gene by approximately 6.7% (Liu et al., unpublished data).

2.3. Test compounds

The colominic acids (Marukin Shoyu Co., Ltd., Kyoto, Japan) were prepared by successive treatment with chlorosulfuric acid in pyridine at 70°C for 1 h or sulfur trioxide trimethylamine complex in *N,N*-dimethylformamide at 25°C for 20 h and then raised to 50°C for 5 h. The reactions were carried out using cation (Amberlite IR-120B) and anion (Amberlite IRA-93ZU) exchange column chromatography. The structure, molecular weight and sulfur content of the sulfated colominic acids are shown in Fig. 1 and Table 1. MW stands for molecular weight and S means surfur content. Heparin and dextran sulfate (average molecular weight, 500 000; sulfite content, 17%) was purchased from Pharmacia (Uppsala, Sweden) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. These compounds were dissolved in 90% PBS(−) and 10% dimethyl sulfoxide (DMSO) at 10 mg/ml and stored at − 20°C until use.

2.4. Assay for anti-HIV-1 activity

The measurement of activities of compounds against HIV-1 replication was based on the inhibition of viral protein expression in MT-4 cells infected with MOLT-4/HTLV-IIIB (Takeuchi et al., 1987) or C8166 cells infected with HIV-1[GUN-1]. Briefly, MT-4 cells (0.5 ml) or C8166 cells were seeded at 1.0×10^5 cells/ml in 48-well plastic plate. The drugs (0.05 ml) were added to the wells containing cells at concentrations ranging from 0.1 μg/ml to 100 μg/ml. After incubation for 1 or 2 h, the cells were infected with HIV-1 at the multiplicity of infection (m.o.i) of 0.05. HIV-1 antigen-positive cells were detected by IFA 4 days later. Serum of an AIDS patient and fluorescein isothiocyanate(FITC)-conjugated rabbit anti-human IgG (Sigma, St. Louis, MO) were used as the first and second antibodies.

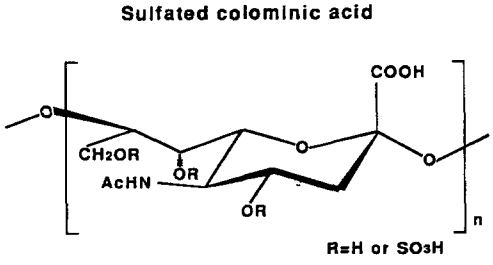

Fig. 1. Chemical structure of sulfated colominic acids.

Table 1
Anti-HIV-1 activities of sulfated colominic acids

Compound	EC_{50} (μg/ml) [a]			
	MT-4 [b]	C8166 [c]	PBLs [d]	Molt-4 [e]
#1-MW7-S 1	>100	>100	>100	>100
#2-MW7-S 3	>100	>100	>100	>100
#3-MW7-S 10	0.50	1.58	16.21	18.18
#4-MW8-S 6	3.16	3.06	>100	4.95
#5-MW9-S 8	0.06	0.50	2.39	1.83
#6-MW12-S 10	0.26	0.07	1.47	1.70
#7-MW14-S 12	0.28	0.27	1.34	0.67
#8-MW16-S 12	0.25	0.07	1.77	1.84
#9-MW8-S 6	0.56	0.43	>100	35.01
#10-MW12-S 9	0.25	0.10	2.45	2.29
Dextran sulfate	0.50	2.51	0.75	2.23

[a] Antivirally effective concentration required to achieve 50% protection of the cells against HIV-1.
[b] MT-4 cells were infected with MOLT-4/III_B in the presence of the sulfated colominic acids. Expression of HIV-1 antigen was detected by IFA after 4 days of cultivation.
[c] C8166 cells were infected with HIV-1[GUN-1] in the presence of the sulfated colominic acids. Expression of HIV-1 antigen was detected by IFA after 4 days of cultivation.
[d] PBLs were infected with HIV-1 and the cell suspension was added to the wells containing the test compounds. The amount of HIV-1 p24 antigen in the cell-free medium was determined by ELISA.
[e] MOLT-4 (clone 8) cells were co-cultured with HIV-1-infected MOLT-4 cells in the presence of the drugs for 24 or 48 h. Syncytia were counted microscopically.

2.5. Reverse transcriptase (RT) assay

The RNA-directed DNA polymerase (reverse transcriptase) enzyme was assayed and determined essentially as described elsewhere (Poiesz et al., 1980; Hoshino et al., 1984). Briefly, 0.5 ml culture medium was centrifuged at 6000 rpm for 5 min. 375 μl of culture medium was mixed with 12 μl of 5 M NaCl and 180 μl of 30% (w/v) polyethylene glycol (Carbowax 6000) and the suspension was placed on ice for 2 h. The suspension

was centrifuged at 15 000 rpm for 20 min at 4°C and the supernatant was removed. The precipitate (virus particles) was resuspended in 20 μl of 50% (v/v) glycerol/25 mM Tris-HCl (pH 7.5)/50 mM KCl/0.025% Triton X-100/5 mM dithiothreitol (DTT) (solution 1) and 10 μl of 0.9% Triton X-100/1.5 M KCl (solution 2). DNA polymerase assays were performed at 37°C for 1 h with a 10-μl aliquot of the disrupted virus solution by addition of 50 μl containing 40 mM Tris-HCl (pH 7.8), 4 mM dithiothreitol (DTT), 45 mM KCl, 1.8 μg oligo(dT)$_{12-18}$ (5.0 U/ml), distilled water, 9 μg template-primer poly(A) and 10 mM $MgCl_2$. The mixture also contained 15 μM of the appropriate labeled deoxyribonucleoside triphosphates and [^{3}H]TTP (5 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) (Radiochemical Center). After incubation, 50-μl samples of each assay mixture were spotted onto DE-81 filter papers. The filters were washed with 5% Na_2HPO_4, distilled water and ethanol. Then the radioactivity of the filter paper-bound samples was counted in cold trichloroacetic acid in a liquid scintillation counter.

2.6. Assay for HIV-1 p24 antigen

PHA-stimulated PBLs were cultured for 3 days and suspended at 2.5×10^5 cells/ml in RPMI 1640 medium. The cell suspension was centrifuged and the supernatant was discarded. Then, the cells were infected with MOLT-4/HTLV-IIIB at m.o.i. of 1.0 for 1 h. After washing once with PBS(−), 20 μl of the test compound at appropriate dilutions were plated in round-bottom 96-well microtiter plates and the cell suspension (5×10^4 cells/well) was added. HIV-1 infection was allowed to continue for 6 days at 37°C. The cell suspension harvested from each well on day 6 were centrifuged and the supernatant was heat-inactivated (56°C, 30 min), lysed (final 0.2% Triton X-100) and diluted by blocking buffer (3% BSA/PBS with 0.02% NaN3) before use.

HIV-1 p24 antigen in a cell-free culture medium was determined by an enzyme-linked immunosorbent assay (ELISA) method. 50 μl of base antibody (anti-p24 mouse monoclonal antibody, Sigma, St. Louis, MO), 50 μl of antigen solution, 50 μl of 1st antibody (anti-HIV-1 human serum) and 50 μl of 2nd antibody (anti-human IgG-alkaline phosphatase, Sigma, St. Louis, MO) were added to each well of the high-binding microtiter plate, respectively. After incubating for 2 h at room temperature, the solution was discarded and the wells were washed twice with PBS(−). 100 μl of substrate solution (Bio Rad, Hercules, CA) was added and the reaction was stopped by adding 100 μl of 0.4 M NaOH. The plates were read at 405 nm wavelength.

2.7. Syncytium and giant cell formation assay

Syncytium formation assay (Nakashima et al., 1988) and giant cell formation assay were performed as follows: 1.5×10^5 MOLT-4 clone 8 cells were co-cultured with 5×10^4 MOLT-4/HTLV-IIIB cells in the presence of the compounds for 24 or 48 h. C8166 cells were inoculated with cell-free culture of MT-4 cells infected with HIV-1[GUN-1] in the presence of the compounds for 24 or 48 h. The number of syncytia or giant cells was counted using a microscope after fixation with 5% formalin.

2.8. Detection of CD4 antigen on the cell surface

The presence of CD4 antigen on the surface of C8166 cells or MT-4 cells was examined by a flow cytometry (CytoACE 150, JASCO Co. Ltd., Tokyo). For this, the cells were seeded at 1×10^6 cells/ml, treated with compounds (100 μg/ml) for 1 h and then reacted with FITC-conjugated NU-TH/I anti-CD4 mouse monoclonal antibody (Nichirei Co., Tokyo). After incubation for 30 min at 4°C, the cells were washed with PBS(+) 1% FCS once and fixed with 1% paraformaldehyde and then subjected to flow cytometry.

2.9. Fetal calf serum (FCS) assay

MT-4 cells were seeded at 1×10^5 cells/ml in Eppendorf tubes in RPMI 1640 medium containing 10% FCS and 90% FCS or FCS only. The compounds were added to the tubes and incubated at 37°C for 1 h. Then the cells were infected with HIV-1 at 37°C for 1 h and the inoculum was removed. The cells were transferred to a 48-well

plate and HIV-1 antigen-positive cells were detected by IFA after 4 days of cultivation.

2.10. Drug combination

The combined effect of the sulfated colominic acid was assayed at various concentrations in the acute infection system of C8166 cells under the previous experimental conditions. To assess whether the drug combination resulted in a synergistic, additive, or antagonistic effect, the isobologram technique was used.

2.11. Activated partial thromboplastin time (APTT)

APTT of plasma from a normal subject was examined in the presence of the sulfated colominic acids by using an automatated machine (Handa et al., 1991). Platerin plus activator was obtained from Organon Teknika Corporation (Durham). Platerin plus activator was mixed with normal human plasma (100 μl) and test compounds (25 μl) and then APTT was measured.

3. Results

3.1. Anti-HIV-1 activities of sulfated colominic acid

MT-4 cells were incubated with MOLT-4/HTLV-IIIB in the absence of drugs for 4 days and expression of HIV-1-antigen was detected by IFA. Over 95% of the cells were positive for viral antigens. Sulfated colominic acids, containing 6–12% sulfur, completely inhibited the expression of HIV-1 antigen in MT-4 cells following exposure to the HTLV-IIIB strain of HIV-1. However, the drugs containing 1–3% sulfur, with a molecular weight of 7000, hardly affected the HIV-1 infectivity (Table 1). No toxicity for the host cells was noted with the agents even at 100 μg/ml. It was also examined whether the agents would affect another HIV-1[GUN-1]. The compounds also completely blocked expression of HIV-1[GUN-1] antigen in C8166 cells (Table 1).

3.2. Effect of sulfated colominic acids on HIV-1 RT and HIV-1 p24 antigen

We further tested whether the drugs inhibited virus production by MT-4 cells exposed to HIV-1 using RT assay. MT-4 cells were cultured in the presence or absence of various concentration of the agents and then exposed to HTLV-IIIB. On day 4, the supernatants were collected and then subjected to RT assay. A substantial level of RT activity could be detected in the supernatant of MT-4 cells exposed to HIV-1 in the absence of the drugs. However, addition of some compounds to the culture completely blocked viral replication. Compounds having a molecular weight of 7000, and containing 1–3% sulfur did not inhibit the production of HIV-1 infection. We then examined whether the RT of HIV-1 would be affected by the drugs directly and noticed no significant inhibition (data not shown). Table 1 also showed that the drugs were highly inhibitory to the production of HIV-1 p24 antigen in the cell-free culture medium when PBLs were infected with HIV-1.

3.3. Effect of sulfated colominic acids on syncytium and giant cell formation assay

HIV-1-infected MOLT-4 cells induced many multinucleated giant cells when they were co-cultured with MOLT-4 cells (clone 8). Syncytium formation was inhibited by most of the agents having anti-HIV-1 activity (Table 1). In addition, C8166 cells were inoculated with HIV-1[GUN-1] in the presence of the compounds for 24 or 48 h, in which the infection was also abolished (data not shown). These results suggest that the sulfated colominic acids would act on at least an early step of HIV-1 infection, namely, adsorption or penetration.

3.4. Effect of sulfated colominic acids on CD4 expression of the T-cells

The compounds were tested for the ability to modulate expression of CD4 antigen on the surface of T-cells. About 80% of C8166 cells were judged to be CD4 antigen-positive. CD4 antigen was still detectable after treatment with the com-

Table 2
Effect of sulfated colominic acids on CD4 expression of C8166 cells

Compound	CD4 expression (%) [a]
#2-MW7-S 3	73
#4-MW8-S 6	75
#6-MW12-S 10	72
#8-MW16-S 12	77
#10-MW12-S 9	76
Dextran sulfate	21
PBS(−)	75

[a] CD4-positive cells (%) detected by flow cytometry after treatment of the cells with one of the compounds at 100 μg/ml for 1 h at 37°C.

pounds for 1 h, but hardly detected on the surface of C8166 cells treated with dextran sulfate (Table 2). Similar results were obtained when MT-4 cells were treated with these drugs.

3.5. Effect of sulfated colominic acids on FCS assay

We also checked the compounds for anti-HIV-1 activity when MT-4 cells were cultured in RPMI 1640 medium containing high percentages of FCS or FCS alone. As shown in Table 3, the compounds could still block the expression of HIV-1 antigens even when the cells were cultured in the presence of high percentages of FCS. On the other hand, Table 3 also demonstrated a similar loss of activity with # 5-MW9-S 8, # 8-MW16-S 12 and dextran sulfate as a result of increasing concentrations of FCS. 100% FCS resulted in a 12.8-fold increase in the EC_{50} (50% effective concentration) for # 5-MW9-S 8, an 8.8-fold increase in the EC_{50} for # 8-MW16-S 12 and a 7.5-fold increase in the EC_{50} for dextran sulfate.

Table 3
Effect of different concentrations of FCS on the inhibitory activity of HIV-1 infection by sulfated colominic acids

Compound	FCS concentrations		
	100%	90%	10%
#5-MW9-S 8	0.9 [a]	0.1	0.07
#8-MW16-S 12	0.8	0.9	0.09
Dextran sulfate	7.5	8.2	1.0

[a] Concentration (μg/ml) of compounds at which 50% of MT-4 cells expressed HIV-1 antigens (EC_{50}).

3.6. Additive effect of sulfated colominic acids on AZT

Since the mode of action of sulfated colominic acids was different from those of nucleotide analogs, suggesting that combinations of the sulfated colominic acids and RT inhibitors may result in synergistic effects. When the sulfated colominic acid was assayed against HIV-1[GUN-1] in combination with AZT, the combination effect was additive and the highest concentrations of compounds did not affect the growth of T-cells (data not shown). The sulfated colominic acids did not interfere with the antiviral activity of AZT and thus it may be possible to use the sulfated colominic acid and AZT at the same time.

3.7. Lack of side effects of sulfated colominic acid

Finally, we examined the effects of the compounds on APTT of human plasma by using an automated machine. The compounds did not prolong APTT at 10 and 1.0 μg/ml. However, heparin and dextran sulfate markedly prolonged APTT at these concentrations (Table 4). These findings suggest that the sulfated colominic acids would have little side effect in vivo.

4. Discussion

In these various assay systems, the sulfated colominic acids displayed a strong anti-HIV-1 activity in vitro. This might be due to the high molecular weight and high sulfite-containing colominic acids. Dextran sulfate blocks the binding of HIV-1 to CD4-positive target cells (Mitsuya et al., 1988; Baba et al., 1988). The drug inhibits the expression of viral antigen and syncytium formation of HIV-1- and HIV-2-infected cells at concentrations between 1 and 10 μg/ml (Mitsuya et al., 1988; Baba et al., 1988; Ueno and Kuno, 1987; Ito et al., 1987). Low molecular weight

Table 4
Effect of sulfated colominic acids on activated partial thromboplastin time

Compound	APTT [a] (s)			
	100 [b]	10	1.0	0.1
#3-MW7-S 10	72.8	42.8	39.1	37.9
#4-MW8-S 6	140.7	41.3	37.6	36.8
#5-MW9-S 8	>280.0	54.3	39.1	37.6
#6-MW12-S 10	>280.0	53.8	40.7	38.2
#7-MW14-S 12	104.6	42.7	38.6	37.7
#8-MW16-S 12	257.5	52.4	39.7	38.3
#9-MW8-S 6	86.4	45.0	39.9	37.6
#10-MW12-S 9	>280.0	66.5	39.8	38.2
Dextran sulfate	>280.0	280.0	94.8	ND
Heparin	>280.0	>280.0	105.3	ND
DMSO [c]	ND	49.6	39.7	ND
No compound	39.4			

[a] Activated partial thromboplastin time of normal human plasma in the presence of compounds.
[b] Concentration (μg/ml) of the compounds.
[c] Dimethyl sulfoxide (DMSO) was tested at 10% and 1%.

(2000) dextran sulfate was reported to have little anti-HIV-1 activity. Analyses of the antiviral activity of dextran sulfate against HIV-1 have indicated that dextran sulfate with molecular weight less than 2300 had no antiviral effect (Hartman et al., 1990). The EC_{50} value of dextran sulfate estimated in this experiment was much smaller than those of dextran sulfate reported in some literature. Molecular weight of the sulfated colominic acids used in this study were ranged from 7000 to 16000.

The compounds also abolished syncytium formation upon co-cultivation of MOLT-4 cells (clone 8) with MOLT-4/HTLV-IIIB cells, suggesting that they would act on at least an early step of HIV-1 infection, namely, adsorption or penetration. It was reported that sulfated gangliosides bind to the CD4 antigen on the surface of T-cells, down-regulate its expression and inhibit the plating of HIV-1 infection (Handa et al., 1991; Kawaguchi et al., 1989). We tested whether the treatment of T-cells by sulfated colominic acids would affect CD4 expression. However, CD4 antigen was detectable after treatment with the compounds. These findings suggest that the sulfated colominic acids might bind to some other glycoproteins or glycolipids on the cell surface. Since CD26 is considered as a co-factor for entry of HIV-1 into CD4-positive cells (Callebaut et al., 1993) and the sulfated compounds interact with the third variable domain (V3 domain) of HIV-1 envelope glycoprotein gp120 (Batinic and Robey, 1992; Okada et al., 1995), it remains to be determined whether the sulfated colominic acids would affect CD26 expression on the cell surface and the V3 domain of HIV-1 gp120.

Sulfated polysaccharides have been shown to have anti-HIV-1 activity (Baba et al., 1990a). However, a phase I/II trial of orally administered dextran sulfate has suggested that this compound has little toxicity but also little clinical effect (Abrams et al., 1989). Further studies showed that dextran sulfate is poorly absorbed after oral administration using changes in partial thromboplastin time as a marker (Lorentsen et al., 1989). Using rats as a model, it has been suggested that this anionic polysaccharide is almost totally degraded in the gastrointestinal tract when given orally and sufficient inhibitory dose is not achieved in the plasma (Hartman et al., 1990). It also needs to be further examined using the bioavailability study whether the oral and intravenous administration of sulfated colominic acids are still able to produce significant anti-retroviral effect against HIV-1 in vivo. Many sulfated polysaccharides having anti-HIV-1 activities are known to prolong APTT (Baba et al., 1990b; Bagasra and Lischner, 1988; Mitsuya et al., 1988; Nakashima et al., 1988). The sulfated colominic acids did not prolong APTT at 10 and 1.0 μg/ml, indicating that they would not have anti-coagulant activity and may have little side effect in vivo. In this report, heparin and dextran sulfate markedly prolonged APTT even at 1 μg/ml.

Furthermore, it has been shown that the higher the concentration of human plasma, the higher the concentration of dextran sulfate required for antiviral effect (Hartman et al., 1990). The sulfated colominic acids also had potent anti-HIV-1 activities even when T-cells were cultured in RPMI 1640 medium containing high percentages of FCS. The ganglioside or sulfated ganglioside has been reported to down-regulate CD4 antigen and inhibit HIV-1 infection in serum-free culture

(Handa et al., 1991; Kawaguchi et al., 1989). This activity is blocked by addition of FCS or bovine and human serum albumin. The colominic acids were sulfated and their anti-HIV-1 activities were examined. The sulfated colominic acids had much more potent anti-HIV-1 activities than non-sulfated colominic acids. The colominic acids also did not affect growth of T-cells at concentrations of up to 100 μg/ml in vitro. These properties may be therapeutically advantageous if these compounds were considered for possible clinical use. The sulfated colominic acids had an additive effect when examined in combination with AZT, and furthermore, had very low cytotoxicity. Thus, they may represent a new class of anti-HIV-1 agents and good candidates for therapeutic use against HIV-1 infection.

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