Reduction of the viral load of HIV-1 after the intraperitoneal administration of dextrin 2-sulphate in patients with AIDS

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Objective: To determine the safety and efficacy of the sulphated polysaccharide, dextrin 2-sulphate, when delivered to the lymphatic circulation by the peritoneal route.

Design: An open Phase I/II dose-escalation clinical study in which six patients with AIDS were treated with seven courses of dextrin 2-sulphate each lasting 1 month.

Methods: During each course of treatment, the drug was administered daily for 28 days using an intraperitoneal catheter. Viral load was measured at frequent intervals using a plasma tissue culture infectious dose (TCID) assay, a cellular TCID assay, p24 antigenaemia, HIV-1 RNA and HIV-1 DNA. Plasma β-chemokine levels were also measured.

Results: Dose escalation was completed without toxicity. A total of 7 patient-months of treatment were completed. With increasing doses of dextrin 2-sulphate, the infectious plasma viraemia, cellular viraemia and p24 antigenaemia all fell during the period of drug administration, but with no significant change in HIV-1 RNA. This was associated with increased plasma levels of macrophage inflammatory protein (MIP)-1α and MIP-1β. Dextrin 2-sulphate accumulated in peritoneal macrophages and induced the release of MIP-1α and MIP-1β from these cells *in vitro*. These β-chemokines could have augmented the cell surface-mediated anti-HIV-1 effect of dextrin 2-sulphate *in vivo* by binding to and blocking the CC-chemokine receptor-5. A second fall in infectious plasma viraemia, cellular viraemia, p24 antigenaemia and HIV-1 RNA was seen at day 100 which was then sustained for several months. A clinical improvement in Kaposi's sarcoma was also seen.

Conclusions: Our results suggest that the intraperitoneal administration of dextrin 2-sulphate can reduce the replication of HIV-1 in patients with AIDS. With increasing doses of dextrin 2-sulphate, the fall in viral load was seen during the period of drug administration and again 2 months after completing treatment.

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Introduction

Although several sulphated derivatives of dextran and dextrin have activity against HIV-1 *in vitro* [1–4], clinical trials using dextran sulphate have shown considerable toxicity and no antiviral effect *in vivo* [5–7]. We have previously described the synthesis, chemical characterization and cell surface binding characteristics of dextrin 2-sulphate [3,8], which blocks the infection of lymphocytes and macrophages by primary viral isolates of HIV-1 *in vitro* [4].

We report the first clinical trial of dextrin 2-sulphate in patients with AIDS. Sulphated polysaccharides are not absorbed from the gut [6] and they are unlikely to achieve an adequate therapeutic concentration in the lymphatic circulation after intravenous administration [7]. Because a substantial proportion of the replication of HIV-1 takes place in CD4+ T lymphocytes and macrophages in lymphoid tissue [9–11], a Phase I/II study (Study in AIDS of Intraperitoneal Therapy; SAINT) was undertaken in which dextrin 2-sulphate was administered daily for 28 days via the peritoneal cavity. This study is the first clinical trial to use the peritoneal route to target an antiretroviral agent to the lymphatic circulation.

Methods

Patients and treatment protocol

The study was approved by the ethics committee of Hammersmith Hospitals Trust. Informed written consent was obtained from each patient. All six patients had a diagnosis of late-stage AIDS (mean ± SEM CD4 cell count, $31 \pm 11 \times 10^6$/l; range, < 10–60×10^6 cells/l); two had disseminated cytomegalovirus infection and three had systemic Kaposi's sarcoma. The patients were receiving various combinations of zidovudine, zalcitabine, didanosine, ganciclovir and foscarnet. All patients continued to take the antiretrovirals prescribed by their clinicians and no new antiretrovirals were prescribed during the study period. Patient A was on zidovudine and didanosine; patient B was on zidovudine, zalcitabine and foscarnet; patient C was on zidovudine; patient D on didanosine; patient E on zidovudine; and patient F was on zidovudine and didanosine.

Tenckhoff catheters were inserted under local anaesthesia and the initial exchanges of fluid into the peritoneal space were performed using 7.5% dextrin (icodextrin, ML Laboratories, Blaby Hall, Leicestershire, UK), which acted as a carrier solution from which dextrin 2-sulphate was absorbed over 24 h. Dextrin 2-sulphate was added to the 7.5% dextrin solution (daily exchange volume of 1.5 l) in a dose-escalation study. Patients A and B were treated with 37.5 mg daily for 5 days, 75 mg daily for 5 days, and 150 mg daily for 18 days. Patients C and D were treated with 37.5 mg daily for 2 days, 75 mg daily for 2 days, 112 mg daily for 2 days, and 150 mg daily for 22 days. Patients E and F were treated with 150 mg daily, as was patient C on retreatment. The drug was administered for 28 consecutive days.

While receiving dextrin 2-sulphate, the patients' clinical condition and haematological, haemostatic and biochemical profiles were monitored daily with less frequent monitoring after drug treatment had been completed. Haemoglobin, white cell count, differential, platelets, blood film, urea, electrolytes, liver function tests, calcium, phosphate, magnesium, plasma osmolality, complement, C-reactive protein, prothrombin time, kaolin partial thromboplastin time, thrombin time, fibrinogen, CD4 cell count and CD8 cell count were measured by the hospital's clinical diagnostic laboratories. An anti-Factor Xa assay was also used to detect heparin or heparin-like anticoagulant activity in blood. In spiking experiments, dextrin 2-sulphate prolonged the thrombin time at > 0.5 μg/ml.

Viral load

This was measured weekly during the period of dextrin 2-sulphate administration and at least once monthly thereafter. Blood was processed within 4 h of collection using methods previously described [12]. HIV-seronegative, phytohaemagglutinin-activated peripheral blood mononuclear cells (PBMC) were cultured in quadruplicate with serial (1 : 4) dilutions of the patient's PBMC (range, 1 : 1 to 1 : 256), and with serial dilutions of the patient's plasma (1 : 1.6 to 1 : 398). The cultures were fed every 5 days with HIV-seronegative PBMC. Cell-free culture supernatants were harvested on days 14 and 21 for measurement of p24 antigen using an enzyme immunoassay (Coulter, Luton, Bedfordshire, UK). The Spearman–Karber formula was used to calculate the median tissue culture infective dose ($TCID_{50}$) [13,14] and viruses were phenotyped using an MT2 assay [15].

Serum was separated and stored in liquid nitrogen in duplicate within 4 h of collection. HIV-1 RNA was measured using the Amplicor Monitor assay (Roche Diagnostics, Branchburg, New Jersey, USA). Serum-based measurements of HIV-1 RNA correlate very well with plasma-based measurements [16]. Samples were freeze-thawed once only. Each patient's samples were batch-tested on at least two separate occasions. In a given run, detection was undertaken on the same batch of microtitre plates in order to minimize variability. In reconstitution experiments, dextrin 2-sulphate (100 μg/ml) in serum did not interfere with the Amplicor Monitor assay. DNA was extracted from cells and polymerase chain reaction (PCR) was performed

on 0.5–1.0 μg DNA using primers for both *gag* and the unintegrated, circular forms of HIV-1 DNA, as previously described [17,18]. The limit of detection was 5 copies/μg, as determined using 8E5/LAV cells.

On a population basis, there is a large variation in the viral load (irrespective of how it is measured) in patients with similar CD4 cell counts. However, on an individual patient basis, there is much less variation for all measures of viral load. For example, the intrapatient variation in HIV-1 RNA is 1.8-fold, whereas that of cellular $TCID_{50}$ is sixfold [14,19]. Duplicating the measurement of HIV-1 RNA reduces variance by 15% and sample size by 14–41% [20].

Plasma p24, β_2-microglobulin, neopterin, macrophage inflammatory protein (MIP)-1α, MIP-1β and RANTES (regulated-upon-activation, normal T-cell expressed and secreted) were measured using assays from Coulter for p24, Pharmacia (Uppsala, Sweden) for β_2-microglobulin, Henning (Berlin, Germany) for neopterin, and R&D Systems (Abingdon, Oxfordshire, UK) for MIP-1α, MIP-1β and RANTES. The limit of detection was 15 pg/ml for MIP-1α and 31 pg/ml for MIP-1β. Each patient's samples were batch-tested on at least two separate occasions. Dextrin 2-sulphate (100 μg/ml) did not interfere with any of these assays.

The absorption of dextrin 2-sulphate from the peritoneal compartment was determined by measuring the concentration of the drug remaining in the peritoneal dialysate after a 24 h dwell (time for which fluid remains in the peritoneal compartment) using a limiting dilution infectivity inhibition assay (C8166 cells and $HIV\text{-}1_{IIIB}$) as previously described [3,8].

Peritoneal macrophages

The peritoneal dialysates from patients in the trial and from HIV-negative patients on continuous ambulatory peritoneal dialysis were collected after a 24 h dwell. Cytospin preparations were either fixed with alcohol and stained using Papanicolaou stain, or air-dried, fixed with methanol and stained using Giemsa stain. All slides were examined by a cytopathologist. The light microscopy findings were confirmed by immunocytochemistry using an anti-CD68 antibody (Dako, High Wycombe, Buckinghamshire, UK) to identify macrophages. Mesothelial cells were highlighted by positive staining for cytokeratin. In these preparations > 80% of the cells were macrophages.

The uptake of dextrin 2-sulphate by HIV-1-negative cells *in vitro* was determined using a biotinylated derivative of the molecule [21]. Cells were cultured for up to 5 days in biotin-free Dulbecco's modified Eagle's medium (Gibco, Paisley, Scotland) containing 5% fetal calf serum, 10% human serum, and 100 μg/ml dextrin 2-sulphate containing a 1 : 10 spike of biotinylated dextrin 2-sulphate. By using a large excess of non-biotinylated dextrin 2-sulphate, approximately 85% of the signal originating from the cell-surface binding of biotinylated dextrin 2-sulphate was eliminated. The presence of intracellular biotinylated dextrin 2-sulphate was identified by alkaline phosphatase-conjugated streptavidin and the colour developed using a fast red/napthol substrate (Sigma, Poole, Dorset, UK). The effect of dextrin 2-sulphate on the release of chemokines from peritoneal macrophages and from blood monocytes *in vitro* was investigated using single donor HIV-1-negative cells, which were cultured in the appropriate media [4,22] containing either dextrin 2-sulphate (100 μg/ml), dextrin 2-sulphate (100 μg/ml) in 7.5% dextrin, or 7.5% dextrin. Cell-free culture supernatants were collected at regular intervals over 72 h. Clinical grade, endotoxin-free dextrin 2-sulphate and dextrin were used for these experiments. They were performed using three different donors.

Results are given as the mean ± SEM, and groups were compared using a two-tailed Mann–Whitney U test (Graphpad, Instat, California, USA).

Results

Clinical outcome

This study started in April 1995. Dextrin 2-sulphate was tolerated up to the maximum daily dose of 150 mg daily. The total dose administered was 3.4–4.3 g and the total dose absorbed was approximately 1.7–2.6 g. The concentration of dextrin 2-sulphate was < 0.5 μg/ml in blood and < 3 μg/ml in urine at all times. No anti-Factor Xa activity was detected in blood at any time. Serum levels of dextrin and its metabolites during the period of treatment were 30–40% of those seen in patients on continuous ambulatory peritoneal dialysis who use dextrin as their dialysis fluid and reflected renal excretion [23]. The exchanges were well tolerated with no episodes of peritonitis. There was no clinical, haematological, haemostatic or biochemical evidence of toxicity. Following insertion of the catheter, analgesia was provided for 1 week using a paracetamol–codeine preparation. Several of the patients reported a clinical improvement of which one manifestation was an increase in their exercise tolerance to walking several miles daily.

Viral load during dose escalation

Four patients (A–D) were treated in this phase of the study. The time to a 10-fold reduction in the plasma $TCID_{50}$ ranged from 25 to 100 days (Figs 1 and 2). This reduction in the infectious plasma viraemia was still seen at 150 days. At the time of death of patient D (day 71), plasma $TCID_{50}$ had fallen from 6 to negative on three consecutive occasions (data not shown).

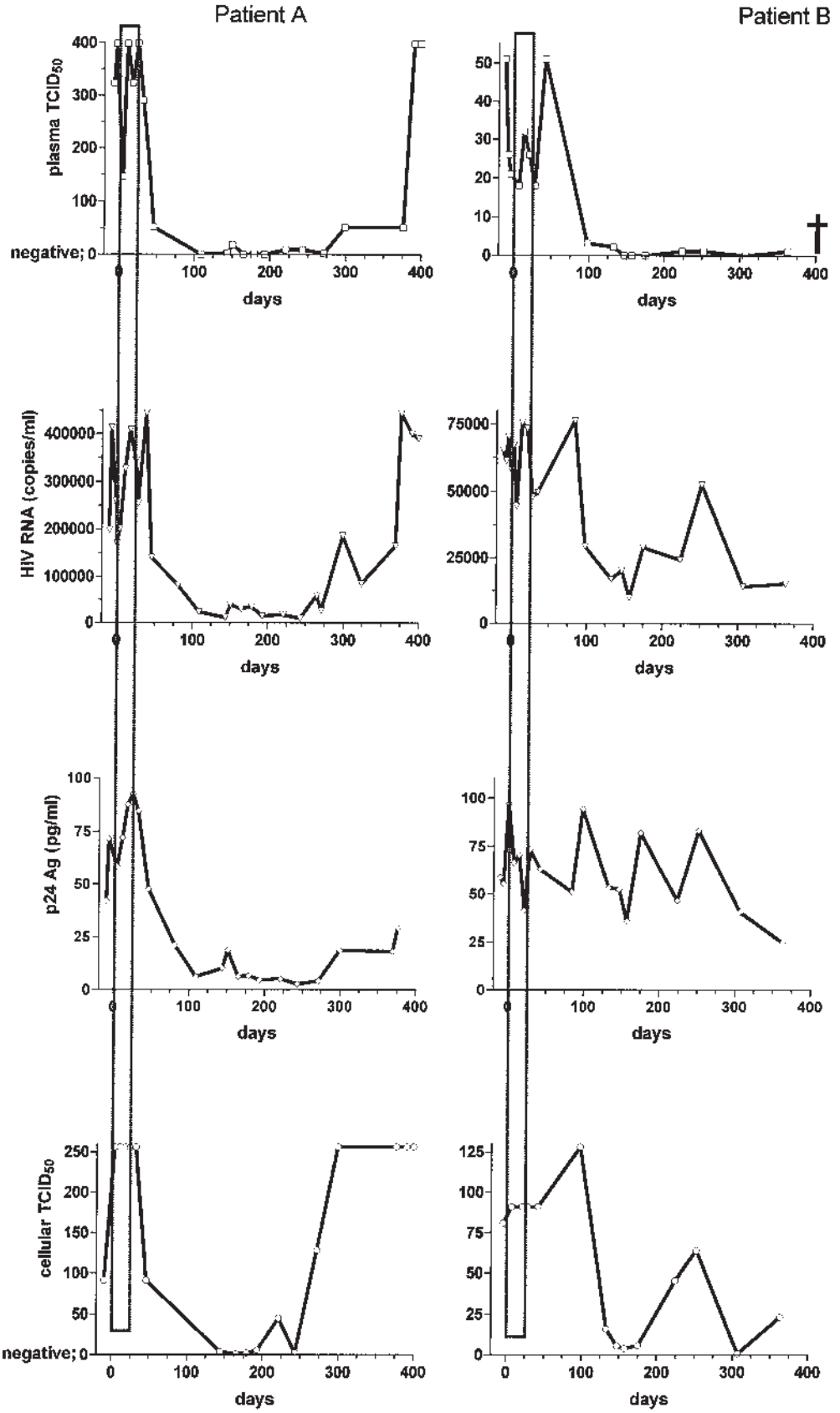

Fig. 1. Viral load during and after the dose-escalation phase of the study in patients A and B. The dotted box shows the period of treatment. †Patient death.

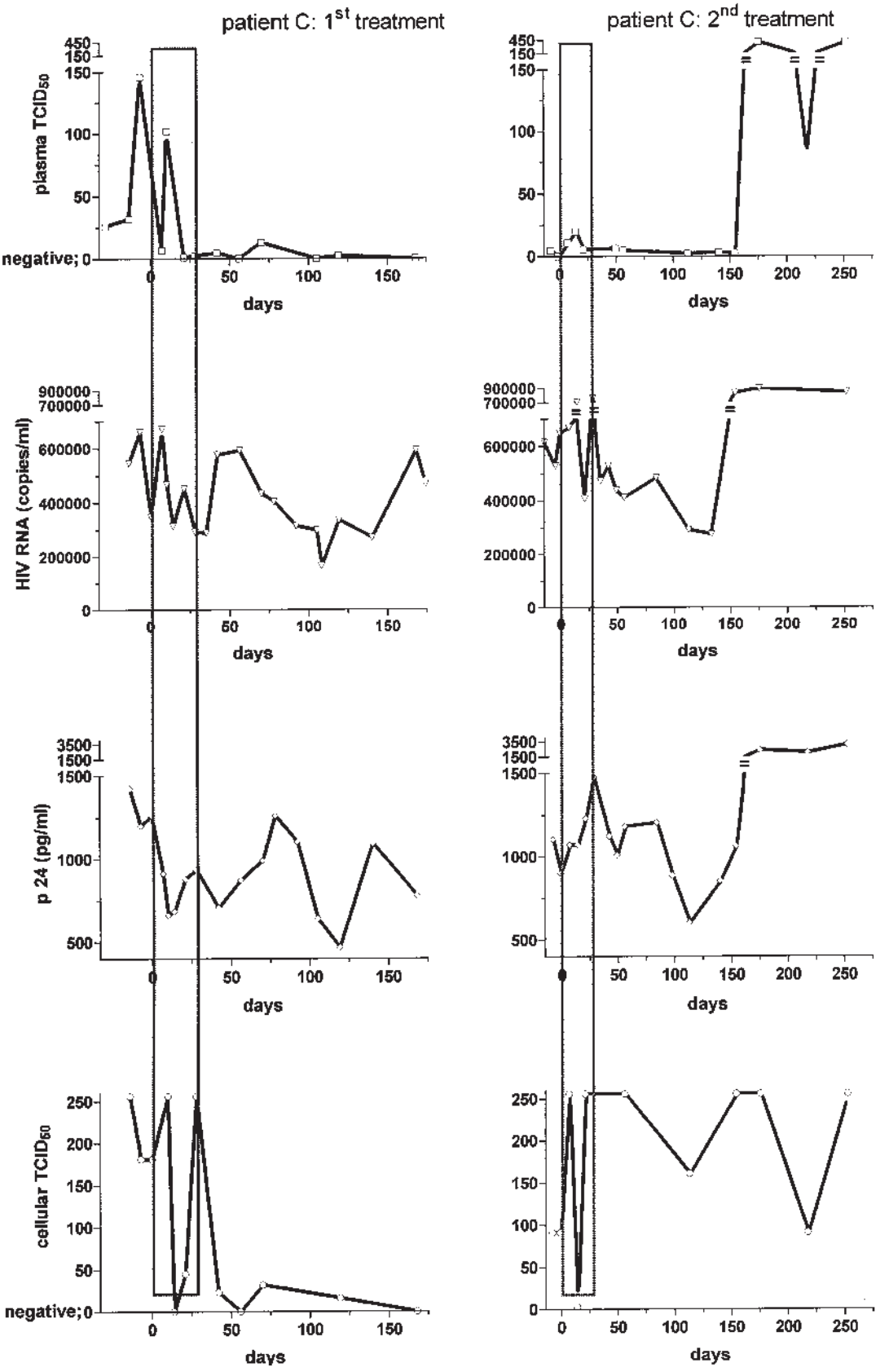

Fig. 2. Viral load in patient C during and after the first and second courses of treatment. The dotted box shows the period of treatment. The second course of treatment started 216 days after the initiation of the first course of treatment.

Table 1. HIV-1 RNA during the pretreatment and post-treatment period for each patient.

Patient	Pretreatment HIV-1 RNA (mean ± SEM copies/ml)	n	90–150 days HIV-1 RNA (mean ± SEM copies/ml)	n	*P*
A	253379 ± 38812	9	24071 ± 5210	9	< 0.0001
B	63660 ± 7515	8	14930 ± 1933	7	0.0003
E	1351072 ± 282981	6	370930 ± 102625	3	0.02
F	61010 ± 10072	6	73883 ± 11444*	7	0.63
C					
First treatment	522425 ± 66205	7	288636 ± 34663	8	0.006
Second treatment	611390 ± 55265	5	304022 ± 41239	5	0.008
D	568726 ± 172776	4	254701 ± 74261†	3	0.23

The sample size (n) represents the total number of timepoints pooled for the comparison. **Mycobacterium avium-intracellulare* and cytomegalovirus were isolated from blood taken on days 60 and 150 respectively. †HIV-1 RNA from days 49 to 63. The pretreatment and post-treatment HIV-1 RNA values were compared using a two-tailed Mann–Whitney U test.

Serum HIV-1 RNA had also fallen significantly in patients A, B and C by day 100 (Table 1). A fall in plasma HIV-1 p24 was seen in patients A (57 ± 9 to < 10 pg/ml; $P = 0.03$) and C (1295 ± 39 to 468 ± 34 pg/ml; $P = 0.001$) by day 100 (Figs 1 and 2), and in patient D (1278 ± 119 to 324 ± 21 pg/ml; $P < 0.0001$) by day 71. Post-mortem examination established the cause of death as *Pneumocystis carinii* pneumonia in patient D. The peritoneum showed no significant abnormality. The liver (1886 g) and spleen (244 g) were moderately enlarged. The liver sinusoids contained an increased number of macrophages with a granular cytoplasm. The lymph nodes and spleen were depleted of lymphoid follicles and showed non-specific reactive changes.

Viral load at maximum dose

Three patients (second treatment of patients C, E and F) were treated with dextrin 2-sulphate at 150 mg daily for 28 days. The results suggest a biphasic reduction in viral load. The plasma and cellular cultures became negative during the period of administration of dextrin 2-sulphate (Figs 2 and 3). Infectious plasma viraemia rose when treatment was stopped, only to fall again by day 100. The long-term follow-up of patient E was complicated by disseminated *Mycobacterium avium-intracellulare* (MAI) infection at day 160 and that of patient F by disseminated MAI infection and cytomegalovirus at days 60 and 150, respectively. Both opportunistic infections are known to cause a substantial increase in HIV-1 replication [24,25]. HIV-1 RNA fell significantly in patients C and E by day 100 (Table 1). Following the retreatment of patient C, his plasma p24 fell again from 1003 ± 60 to 604 ± 43 pg/ml ($P = 0.004$; Fig. 2) by day 100. Plasma p24 was not detectable in the plasma of patients E or F even after immune complex dissociation.

Changes in the viral load were also seen using the cellular $TCID_{50}$ assay. Table 1 summarizes the pretreatment and post-treatment (90–150 days) HIV-1 RNA. A statistically significant fall in HIV-1 RNA was seen after 100 days in six of the seven treatments undertaken. There was no significant change in the percentage or absolute CD4/CD8 lymphocyte count (data not shown), plasma β_2-microglobulin [pretreatment, 5.36 ± 0.35 mg/l (n = 8), versus treatment, 5.22 ± 0.25 mg/l (n = 12); $P = 0.75$] or plasma neopterin [pretreatment, 31 ± 6 mg/l (n = 17), versus treatment, 42 ± 7 mg/l (n = 28); $P = 0.11$].

The viral isolates from the PBMC and plasma of patients A, B and F had a syncytium-inducing (SI) phenotype, whilst those from patients C, D and E had a non-SI (NSI) phenotype. The circulating viral phenotype had not changed 6 months after treatment with dextrin 2-sulphate. When peritoneal macrophages from the patients were cultured *in vitro* for 28 days, p24 was not detected [22]. When peritoneal macrophages were cocultured with HIV-negative PBMC or with monocyte-derived macrophages (MDM), only NSI viruses were isolated. The pretreatment HIV-1 DNA proviral load in peritoneal macrophages from these patients was < 300 copies/10^6 cells [17,18].

Kaposi's sarcoma

Three patients (A, C and E) had extensive Kaposi's sarcoma. All showed clinical evidence of improvement despite receiving no other concurrent therapy specific for Kaposi's sarcoma. This improvement was gradual; it was not like the rapid clinical response seen after doxorubicin, bleomycin or vincristine therapy. The nodular lesions became flatter and developed a brown/tan halo. Tumour-associated oedema decreased and ulcerated lesions started to heal with the formation of a hard, white plaque-like surface. All these changes were compatible with a response to treatment of Kaposi's sarcoma as described by the AIDS Clinical Trials Group Oncology Committee [26].

Uptake of dextrin 2-sulphate by peritoneal macrophages

Peritoneal macrophages from the trial patients were collected after each 24 h peritoneal dialysate dwell and examined. Three days after starting treatment with dextrin 2-sulphate, striking coarse granularity of the

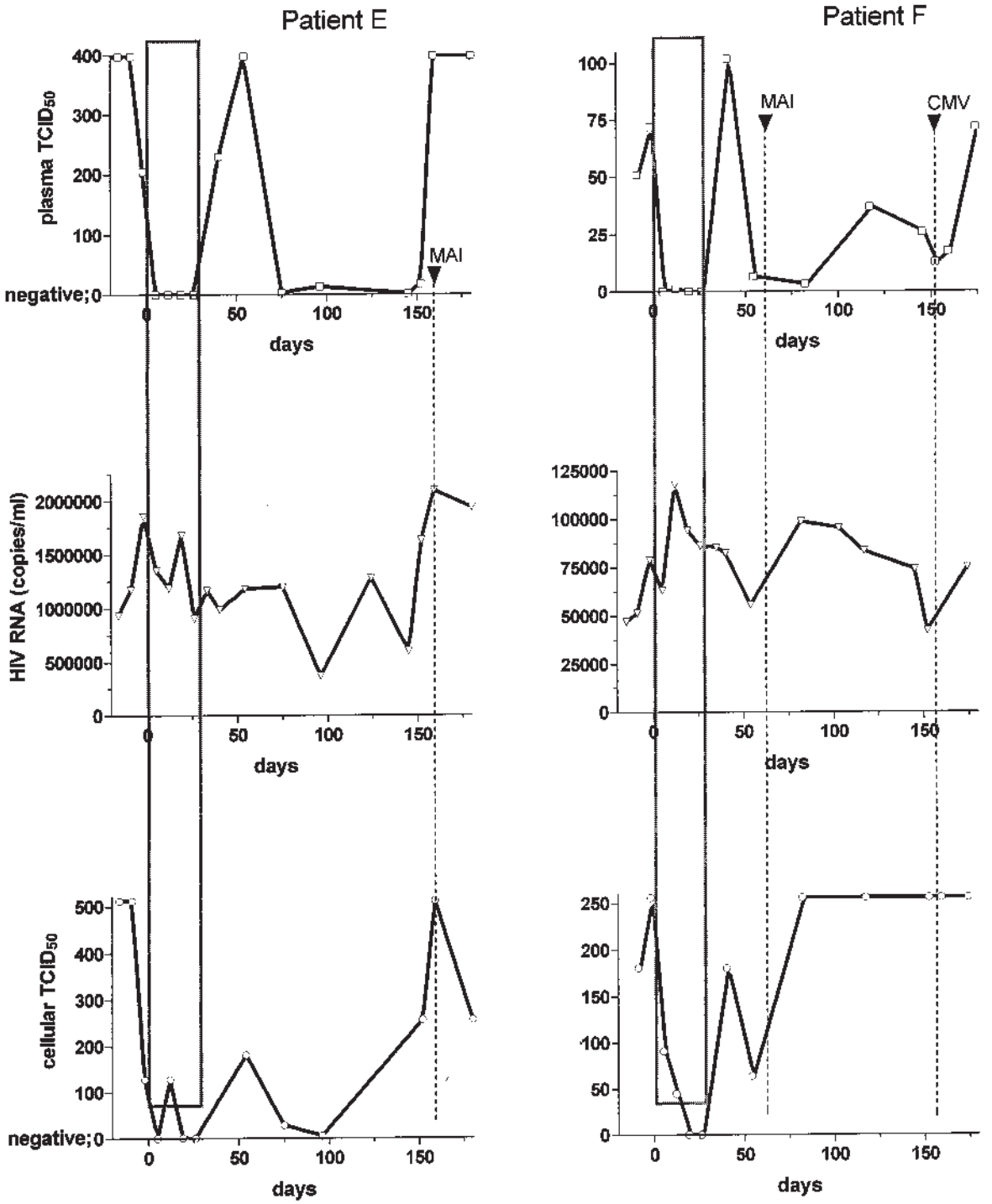

Fig. 3. Viral load during and after maximum dose treatment in patients E and F. Plasma p24 was not detectable in either patient, even after immune complex dissociation. The dotted box shows the period of treatment. MAI, Disseminated *Mycobacterium avium-intracellulare* infection; CMV, disseminated cytomegalovirus infection.

cytoplasm of peritoneal macrophages and mesothelial cells was seen (Fig. 4a). Cytoplasmic granules were not seen in neutrophils, lymphocytes or erythrocytes. These peritoneal macrophages were successfully maintained in culture *in vitro* for up to 4 months. Treatment with dextrin during the first few days of the trial did not result in the accumulation of granules in any cell type. No abnormal features were seen in the PBMC of any patient at any time.

Plasma levels of MIP-1α rose significantly during the period of drug administration [pretreatment, 37 ± 6 pg/ml (n = 15), versus treatment, 144 ± 33 pg/ml (n = 24); $P = 0.04$], as did those of MIP-1β [pre-treatment, 71 ± 12 pg/ml (n = 25), versus treatment,

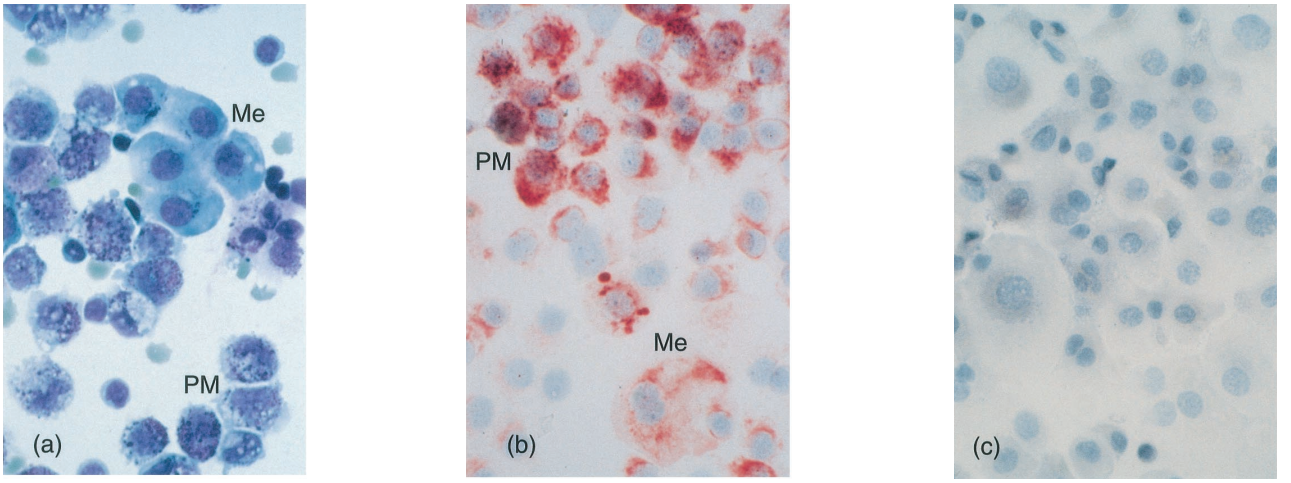

Fig. 4. (a) Cytospin preparation of the peritoneal dialysate after 4 days treatment with dextrin 2-sulphate. Striking cytoplasmic granularity of peritoneal macrophages (PM) and mesothelial cells (Me) is seen. Cytoplasmic granules are not seen in lymphocytes, neutrophils or erythrocytes (Giemsa stain, magnification × 250). (b) The red-staining reaction in the cytoplasm of peritoneal macrophages (PM) and mesothelial cells (Me) indicates uptake of the biotinylated derivative of dextrin 2-sulphate. There is no positive staining in lymphocytes, neutrophils or erythrocytes (magnification × 250). (c) As a control, slides were processed as for (b) after the cells had been incubated with non-biotinylated dextrin 2-sulphate (magnification × 250).

116 ± 18 pg/ml (n = 34); $P = 0.02$]. By the time of the second fall in viral load (i.e., days 90–150), plasma levels of MIP-1α had fallen to 51 ± 12 pg/ml (n = 19) and plasma levels of MIP-1β had fallen to 70 ± 12 pg/ml (n = 30). Plasma levels of RANTES were between 30 and 50 ng/ml at all times (data not shown) and almost certainly reflected the rapid release of this chemokine from platelets after venipuncture [27].

Release of MIP-1α and MIP-1β by peritoneal macrophages

In further *in vitro* immunohistochemical studies, a biotinylated derivative of dextrin 2-sulphate was used to demonstrate that the cytoplasmic granules seen in peritoneal macrophages and mesothelial cells *in vivo* represented the accumulation of dextrin 2-sulphate (Fig. 4b, c). No staining for biotinylated dextrin 2-sulphate was seen *in vitro* in HIV-1-negative neutrophils, lymphocytes, freshly isolated monocytes, cultured monocytes or MDM. Cell-free culture supernatants from HIV-1-negative peritoneal macrophages showed a large increase in MIP-1α and MIP-1β when cultured with dextrin 2-sulphate (100 μg/ml), or dextrin 2-sulphate dissolved in dextrin, but not when cultured with 7.5% dextrin (Fig. 5). No significant change in MIP-1α or MIP-1β was seen when the same experiments were performed using either PBMC or blood monocytes.

Discussion

Our results suggest that short-term intraperitoneal administration of dextrin 2-sulphate can result in a significant and sustained fall in the viral load in patients with AIDS. The fall in viral load seems to be biphasic. During the 28-day period of drug administration, the titre of infectious virus (as measured using virus culture assays) in plasma and PBMC decreased with incremental increases in the dose of dextrin 2-sulphate administered. Given the limits set at the start of the study for measuring the titre of infectious virus, it is possible that the fall was larger than that shown. It was associated with a fall in plasma p24 antigenaemia. The change in these three viral markers was not associated with a significant change in either serum HIV-1 RNA or PBMC HIV-1 DNA (data not shown) during the period of administration of dextrin 2-sulphate. The reason for this discordance between virus measured by culture and p24 antigenaemia, with virus measured by PCR amplification for HIV-1 DNA and HIV-1 RNA remains to be established. It may relate to either the total dose of drug that needs to be administered over a given period of time or the frequency with which the drug should be administered. It is also possible that, by analogy with the clinical trials of interleukin-2, immunotherapy can produce a clinical benefit which is not reflected by measuring HIV-1 RNA or HIV-1 DNA [28].

We have previously shown that dextrin 2-sulphate blocks HIV-1 infection *in vitro* [3,4]. It also binds to a cell surface protein that is expressed on T-cell lines and on activated PBMC [3,8,29,30]. Dextrin 2-sulphate does not bind to the β-chemokines (RANTES, MIP-1α or MIP-1β [21]), does not stimulate the release of MIP-1α or MIP-1β from blood monocytes, and does not induce the chemotaxis of blood monocytes (unpublished observations using HIV-negative blood

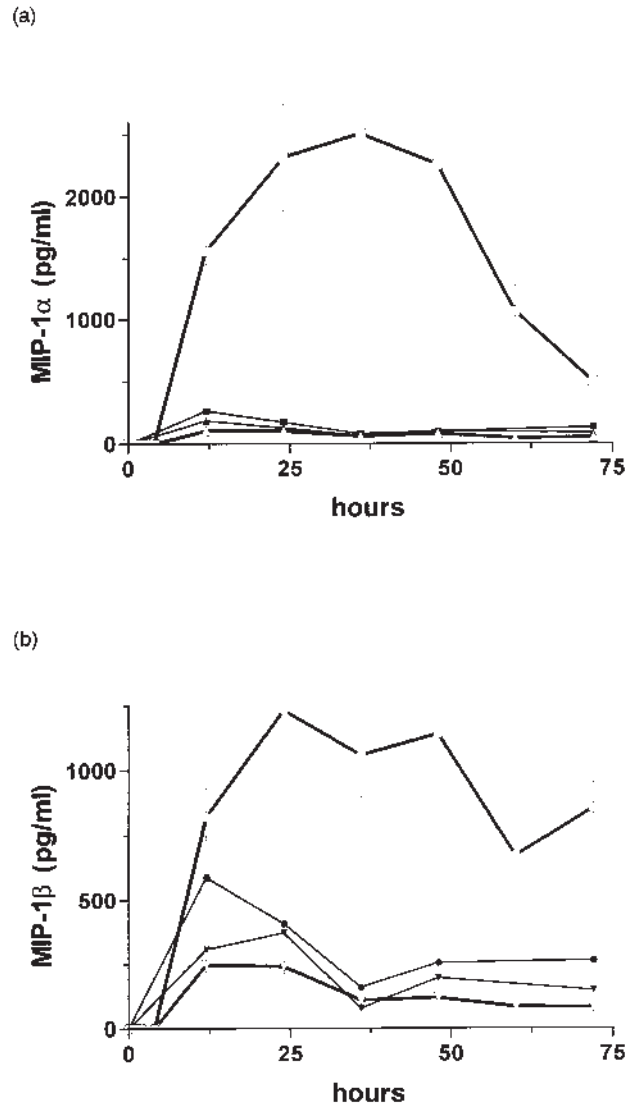

Fig. 5. (a) Macrophage inflammatory protein (MIP)-1α in cell-free culture supernatants. HIV-1-negative human peritoneal macrophages were cultured with either dextrin 2-sulphate at 100 μg/ml (□) or 7.5% dextrin (Δ; n = 4; $P < 0.0001$). HIV-1-negative human blood monocytes were cultured with either dextrin 2-sulphate at 100 μg/ml (■) or 7.5% dextrin (▲; n = 4; $P = 0.44$). (b) MIP-1β in cell-free culture supernatants. HIV-1-negative human peritoneal macrophages were cultured with either dextrin 2-sulphate at 100 μg/ml (○) or 7.5% dextrin (▽; n = 4; $P < 0.0001$). HIV-1-negative human blood monocytes were cultured with either dextrin 2-sulphate at 100 μg/ml (●) or 7.5% dextrin (▼; n = 4; $P = 0.16$). The re=sults are representative of experiments performed using three different donors.

monocytes and a Boyden chamber). It blocks the entry of low passage, SI and NSI primary viral isolates of HIV-1 in both activated PBMC and MDM with a 90% inhibitory concentration of 69 μg/ml (geometric mean) [4]. We had therefore expected to see a fall in the patients' viral load during the period of treatment, and this was detected using virus culture assays for plasma and PBMC, as well as p24 antigenaemia. Our *in vivo* observations seem to confirm the hypothesis that blocking viral entry into cells (by dextrin 2-sulphate itself and following the release of MIP-1α and MIP-1β) can reduce the infectious viral load significantly, provided the drug is delivered in sufficient concentration to the lymphoid tissue reservoir in which a substantial proportion of viral replication takes place.

Plasma levels of MIP-1α and MIP-1β increased during the period of drug administration. These *in vivo* observations could be explained by the accumulation of dextrin 2-sulphate in peritoneal (i.e., tissue) macrophages, which then induced the release of MIP-1α and MIP-1β from these cells. Given that only NSI viruses were isolated from the peritoneal macrophages of our patients (even when SI viruses were circulating simultaneously in the peripheral blood), these chemokines would have blocked the entry of NSI viruses into CD4+ T lymphocytes and macrophages by binding to the CC-chemokine receptor 5 (CCR-5) [31–35]. Local concentrations of MIP-1α and MIP-1β would have been much higher in the microenvironment of tissue macrophages in lymphoid tissues than were measured in the peripheral circulation [36].

Verani *et al.* [35] recently showed that human MDM can be induced to release β-chemokines by lipopolysaccharide *in vitro*, and that these chemokines block the infection of human MDM by primary NSI viruses. As most of the peritoneal macrophages from our patients were not infected (pretreatment HIV-1 DNA <300 copies/10^6 cells), it is possible that tissue macrophages have a dual role. Whereas some cells act as a reservoir for the virus [18], the majority of uninfected tissue macrophages can be stimulated to release β-chemokines, which then block new rounds of viral infection in their local microenvironment.

The second and more sustained fall in viral load was seen between 90 and 150 days using all five measures of viral load. The fall in the plasma $TCID_{50}$ was often sustained for even longer. The mechanism underlying this unexpected and late fall in viral load may relate to the accumulation of dextrin 2-sulphate in peritoneal macrophages and is currently under investigation.

The clinical improvement in Kaposi's sarcoma was gradual and was sustained until the viral load returned to pretreatment levels. Two sulphated polysaccharide-peptidoglycan compounds have previously been shown to inhibit angiogenesis *in vitro* using cells derived from Kaposi's sarcoma lesions [37,38]. However, no clinical benefit was seen when these compounds were administered to patients. The highly vascular nature of Kaposi's sarcoma has led to the suggestion that this lesion devel-

ops from aberrant lymphaticovenous connections, which in early macular lesions are lined by cells that are phenotypically similar to lymphatic endothelium [39–41]. Spindle cells, which are typical of Kaposi's sarcoma, express several endothelial cell markers [42]. It is therefore possible that these compounds will only be clinically effective if they are delivered in sufficient concentration to the lymphatic circulation, and if they accumulate within cells that have the phenotypic characteristics of endothelial cells. Dextrin 2-sulphate accumulates in tissue macrophages and mesothelial cells *in vivo*, and we have preliminary evidence that it also accumulates in human umbilical vein endothelial cells *in vitro* (unpublished observations).

Our results suggest that the intraperitoneal administration of dextrin 2-sulphate in patients with AIDS may reduce the viral load of HIV-1. Three different mechanisms may operate: (i) binding of the drug to a cell surface protein on lymphocytes and MDM to block viral entry, (ii) inducing the release of MIP-1α and MIP-1β from tissue macrophages, which then block viral entry into CD4+ T lymphocytes and macrophages by binding to CCR-5, and (iii) an intracellular mechanism in tissue macrophages. Increasing the length of treatment may result in a significant and sustained fall in viral load without the need for continuous treatment. A multicentre study to answer this question is now in progress using a subcutaneous peritoneal 'portacath' and a simpler treatment protocol.

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