Orally active prostacyclin analogue in primary pulmonary hypertension

Yoshiaki Okano, Takao Yoshioka, Akito Shimouchi, Toru Satoh, Takeyoshi Kunieda

Primary pulmonary hypertension is a disease of unknown aetiology that is progressive and uniformly fatal. However, substantial improvement in the duration and quality of life has been reported in patients who have received long-term intravenous prostacyclin (PGI₂) infusion. But this treatment is more uncomfortable for patients and potentially more hazardous than oral therapy. On the other hand, the efficacy of oral vasodilators is not clear, apart from high-dose calcium-channel blockers in selected patients with mild symptoms. Beraprost sodium is an orally active PGI₂ analogue with a stable structure due to its cyclopentabenzofuranyl skeleton. We assessed the efficacy of this drug in 12 patients with severe primary pulmonary hypertension who were unresponsive to calcium-channel blockers and inhalated nitric oxide.

A diagnosis of primary pulmonary hypertension was made according to NIH registry criteria. After the patients had given their informed consent to take part, we did haemodynamic investigations with pulmonary and peripheral arterial catheters. We assessed acute responses after the first six patients had received one dose of beraprost sodium (about 2 µg/kg). Measurements were taken again after ten patients had received a daily dose of 80–180 µg for an average of 2 months. Pulmonary arterial pressure and resistance decreased by 12% and 26%, respectively (table), although one patient did respond more acutely. Three patients (cases 1, 2, and 10) did not show any improvement in their symptoms and died within 6 months. Another patient (case 4) died suddenly after 18 months. In the remaining eight patients there were improvements in functional class, as defined by New York Heart Association functional class. All these patients were still alive with the same dose of beraprost sodium during a mean of 5 (range 12–38; SD 8) months of follow-up. At the start of treatment, there were some minor complications such as flushing and headaches.

A quarter of the patients with severe symptoms did not show any improvement. However, most patients responded well to long-term beraprost sodium therapy, although there were few acute responses. All our patients had a functional status of III or IV, and ten of them had a mixed venous oxygen saturation below 63%, which is associated with a poor prognosis. Thus, the long-term efficacy of beraprost sodium may be partly attributable to its effects on vascular growth or remodelling. We believe that beraprost sodium may be the first treatment option in patients with severe symptoms of primary pulmonary hypertension before resorting to intravenous PGI₂, with its inherent risks and increased medical costs. But before such treatment can be recommended, further multicentre clinical trials are needed to investigate the long-term effects of the drug.

We thank K oshu Fujii (Toray Industries Inc, Tokyo, Japan) for providing information on the pharmacology of beraprost sodium, and all the nurses, physicians, and residents at our centre, especially Shingo K yotani and Yasunori N akayama for their referrals of patients.


Table 1

<table>
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<th>Baseline CI</th>
<th>PAP Baseline</th>
<th>PAP BPS</th>
<th>PVR Baseline</th>
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<th>SO₂ Baseline</th>
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RAP: mean right atrial pressure (mm Hg). CI: cardiac index (L min⁻¹ m⁻²). PAP: mean pulmonary arterial pressure (mm Hg). PVR: pulmonary vascular resistance (mm Hg L⁻¹ m⁻²). SO₂: mixed venous oxygen saturation (%). NYHA: New York Heart Association functional class. BPS: after long-term therapy with beraprost sodium.

Clinical characteristics and haemodynamics

Number of patients for means=10, except for age, RAP, and CI.
Efficacy of camptothecin in progressive multifocal leucoencephalopathy

Juliane Vollmer-Haase, Peter Young, E Bernd Ringelstein

With HIV and the increasing use of immunosuppressive agents in clinical medicine, neurologists are faced with many patients who have progressive multifocal leucoencephalopathy (PML), an opportunistic papovavirus (JC virus) infection of the central nervous system. Patients commonly present with focal neurological deficits and progressive dementia. At present, no standard treatment is known. Therapy with cytarabine has been suggested by a few case reports, but only minimal benefit from cytarabine in AIDS patients with PML has been reported.

Camptothecin is a human topoisomerase I inhibitor which blocks DNA replication in human cancer cells. It is used for the treatment of colorectal carcinomas if fluorouracil is ineffective. Common side-effects are neutropenia, loss of hair, and delayed diarrhoea. Kerr and colleagues reported the inhibition of DNA replication of the human neurotropic virus (JC virus) in glia cells by camptothecin. To our knowledge, there is no report on the treatment of PML or other infectious diseases in people with camptothecin. We report a 32-year-old woman who developed severe PML after several years of cyclophosphamide treatment for systemic lupus erythematosus (SLE). After intravenous camptothecin the severe neurological deficits improved and the extent and size of the previous enlarging white matter lesions on magnetic resonance imaging remained stable.

The patient had developed SLE 3 years ago, presenting with two episodes of acute renal failure. She had not responded to high-dose glucocorticosteroids. Therapy with cyclophosphamide was started with 100 mg per day and was reduced to 70 mg per day. She remained symptom-free for 2-5 years. In September, 1996, she developed a slight left-sided hemiparesis with brisk tendon reflexes and a Babinski sign. On T1 and T2 weighted images magnetic resonance tomography showed a non-enhancing white matter lesion on the right side. Cerebrospinal fluid was normal. HIV test was negative. Due to presumed involvement of the central nervous system by SLE, therapy with cyclophosphamide was continued. In the following 2 months, neurological symptoms progressed and she developed a left-sided hemiplegia, a mild right-sided hemiparesis, aphasia, and mutism. Magnetic resonance imaging showed enlargement of the bilateral white matter lesions, in number and size, without mass-effect. No enhancement was found. Since she failed to respond to immunosuppressive therapy, a biopsy sample of the brain was taken. This sample permitted the histological diagnosis of PML, subsequently confirmed by repetitive PCR for JC virus in cerebrospinal fluid. There was no suggestion of cerebral SLE, and therapy with cyclophosphamide was stopped. Neurological symptoms, however, rapidly increased. With informed consent, single-dose camptothecin 350 mg/m2 body surface was started every 3 weeks. During therapy she developed mild neutropenia and delayed diarrhoea but without systemic infection. After two cycles with camptothecin, she could speak in full sentences and was able to perform easy tasks. Magnetic resonance tomography showed steady state of the lesions. Further follow-up has shown little clinical improvement.

We conclude that camptothecin might block viral DNA (JC virus) replication not only in vitro but also in vivo. Clinical improvement and radiological steady state indicate a beneficial effect of camptothecin in our patient. Our findings could be important for the treatment of PML in non-AIDS patients and in AIDS patients. The dose and duration of therapy with camptothecin in PML patients need to be defined.


Bone-marrow transplantation in aspartylglucosaminuria

T Autti, P Santavuori, R Raininko, M Renlund, J Rapola, U Saarinen-Pihkala

Aspartylglucosaminuria (AGU) is a hereditary lysosomal storage disorder caused by defective activity of the enzyme aspartylglucosaminidase (AGA) and leads to progressive mental impairment. Magnetic resonance imaging (MRI) studies have shown delayed cerebral myelination; differentiation between cortical grey and white matter is poor, and the basal ganglia and thalamus have an abnormally hypointense appearance. Beneficial effects of bone-marrow transplantation (BMT) in other lysosomal diseases have been seen mainly in the visceral organs but not in the brain. Three patients homozygous for the Fin mutation in AGA, aged 1-5, 2-0, and 2-6 years, underwent allogeneic BMT, one from the father, one from a healthy sibling, and one from a matched unrelated donor. Two patients became heterozygous for the Fin mutation indicating successful engraftment, but the graft of the third patient was lost during the first post-transplant year. Follow-up ranged from 1-0 to 5-6 years. MRI was done shortly before BMT and repeated every 1 to 2 years by methods published previously. In the two transplanted patients with functioning marrow grafts, and the last MRI images taken showed the ratios between the signal intensities of the deep grey matter structures and those of white matter (figure). The thalamus showed lower signal intensity than the basal ganglia. Fair or evident cortical grey versus white matter differentiation was seen in the two transplanted patients with functioning marrow grafts, and the last MRI images taken showed the ratios between the signal intensities of the deep grey matter and white matter to be near normal (figure). In the patient with the failing graft post-transplant, MRI showed poor grey versus white matter differentiation at the age of 2-6 years.

These radiological findings were supported by histopathological and biochemical tests. In two patients storage lysosomes disappeared from rectal biopsy specimens 3 years post-transplant and bone leucocyte AGA activity
reached heterozygous levels. Information about neuropsychological improvement cannot be evaluated for some years because of the slow natural course of this condition. We conclude that this intervention may have beneficial effects on the central nervous system in these patients.

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Human herpesvirus 7 in pityriasis rosea

Francesco Drago, Emilia Ranieri, Fabiana Malaguti, Enzo Losi, Alfredo Rebora

Relapses and occurrence of pityriasis rosea during states of altered immunity suggest viral reactivation. Human herpesviruses, which can become latent and reactivate, are possible culprits.

We took blood samples from 12 patients with pityriasis rosea and 25 healthy people; in five patients, blood was also taken 10–14 months after recovery, and in one during a recurrence. Biopsy samples of involved skin of the patients and of 12 healthy people were taken. Interferons (IFN) alpha and gamma were assayed with ELISA. Viral isolates were identified by morphological abnormalities of cocultivated peripheral blood mononuclear cells (PBMCs) and electron microscopy. Nested PCR of HHV7 DNA sequences was done on the plasma, PBMC, and skin samples of the patients and controls. Outer primers were 5'-AGTTCCAGCAGCTGCACTG-3' and 5'-CACAAGGACTGCTTATC-3'. Inner primers were 5'-CGCATACACCAAACCTACTG-3' and 5'-GACTCATATTGCGGATCGAC-3'. Control DNA was extracted from HHV7 infected and non-infected Sup-T1 cells. The size of the products was expected to be 264 pb.

Serum samples from patients with pityriasis rosea contained IFN-alpha and IFN-gamma; serum from controls did not. Patients' cocultivated PBMCs showed ballooning cells and syncytia after 7-day cultivation; PBMCs from controls and recovered patients did not.

Nested PCR analysis of DNA from HHV7

Products of HHV7 were visualised by electrophoresis on 2% agarose gels (in TBE buffer and containing 1 µg ethidium bromide). M: molecular-weight marker: 4x 174-RF-DNA Ha e III DIGEST. Lanes 1 and 2: DNA extract from Sup-T1 infected by HHV7. Lane 3: uninfected Sup-T1. Lane 4: umbilical cord blood mononuclear cells activated by PHA. Lane 5: skin specimens from patient with acute pityriasis rosea. Lane 6: plasma from healthy control. Lane 7: plasma from patient with acute pityriasis rosea. Lane 8: PBMC from patient with pityriasis rosea during a relapse after 1 year. Lane 9: PBMC from patient with pityriasis rosea during acute pityriasis rosea.
There was also a cytopathic effect in a relapsing patient and in Sup-T1-cell cultures inoculated with the cell-free supernatant from centrifuged cultivated PBM C's. Electron microscopy of the supernatant showed virions with a tegument layer similar to that seen in HHV7.1 PCR identified HHV7 DNA in PBM C's, plasma, and skin from all patients and in PBM C's of the five patients who were re-examined (figure). Weaker signals of HHV7 DNA were found in PBM C's of 11 (44%) controls, but not in their plasma. Skin was negative in the control diseases.

Evidence supporting the role of HHV7 in pityriasis rosea are its DNA sequences isolated from patients' PBM C's, skin, and plasma and the absence of HHV7 DNA in convalescents' plasma and controls' skin. Although HHV7 DNA is detectable in PBM C's and saliva in most healthy adults,3 the role of HHV7 in human disease is unclear. HHV7 DNA has not been detected in plasma of healthy people or of patients with other illnesses suspected to be due to HHV7.4 The finding of HHV7 DNA in plasma, which reflects viral replication and virulence, strongly supports its causative role in pityriasis rosea.


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Antibodies to human herpesvirus 8 in women and infants born in Haiti and the USA

James J Goedert, Dean H Kedes, Don Ganem

Studies of HIV-1-infected individuals have reported an association between serological evidence of HHV8 infection and the risk of developing Kaposi's sarcoma. HIV-1-infected homosexual men have a substantially higher prevalence of anti-HHV8 antibodies than do HIV-1-infected haemophiliacs or non-HIV-infected people.1,2 We examined HIV-1-positive and HIV-1-negative pregnant women and their children. Women with AIDS are much less likely to develop Kaposi's sarcoma than are homosexual men with AIDS, and vertically infected children have a very small risk of Kaposi's sarcoma.

We studied 478 individuals from the Mothers and Infants Cohort Study in New York City, USA, including 146 HIV-1-positive pregnant women and 143 matched HIV-1-uninfected pregnant women.3 Of the 189 evaluable infants born to these women, 98 were born to HIV-1-positive mothers; of these, 26 (27%) were infected with HIV-1. Maternal serum samples were available for all but 18. Sera were diluted 1:40 and screened for antibodies to the HHV8 latency-associated nuclear antigens (LANA) expressed in the BCBL-1 cell line.1 Coded sera that were randomly included from ten healthy blood donors were negative for anti-LANA and sera from two patients with AIDS and Kaposi's sarcoma were positive at titres of 1:400 and 1:4000.

Group | HIV-1 status | HHV8 prevalence
--- | --- | ---
US-born women | Infected | 2/118 (1.7%)
Uninfected | 1/80 (1.3%)
Total | 3/198 (1.5%)
Haitian-born women | Infected | 1/28 (3.6%)
Uninfected | 8/63 (12.7%)
Total | 9/91 (9.9%)
Infants | Infected | 0/26
Uninfected | 0/158
Indeterminate | 0/5
Total | 0/189

Prevalence rates in US-born and Haitian-born pregnant women and their offspring

Of the 289 pregnant women, 12 (4.2%) were seropositive for anti-HHV8. This proportion is in good agreement with findings in high-risk non-pregnant women, in whom a prevalence of 3-4% was found.1 The present cohort included 91 women of Haitian origin. Based on earlier work indicating a higher prevalence of Kaposi's sarcoma in Haitian-born homosexuals with AIDS compared with those born in the USA (6.3% vs 1-6%),1 we tested the prevalence of HHV8 seropositivity in the subgroup. Nine (10%) of 91 Haitian women were positive, significantly higher than the proportion (3/198, 1.5%) of other women (table, p=0.002, two-tailed Fishers exact test). HHV8 seropositivity was not increased in women with HIV-1 infection, either among Haitian (1/28, 3-6%) or non-Haitian (2/118, 1.7%) women. All 189 infants were seronegative for HHV8, including 26 who were infected with HIV-1. Notably, none of the nine children born to HHV8-seropositive mothers were HHV8-seropositive when tested at a median age of 12 months.

These findings add to the growing evidence associating HHV8 infection with risk of Kaposi's sarcoma. Rates of seropositivity (0-4%) in HIV-1-infected women and children were substantially lower than those (25-35%) observed in HIV-1-positive homosexual men. In addition, the increased prevalence of the infection observed among women of Haitian origin accords well with the increased risk of Kaposi's sarcoma in HIV-1-infected Haitians. Since the serological test for HHV8 used here detects only about 80% of HHV8-infected individuals with Kaposi's sarcoma, our estimates of HHV8 prevalence are likely to be underestimates. Higher estimates of HHV8 prevalence have been suggested on the basis of one serological study measuring antibodies to lytic viral antigens. Nonetheless, the relative prevalence rates in different populations as measured by a single test, can still be reliably compared with one another. Our data show that such comparisons strongly and consistently linked anti-LANA HHV8 seropositivity to risk of Kaposi's sarcoma.

We thank the study individuals, staff, and principal investigators of the Mothers and Infants Cohort Study, Sheldon Landesman, Arye Rubinstein, and Anne Willoughby, for their advice.


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High incidence of meningioma in survivors of Hiroshima

T Shintani, N Hayakawa, N Kamada

A high incidence of meningioma among Nagasaki atomic-bomb survivors, reflecting the distance from the hypocentre, was reported by Shibata et al.\(^1\),\(^2\) We investigated the incidence of meningioma among survivors of Hiroshima’s atomic bomb in relation to radiation exposure. 68 patients surgically treated for meningioma who had been within 2 km of the atomic bomb explosion were identified from the database of the Hiroshima Tumour Registry. T treatment dates were between 1975 and 1992. 607 non-exposed patients with meningioma were also studied.

The incidence of meningioma among survivors of Hiroshima in 5-year intervals since 1975 were 5·3, 7·3, 10·1, and 1·9 cases per 10\(^5\) population, respectively. The relation between the incidence and distance from the hypocentre is shown in the figure (A). In 1990–92, the incidence of meningioma in people who had been exposed within 1 km was six times higher than among those not exposed. The relation between the incidence of meningioma and radiation exposure to the brain is shown in the figure (B). We defined relative biological effectiveness (RBE) of neutron beams as 10. Exposure radiation doses (Sv) were calculated as a total sum of \(\gamma\)-rays and 10 times the neutron doses. The incidence of meningioma among survivors of Hiroshima’s atomic bomb increased between 1975 and 1994. Acute leukaemia, thyroid cancer, breast cancer, lung cancer, gastric cancer, colon cancer, and skin cancer among atomic-bomb survivors are well known as radiation-induced tumours.

The high incidence of meningioma presented here and in reports from the Nagasaki group strongly suggest that meningioma is the eighth radiation-related tumour occurring in atomic-bomb survivors.


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DOBrava hantavirus in Estonia: does the virus exist throughout Europe?

Alexander Plyusnin, Olli Vapalahti, Vera Vasilienko, Heikki Henttonen, Antti Vaheri

Hantaviruses cause haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Each hantavirus is maintained in its own natural rodent host, and human beings get infected by inhalation of rodent excreta.\(^1\) HPS is caused by Sin Nombre and related viruses, which are carried by sigmodontine rodents endemic in the New World; HFRS is associated with Hantaan (HTN) and Seoul (SEO) viruses in Asia and with Puumala virus in Europe.\(^1\) Dobrava (DOB) virus has recently been shown to cause severe HFRS in Slovenia, Albania, Greece, and Bosnia.\(^2\)\(^-\)\(^4\) The prototype DOB strain was isolated from the yellow-necked field mouse, Apodemus flavicollis, in Slovenia.\(^2\) We report detection of DOB genome sequences in the striped field mouse, A. agrarius, from Estonia.

A. agrarius and A. flavicollis, were trapped in seven localities during September, 1996. Lung samples were screened for HTN/DOB-related antigen by immunoblotting. Three A. agrarius, all from the same locality (Hääka, Saaremaa island), were found positive and subsequently analysed by reverse transcription and nested PCR. Amplicons (354 nt in length) from the S(mall) genomic segment were prepared from two specimens and sequenced. Analyses of the virus-specific sequences, identical in both animals, revealed that they belong to the DOB genotype and share an ancient ancestor with the virus from Slovenia (nt identity 88·4%). Strain(s) from Saaremaa share a more recent ancestor with another Estonian strain originating from mice trapped in Vormsi island in 1994. Strains from the two islands are diverged from each other (9-6% nt difference), but, taken together, represent a distinct sublineage within the DOB genotype. This picture is consistent with the view on geographical clustering of hantavirus genetic variants.\(^5\)

Our data agree with earlier findings of hantaviral antigen in A. agrarius in Estonia\(^6\) and suggest the presence of DOB hantavirus as far to the north of Europe as Estonian islands. The results raise the questions of whether DOB can be maintained in both Apodemus species and whether the virus exists throughout Europe, from Balkans to the Baltic area. Although A. flavicollis has been considered the main host for DOB in Balkans,\(^2\) studies of hantaviruses enzootic in Apodemus mice in other parts of Europe are still to be performed. Whether the occurrence of DOB in its natural host(s) is continuous or not, requires further studies.

Since DOB is thought to cause a more severe form of HFRS than Puumala, similar to HTN-infection,\(^1\),\(^4\) the most
intriguing implication of our findings is the possibility of human DOB-infection in Europe outside the Balkans. Several reports based on either immunofluorescent or enzyme immunoassays, showed the presence of HTN-like antigen/antibodies in human beings and rodents in several European countries, including former Czechoslovakia, Germany, and Russia. However, definite hantavirus typing requires either a specific neutralisation test or genetic analysis of viral sequences from human or rodent samples. Moreover, it has been shown that even neutralising antibodies in early serum specimens from human hantavirus infection can be misleadingly cross-reactive. For instance, several acute phase HFRS sera showed the highest titres to HTN virus, but convalescent sera from these patients showed high specificity for DOB or Puumala hantaviruses.

We conclude that more attention needs to be paid to the surveillance of DOB hantavirus in Europe.


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Trypanosoma cruzi DNA in human mummies
Felipe Guhl, Carlos Jaramillo, Roxana Yockteng, Gutavo Adolfo Vallejo, Felipe Cárdenas-Arroyo

Chagas’ disease (American trypanosomiasis) infects 18–20 million Latin Americans. This incurable disease has been the focus of our research at the Parasitology Centre in the University of the Andes in Bogota, Colombia. Sharing our expertise and facilities with the Paleobiology Laboratory at the University of Minnesota, Duluth, USA, and the Department of Pathology at the University of Pisa, Italy, we studied a large number of soft tissue and skeletal specimens from spontaneously mummified human remains in the Atacama desert (figure).

Because gross pathological changes of Chagas’ disease are inconsistent and not specific, we used molecular biology methods to identify Trypanosoma cruzi in samples of human tissue, an approach similar to that used by Salo et al to identify M ycobacterium tuberculosis in ancient mummified human lung. Avila et al have shown that the kinetoplast has multiple repeat minicircle DNA segments that can be amplified in modern trypanosomes by PCR using primers S35 and S36 to target a 330 base-pair segment. We used this strategy on extracts of the rehydrated, mummified ancient skeletal and visceral organ tissues from 27 mummified bodies of seven cultures over the last 4000 years in southern Peru and northern Chile.

Sample extracts of human mummy tissues were prepared similar to the methods described by Salo et al and amplified by PCR with primers S35 and S36. A positive test was described as the isolation of a 330 bp band on gel electrophoresis from the amplified PCR product. Mummies had positive tests on at least one sample. Seven of the 21 heart samples and four of four oesophagus samples tested were positive. One mummy had positive reactions in samples of heart, oesophagus, colon, and rectum but negative results in ileal and lung samples.

Brazilian workers infected a mouse with T cruzi, killed and dehydrated it. After rehydration of a sample they were able to recover the same DNA target we sought. We have, however, been unable to find a report of successful recovery of T cruzi DNA from any ancient body, either human or animal. Our results indicate that human residents at coastal sites in southwestern South America suffered from American trypanosomiasis as long as 4000 years ago.

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