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# Antiviral treatment of Coxsackie B virus infection in human pancreatic islets

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**Abstract**

Enterovirus infections of the pancreatic islets are believed to trigger or precipitate the near total destruction of β-cells that constitutes type 1 diabetes (T1D). This study investigated the ability of an anti-picornaviral compound, pleconaril, to block the replication of two β-cell tropic Coxsackie B4 virus (CBV-4) strains in isolated human islets. The two strains, VD2921 and V89 4557, with demonstrated abilities to cause non-lytic persistence or lytic infection, respectively, in islets, represented two different potential mechanisms behind virus-induced T1D. The virus replication in the islets was studied with and without addition of pleconaril. In addition, islet morphology was studied every day. To test the effects of pleconaril and/or DMSO on the β-cells' insulin secretion, glucose perifusions were performed on treated and untreated islets. Virus titrations showed a clear reduction of the replication of both strains after pleconaril treatment. The VD2921 strain was inhibited to undetectable levels. The V89 4557 strain, however, showed an initial reduction of titers but virus titers then increased despite the addition of a second dose of pleconaril. This incomplete inhibition of viral replication suggested the existence of a resistant subtype within this strain. Pleconaril treatment reduced the β-cells' insulin secretion in response to glucose stimulation in some experiments and induced slight morphological changes to the islets compared to untreated controls. In summary, pleconaril reduced the replication of the two β-cell tropic CBV-4 strains in human islets. However, genetic differences between these strains influenced the effectiveness of pleconaril treatment. This stresses the importance of using multiple viral strains in antiviral tests.


*Keywords:* β-Cell; Islet of langerhans; Enterovirus; Picornavirus; Pleconaril; Type 1 diabetes

## 1. Introduction

Enteroviruses (EV) are small non-enveloped positive-stranded RNA viruses belonging to the Picornavirus family. The EV have been classified into five species (A–D and the polioviruses) based on the sequences of the viral capsid proteins (VP1–4) (King et al., 2000). The B species includes Coxsackie B viruses (CBV) 1–6, Coxsackie A9 virus, all echoviruses, and EV 69 and 73. EV mostly cause asymptomatic infections but are also frequently the cause of severe neonatal infections and aseptic meningitis (Sawyer, 2002) and have been associated with several autoimmune disorders: type 1 diabetes (T1D) [reviewed in Tauriainen et al., 2003], idiopathic dilated cardiomyopathy [reviewed in Kim et al., 2001] and Sjögren's syndrome (Triantafyllopoulou et al., 2004).

In patients with T1D, a high frequency of EV infections has been found around the time of clinical diagnosis (Frisk et al., 1992; King et al., 1983; Otonkoski et al., 2000; Yin et al., 2002a) and also during the prediabetic period months to years before symptoms appear (Lönnrot et al., 2000). Different serotypes of EV have also been isolated from a few patients at disease onset (Champsaur et al., 1982; Hindersson et al., 2005; Vreugdenhil et al., 2000; Yoon et al., 1979).

More direct evidence for EV involvement is the detection of EV genome by in situ hybridization in the pancreatic islets of four T1D patients (Ylipaasto et al., 2004). This would suggest that an EV infection of the islets, and possibly persistence of the virus is an important step in the pathogenesis of T1D. The tropism of EV for human islets and β-cells has been convincingly shown both in vivo and in vitro. EV-positive islet cells were found in 7/12 pancreases from infants who had died of fulminant coxsackievirus infections (Foulis et al., 1990; Ylipaasto et al., 2004). Furthermore, 6/7 of these EV-positive pancreases also showed insulitis while no insulitis was seen in any of the

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EV-negative pancreases, indicating not only enteroviral tropism for the islets but also a strong correlation of such infections with insulitis. Insulitis is one of the key features found in the pancreas of T1D patients in pathology studies (Foulis et al., 1986). A number of EV strains have been found to be islet and β-cell tropic in vitro and can infect isolated human pancreatic islets. The consequence of EV replication in human islets ranges from rapid cell lysis to subtle or no effects on morphology, in vitro, and depends primarily on the genetics of the EV strain (Frisk and Diderholm, 2000; Paananen et al., 2003; Roivainen et al., 2002). Overall these data show that EV infection of islets could contribute to β-cell death either directly or by attracting infiltrating immune cells which is compatible with the hypothesis that EV infection of the islets can lead to T1D in susceptible individuals. It is currently not known what viral phenotype characterises a potentially 'diabetogenic' EV.

Thus far, no interventions have successfully been able to reduce the incidence of T1D (Gale et al., 2004; Skyler, 2002), but considering the large amount of evidence for EV involvement in T1D pathogenesis, antiviral therapy could be considered as a novel approach. Several anti-picornaviral substances are available for in vitro studies but at the start of this study only one (pleconaril) was at the stage of clinical trials. Pleconaril (3-(3,5-dimethyl-4((3-(3-methyl-5-isoxazolyl)propyl)oxy)phenyl)-5-trifluoromethyl-1,2,4-oxadiazole) is an orally bioavailable antiviral compound designed to broadly inhibit picornaviruses (including EV and rhinoviruses). It belongs to a series of structurally related antiviral compounds, the WIN compounds, which bind into the hydrophobic pocket of VP1 beneath the floor of the canyon of the picornavirus capsid (Smith et al., 1986). The binding of these compounds displaces the unidentified hydrophobic molecule, the 'pocket factor' (Muckelbauer et al., 1995), and stabilizes the capsid, which interferes with the uncoating step necessary to release the viral RNA from the capsid during infection (Fox et al., 1986). WIN compounds have also been shown to block the cellular attachment of some viruses which use the canyon as the major binding site for receptors (Pevear et al., 1989) and this second mechanism of action may therefore be applicable to CBV. The in vitro efficacy of pleconaril has been shown using laboratory viral strains as well as clinical isolates (Kaiser et al., 2000; Ledford et al., 2004; Pevear et al., 1999). Pleconaril has also been used in clinical trials to treat common colds (Hayden et al., 2002), enteroviral meningitis (Abzug et al., 2003) and infections in neonates (Bryant et al., 2004).

To evaluate the possibility of using pleconaril to prevent T1D, we have tested the in vitro effect of a maximal pleconaril concentration (10 μM) on the replication of two different β-cell tropic CBV-4 strains. The study was performed using cultured human pancreatic islets isolated from organ donors. The two virus strains were chosen based on their previously shown ability to infect human pancreatic islet cells (Frisk and Diderholm, 2000; Yin et al., 2002b). A high pleconaril concentration was used to achieve maximum suppression of the virus based on the highest tolerated doses shown in other cell culture models (Ledford et al., 2004; Pevear et al., 1999). The effect of pleconaril on β-cell-specific function was also studied.

## 2. Materials and methods

### 2.1. Cell culture

Human pancreases were procured from heart-beating organ donors at transplantation units in Sweden, Norway, Finland and Denmark. The pancreatic islets were then isolated in Uppsala, Sweden, by an automated method (Ricordi et al., 1989). After isolation the islets were cultured in 9 cm Petri dishes in 10 ml RPMI supplemented with 10% human serum for 3–7 days before the virus inoculations. During the studies of virus replication, the islets were cultured in RPMI containing 5.5 mM glucose (SVA, Uppsala, Sweden) and supplemented with L-glutamine and 10% newborn bovine serum (Hyclone, Logan, UT). The study was approved by the Uppsala University Ethics Committee (Registration number 02-271, 8th April 2002). Green Monkey Kidney cells, used for cell culture infectious dose-50 ($CCID_{50}$) titrations, were cultured in 96-well culture plates (Nunc) in Eagle's Minimum Essential Medium (SVA, Uppsala, Sweden) supplemented with 10% newborn bovine serum. All cells were cultured at 37 °C, in 5% $CO_2$.

### 2.2. Virus strains and test compound

Two well-defined strains of CBV-4 (Frisk and Diderholm, 1997, 2000; Frisk et al., 2001a, 1994; Frisk and Tuvemo, 2004; Hindersson et al., 2004; Yin et al., 2002b) were used to infect the human islets: the VD2921 strain, and the V89 4557 strain. Both strains have previously been shown to infect human islets and to cause no cytopathic effects (CPE) or extensive CPE, respectively, during replication in islets (Frisk and Diderholm, 2000; Yin et al., 2002b). Pleconaril (3-(3,5-dimethyl-4((3-(3-methyl-5-isoxazolyl)propyl)oxy)phenyl)-5-trifluoromethyl-1,2,4-oxadiazole) (MW: 381.4 g/mol) was supplied by ViroPharma Incorporated and was dissolved in 100% dimethyl sulphoxide (DMSO) to a 200× stock solution (2 mM). The final concentrations of pleconaril and DMSO after addition to the islet cultures were 10 μM and 0.5%, respectively. This concentration of pleconaril has been shown to inhibit most strains in vitro (Kaiser et al., 2000; Pevear et al., 1999) while being below the 50% cytotoxic concentration for common cell lines (Pevear et al., 1999).

### 2.3. Virus inoculation of cells and pleconaril treatment

The effect of pleconaril on virus replication was studied in human islets cultured free-floating in six-well culture plates (Greiner Bio-One Gmbh, Frickenhausen, Germany). Each experiment used islets from a single donor and comprised five experimental conditions in duplicate. The following conditions were used: (a) uninfected controls; (b) pleconaril-treated uninfected islets; (c) virus-infected islets; (d) virus-infected islets treated with pleconaril 30 min post inoculation (p.i.); (e) islets inoculated with pleconaril-pretreated virus. For each condition, 50 islets were first handpicked into 1 ml of culture medium. In conditions 'c' and 'd' the islets were inoculated by addition of 0.2 ml of virus suspension containing $10^3$–$10^5$ $CCID_{50}$ of the

VD2921 strain or the V89 4557 strain followed by incubation at 37 °C for 30 min. In condition 'e', the islets were inoculated by addition of pleconaril-pretreated virus followed by incubation as above. Pretreatment of the virus with pleconaril was achieved by addition of 15 μl of a pleconaril stock solution (2 mM) in 100% DMSO to the 0.2 ml virus inoculum (VD2921 or V89 4557) and preincubation of this mixture at room temperature for 1 h before the inoculations. Uninfected islets (a and b) were mock-infected. After the 30 min incubation at 37 °C, an additional 2 ml of culture medium were added to all islet cultures and 15 μl of pleconaril 200× stock solution (2 mM) were added to (b and d) cultures. The final concentration of pleconaril (in b, d and e) was 10 μM and the final concentration of the solvent, DMSO, was 0.5%. On day 3 the culture medium was changed in all wells by careful aspiration followed by addition of fresh medium. Therefore two measurements of virus titer were made using the samples of culture medium collected before and after the change of medium. In some of the experiments, a second dose of pleconaril was added after the change of medium to conditions (b and d), but not to those inoculated with the pleconaril-pretreated virus (e). Virus replication was studied during 6 days p.i. by $CCID_{50}$ titration of infectious virus in samples of the culture medium collected at 1 h p.i. (day 0) and on days 1, 3, 4 and 6 p.i.

### 2.4. Insulin content

On day 6, the islets that had been cultured under the experimental conditions described above (a–e) were counted and handpicked into 200 μl acid ethanol (1.5 ml of 12 M HCl added to 98.5 ml of 70% ethanol) and kept at −20 °C. Insulin content was measured by Insulin ELISA (Mercodia, Uppsala, Sweden).

### 2.5. Pleconaril resistance test

To test if pleconaril-resistant virus had accumulated after passage of virus in islet cultures undergoing treatment with pleconaril, a second islet infection and pleconaril treatment was performed with pleconaril-passaged virus. Virus was selected from three separate islet infections where the V89 4557 strain had replicated in the presence of pleconaril. Culture medium containing infectious virus collected on day 6 was used for inoculations. Samples of 200 μl were preincubated with 15 μl of pleconaril 200× stock solution (2 mM) for 1 h at room temperature before inoculation onto new islets. The virus-pleconaril suspensions were added to 50 human islets in 1 ml of culture medium and incubated at 37 °C for 2 h. As a control, 50 islets were infected with a virus-pleconaril suspension of pleconaril-unpassaged V89 4557 treated as above. As a second control, 50 islets were infected with pleconaril-unpassaged V89 4557 without addition of pleconaril. After the incubation, 2 ml of culture medium were added and samples of the culture medium were collected for virus titrations (day 0). On day 3, 1 ml of fresh culture medium was added to all islet cultures and 5 μl of pleconaril 200× stock solution (2 mM) were added to the two pleconaril-treated cultures. The final concentration of pleconaril in islet cultures infected with pleconaril-passaged or pleconaril-unpassaged virus was 10 μM. Virus replication was studied for 7 days by $CCID_{50}$ titration of samples of the culture medium collected at 2 h p.i. (day 0) and on days 1, 3, 5 and 7 p.i.

### 2.6. Light microscopy

All islets were studied by light microscopy every day during the experiments for the appearance of cytopathic effects (CPE). The degree of CPE was graded from 0 to 4+, with 0 being intact islets and 4+ being a total destruction of the islets.

### 2.7. Virus titrations

Virus titers in the culture medium from infected islets were determined by the $CCID_{50}$ titration method on GMK cells. Briefly, 10-fold dilutions (1:10 to $1:10^6$) of samples of the culture medium which had been collected on days 0–7 p.i. were added in triplicate to GMK cells cultured in 96-well plates. The $CCID_{50}$ titer/0.2 ml was determined by the cytopathic effects visible after 7–10 days.

### 2.8. Islet function after pleconaril and DMSO treatment

Islets normally increase their insulin secretion when exposed to high glucose. To study the effects of pleconaril and/or DMSO treatment on this islet function the islets were perifused with low and high glucose concentrations and the insulin secretion was measured during 2 h. Twenty islets which had been cultured for 2–4 days in culture medium with addition of solvent only (0.5% DMSO), 10 μM pleconaril (and solvent, 0.5% DMSO) or without additions were placed in a perifusion chamber. The islets were first stabilized to reach basal levels of insulin secretion by perifusion with a buffer (11.5 mM NaCl, 0.5 mM KCl, 2.4 mM $NaHCO_3$, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM Hepes, 0.5 mM Phenol Red, 2% (w/v) albumin, pH 7.4) containing a non-stimulatory (1.7 mM) glucose concentration for 30 min. The first sample for insulin measurement was then collected. At 36 min, the perifusion buffer was changed to one containing a stimulatory (16.7 mM) glucose concentration. The perifusate was collected in 6 min fractions and analyzed for insulin content by ELISA (Mercodia, Uppsala, Sweden). After 42 min of stimulation, the islets were again perifused with a non-stimulatory glucose concentration for 48 min to study if the insulin secretion returned to basal levels. The stimulation index was calculated as the ratio of the total amount of insulin secreted during the high glucose stimulation and that released during low glucose.

In addition to islet function, cell viability was studied in islets cultured in the presence of DMSO since a loss of viability would indirectly affect virus replication and the loss of live cells would also affect the result of the glucose stimulation test. Islet cells were stained by trypan blue after 6 days of culture in medium containing 0.5% DMSO.

### 2.9. Virus thermostability test

Thermostability of the V89 4557 strain from the original inoculum was compared to that of progeny virus from islet infections where the V89 4557 strain had replicated in the presence

of pleconaril. Samples of the original inoculum suspension and samples of culture medium containing pleconaril-passaged V89 4557 from day 6 of islet experiments were incubated at 46 °C for 30 min. As a control, samples of the same virus suspensions were incubated at 4 °C for 30 min. The samples were then titrated by the $CCID_{50}$ method and loss of infectivity was calculated by subtracting the titers of the 46 °C-incubated virus from the titers of the 4 °C-incubated virus.

### 2.10. Statistical analysis

Insulin release (stimulation index) in the perifusion assay was compared between DMSO-treated, pleconaril-treated and untreated islets using Friedman test. Insulin contents of islets were compared using one-way ANOVA. $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Effect of pleconaril on virus replication

The replication of both CBV-4 strains in the islet cells was clearly affected by the presence of pleconaril (Fig. 1). The titers of the VD2921 strain were reduced to undetectable levels after treatment with 10 μM pleconaril (Figs. 1 and 2, $n = 5$) and did not rise again even when fresh culture medium without pleconaril was added on day 3 ($n = 3$), demonstrating the total inhibition of the replication of this strain. In GMK cells, pretreatment with only 0.1 μM pleconaril reduced the VD2921 strain to undetectable titers (data not shown). In comparison, the replication of the V89 4557 strain was reduced until day 3 in the presence of 10 μM pleconaril ($n = 6$), as evidenced by the lower virus titers in treated compared to untreated V89 4557, but after the change of culture medium on day 3 the V89 4557 strain resumed replication at a rate similar to that of untreated virus ($n = 6$). In addition a titer rise was detected between days 3 and 6 in the experiments where the pleconaril-treated V89 4557 strain was treated with a second dose of pleconaril (to a final concentration of 10 μM) after the change of culture medium on day 3 ($n = 4$) (Fig. 1). The inability of a second dose of pleconaril to inhibit the replication of the remaining low titers of the V89 4557 strain on day 3 strongly suggested the presence of drug-resistant virus.

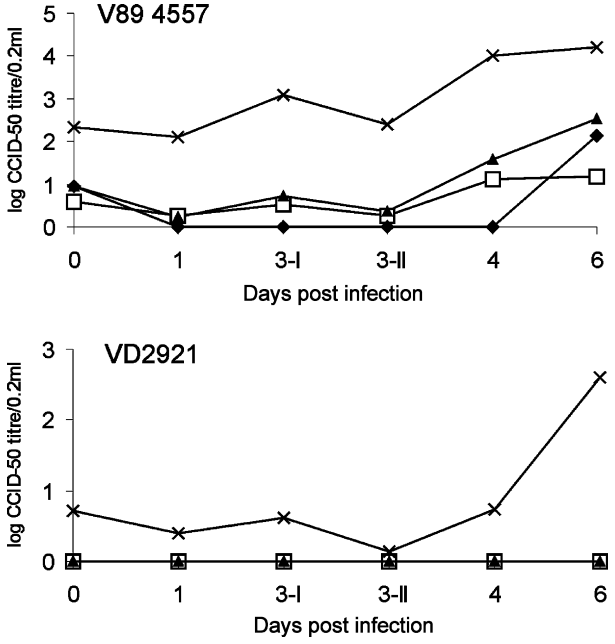


Fig. 1. Virus replication in the presence or absence of 10 μM pleconaril in 50 cultured human islets. Islets were infected with two different CBV-4 strains: the V89 4557 strain ($n = 6$), or the VD2921 strain ($n = 3$). Virus titers are presented as means (log $CCID_{50}$/0.2 ml). Samples for virus titrations were taken at two time points on day 3. These are denoted as day 3-I and day 3-II. Day 3-I indicates the sample taken before the change of culture medium on day 3. Day 3-II indicates the sample taken after the change of culture medium on day 3. (×) Virus only; (♦) virus and pleconaril; antiviral added 30 min p.i.; (□) virus and pleconaril; antiviral added 30 min p.i. and on day 3 after medium change; (▲) pleconaril-pretreated virus.

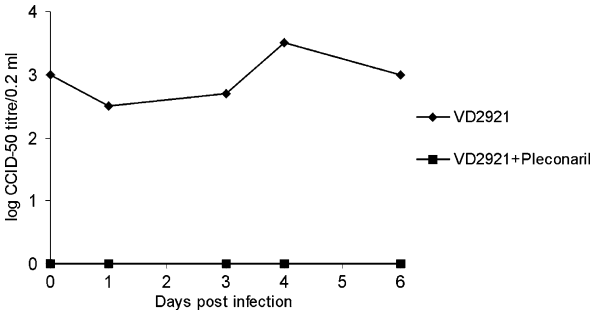


Fig. 2. Virus replication of the VD2921 strain with a high input titer in human islets (mean values, $n = 2$), in the presence or absence of 10 μM pleconaril. Pleconaril was added as pretreatment to the virus before the inoculations.

To confirm the existence of a pleconaril-resistant subpopulation within the V89 4557 strain, a second islet infection was performed to compare the effect of pleconaril treatment on progeny virus derived from pleconaril-passaged V89 4557 (Fig. 3) and on virus from the original inoculum. In the presence of pleconaril, a faster titer rise was seen with pleconaril-passaged virus than with virus which had not been

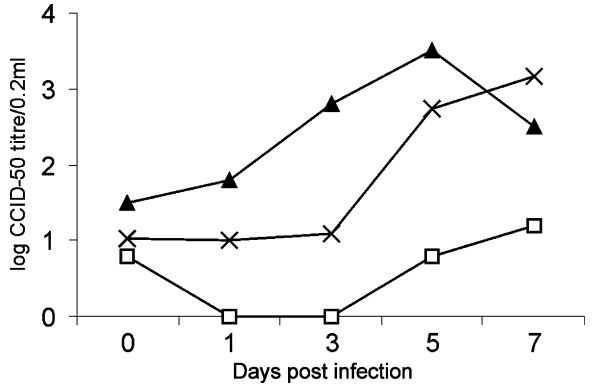


Fig. 3. Virus replication in the presence or absence of 10 μM pleconaril in islets infected with the V89 4557 strain: pleconaril-passaged virus pretreated with pleconaril before inoculation, $n = 3$ (×); unpassaged virus without addition of pleconaril, $n = 1$ (▲); unpassaged virus pretreated with pleconaril before inoculation, $n = 1$ (□). The $CCID_{50}$ titers (log $CCID_{50}$/0.2 ml) are presented as means where applicable.

passaged with pleconaril (i.e. virus from the original inoculum) indicating an increased number of viral particles of the resistant phenotype in samples of pleconaril-passaged virus. In the absence of pleconaril the original inoculum virus replicated well, similarly to what could be seen the first set of experiments.

### 3.2. Virus thermostability test

Thirty minutes of incubation at 46 °C, compared to incubation at 4 °C, reduced the $CCID_{50}$-titers of the V89 4557 strain original inoculum and of the pleconaril-passaged virus from day 6 after treatment, to similar degrees (mean decrease in titer 1.2 $\log_{10}$ ($n = 2$) and 0.9 $\log_{10}$ ($n = 4$), respectively).

### 3.3. Morphology of the islets

As can be seen from the mean degrees of CPE in Table 1, the morphology of the islets was more severely affected by the infection with the V89 4557 strain ($n = 6$) than by the VD2921 infection ($n = 3$), which caused no change in islet appearance compared to uninfected controls. This confirmed previous findings from islet infections with these two strains (Frisk and Diderholm, 2000). Islet destruction caused by virus was characterised by a loss of islet integrity and release of cells into the culture medium. The condition of the uninfected untreated control islets varied between donors and between individual islets from the same donor. In addition, some deterioration was often seen during the 6 days of culture of uninfected untreated islets. The deterioration was slightly greater in islets treated with pleconaril (together with 0.5% DMSO) (1.5+ on day 6) compared to untreated controls (1+ on day 6) (Table 1). In a subsequent experiment, islets were cultured in medium with addition of 0.5% DMSO without pleconaril. The addition of DMSO to the cultures did not affect the appearance of the islets during 6 days of culture ($n = 3$, data not shown) and did not cause a loss of cell viability as determined by trypan blue staining, 78% live cells in the DMSO-treated islets compared to 79% live cells in the control islets ($n = 1$) on day 6. Pleconaril-treated V89 4557-infected islets showed a similar degree of damage as islets infected by V89 4557 alone (Table 1).

Table 1
CPE on day 6 (mean values)

| | CPE on day 6 |
|---|---|
| Uninfected untreated control | 1+ |
| Pleconaril | 1.5+ |
| VD2921 | 1+ |
| VD2921 + pleconaril p.i. | 1+ |
| VD2921 pleconaril pretreatment | 1.5+ |
| Uninfected untreated control | 1+ |
| Pleconaril | 1.5+ |
| V89 4557 | 2+ |
| V89 4557 + pleconaril p.i. | 2+ |
| V89 4557 pleconaril pretreatment | 2+ |

Pleconaril (10 μM) was added to some of the infected islets and to some uninfected islets. Some islets were left untreated with no additions to the culture medium as a second control. CPE was graded from 0 to 4+, where 4+ indicates a total destruction of the islets. Islets were infected with the VD2921 strain ($n = 3$, in duplicate) or the V89 4557 strain ($n = 6$, in duplicate).

Table 2
Dynamic insulin release during glucose perifusion of islets

| | Perifusion assay (stimulation index) |
|---|---|
| Untreated islets | 3.9 ± 2.0 |
| DMSO-treated islets | 3.1 ± 0.5 |
| Pleconaril-treated islets | 5.4 ± 4.7 |

The stimulation index was defined as the ratio of the total amount of insulin secreted during the high glucose stimulation and that released during low glucose uninfected islets had been cultured with addition of 10 μM pleconaril and/or 0.5% DMSO or left untreated for 2–4 days before the perifusion assay ($n = 4$). Means ± S.D.

### 3.4. Response to high glucose after pleconaril or DMSO treatment

To test if addition of 0.5% DMSO or 10 μM pleconaril together with 0.5% DMSO to the islet cultures had any effect on the function of the islets, insulin release from islets perifused by glucose was measured. Four perifusion assays were performed and the mean stimulation indexes are presented in Table 2. The degree of insulin response differed between islet donors and there were no statistically significant differences between untreated controls and DMSO or pleconaril-treated islets (Friedman test). Donor variation is unavoidable due to the genetic diversity of the human donors and is also a consequence of the individual isolation procedures performed on each pancreas.

### 3.5. Insulin content of islets

Insulin content was measured on day 6 p.i. in islets from all experimental conditions. Insulin was detectable in islets from all experimental conditions but due to a high degree of variability no statistically significant differences were seen between virus-infected or pleconaril-treated islets and controls (ANOVA) (data not shown).

## 4. Discussion

Our results demonstrate that it is possible to inhibit the replication of β-cell tropic strains of EV in human islets in vitro by treatment with an antiviral drug. We also found that there is sufficient variation within the group of β-cell tropic EV strains to affect the sensitivity to pleconaril treatment. The replication of the VD2921 strain was completely inhibited by 10 μM pleconaril, while the V89 4557 strain maintained some replication in the presence of this high drug concentration. The accumulation of resistant V89 4557 variant(s) during pleconaril treatment was demonstrated in a second islet infection in which a faster titer rise was obtained with pleconaril-passaged virus compared to unpassaged virus in the presence of pleconaril (Fig. 3).

From clinical studies it is known that pleconaril has good oral availability (Kearns et al., 1999). The drug has been generally well tolerated by patients in clinical trials, but some headaches, diarrhoea and nausea occurred and it may decrease the effect of

oral contraceptives through an induction of cytochrome P-450 3A enzyme activity (Abzug et al., 2003; Hayden et al., 2002; Ma et al., 2006) and has therefore not been approved for use against the common cold. It is also no longer used in clinical trials for treatment of enteroviral meningitis since consistent efficacy has not been shown for this indication. In this study pleconaril treatment (together with the solvent DMSO) slightly altered islet morphology, but not to a degree that could account for the total block of replication of the VD2921 strain since pleconaril-resistant V89 4557 was able to replicate in pleconaril-treated islets. Antiviral treatment together with DMSO in one case also hampered the β-cells' response to high glucose.

The difference in resistance between the virus strains emphasizes the importance of virus genetics and the importance of choice of virus strains to study even when studying strains sharing a similar tropism for β-cells. This variation is exemplified for the two CBV-4 strains also by their different effects on human islets, although those characteristics are probably not related to the susceptibility to inhibition by a capsid binding antiviral. The two strains do, however, show different patterns of receptor usage in other cell types (Frisk et al., 2001b) which may affect the efficacy of an antiviral drug aimed at inhibiting the attachment/uncoating step of the viral infection. Even though the viral titer in most experiments (Fig. 1) was 10-fold higher in the inoculum with the V89 4557 strain, this was not the reason for the resistance of the V89 4557 strain, since further experiments showed that pleconaril was also able to completely inhibit the replication of a higher titer VD2921 inoculum (Fig. 2). Rhinovirus resistance to high concentrations of WIN compounds has been attributed to mutations in the hydrophobic pocket, leading to loss of ability to bind the antiviral (Heinz et al., 1989). Mutations in the hydrophobic pocket may also reduce the stability of the capsid by affecting the binding of the pocket factor which leads to a phenotype with increased sensitivity to heat (46–50 °C) (Groarke and Pevear, 1999; Nikolova and Galabov, 2003). The fact that this characteristic was not found in the V89 4557 strain suggests that, in this case, the resistance was not due to a mutation compromising the function of the hydrophobic pocket. Mutations resulting in bulkier amino acids in this cavity may however increase the stability of the virus capsid as well as blocking binding of the antiviral (Reisdorph et al., 2003).

In conclusion, this study shows that it is possible to inhibit the replication of β-cell tropic EV in human islets in vitro. However, the resistance that was found in one of the virus strains underscores the importance of testing multiple virus strains. It also shows the genetic diversity of β-cell tropic EV that exist and that must be targeted by an antiviral approach until further characterization defines diabetogenicity.

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