Inhibition of high risk HPV-31 in human cervical epithelial cells in vitro by the PC-PLC inhibitor LMV-601

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**Background:** Expression of early genes and episomal DNA replication of human papilloma virus (HPV) is dependent from an active AP1 complex. Activation of AP1 was shown to be precluded by inhibition of phosphatidylcholine specific phospholipase C (PC-PLC). We studied the effect of the PC-PLC inhibitor LMV-601 on HPV-31 infected 9E cervical epithelial cells (CIN 612 9E).

LMV-601 is (-)-exo/exo-O-Tricyclo-[5.2.1.0(2,6)]-dec-9-yl-dithiocarbonate potassium salt. Tricyclo-[5.2.1.0(2,6)]-dec-9-yl-dithiocarbonate potassium salt consists of 8 isomers (4 diastereomers, each having 2 enantiomers) and became known under the code D609, first synthesized in 1984 by Merz and Co in co-operation with the German Cancer Research Centre (DKFZ). The pure (-)-exo/exo isomer was first isolated in 2006 and is developed by Lumavita AG as an antiviral drug.

**Methods:** 9E HPV-31 infected cervical epithelial cells were from L.A. Laimins, Chicago.

(a) **Short term study:** After 72 h treatment, effect on cell growth, HPV-31 specific DNA (Southern Blotting) and RNA (Northern Blotting) was assessed.

(b) **Long term treatment** (9 passages): After each passage, viral RNA and DNA levels, and cell morphology were assessed.

**Results:** (a) Short term study: LMV-601 displayed a dose dependent inhibitory effect on cell growth (IC50 16 lg/mL), HPV-31 specific RNA expression (IC50 10.69 lg/mL) and DNA content (62.5% reduction at the highest dose tested, i.e. 32 lg/mL).

(b) Long term treatment: The number of passages required to reduce the amount of HPV-31 specific RNA by 50% (T50RNA) was 2.23 at 3.3 lg/mL LMV-601 and < 1 at 10 lg/mL LMV-601. The corresponding T50DNA values were 3.28 and < 1, respectively.

After six passages the growth rate of the cells was reduced and the morphology of the cells changed from the spindle form to a normal phenotype. After passage 9, cells were enlarged, became senescent (identified by expression of the senescence marker beta-gal), and ceased to grow.

When human, non-HPV immortalized HaCat keratinocytes were treated with the same concentrations of LMV-601, neither cumulative inhibition of growth rate nor induction of senescence could be observed.

**Conclusion:** LMV-601 inhibits HPV-31 specific RNA expression and DNA replication. Furthermore, these results support the hypothesis that chronic treatment with LMV-601 “cures” pre-cancerous 9E keratinocytes by elimination of HPV genomes.

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**Background:** In Herpes Zoster, the pain precedes dermatome manifestation from 10–12 hours to 2–5 days even to 10 days. Zosterian pain last, however it’s intermittent. Our goal in this study is in highlighting the topographic variety of Zosterian pain and initial misdiagnose related with it.

In this study we have included 202 cases of Herpes Zoster. 97 of the them were initially not identified as Herpes Zoster. The group age was 19–78 years old, time period from 1998–2009. 37 of them were HIV positive. In our cases pain precedes exantematic manifestation from 18 to 68 hours.

**Methods:** The cases were assessed based on correlation between neurotics zosterian pain and initial nosology.

**Results:** According to pain location we distinguished these initial misdiagnoses:

<table>
<thead>
<tr>
<th>Location</th>
<th>Diagnosis</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>migraine</td>
<td>8</td>
</tr>
<tr>
<td>sinusitis</td>
<td>frontal</td>
<td>2</td>
</tr>
<tr>
<td>otitis</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>ophtalmitis</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>arthiritis temporo — mandibula</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>odontalgia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thorax</td>
<td>angina pectoris</td>
<td>3</td>
</tr>
<tr>
<td>pericarditis</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>pleuritis</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>pneumonia</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Abdomen</td>
<td>abdominal colic</td>
<td>6</td>
</tr>
<tr>
<td>kidney colic</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>hepatic colic</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>cholecystitis</td>
<td></td>
<td>2</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>orchitis</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Upper extremites</td>
<td>cervical racialgya</td>
<td>5</td>
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<tr>
<td>cervical spondylarthrose</td>
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<td>7</td>
</tr>
<tr>
<td>thorax racialgya</td>
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<td>6</td>
</tr>
<tr>
<td>scapulo-humeral bursitis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Lower extremites</td>
<td>ischialgya</td>
<td>7</td>
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<tr>
<td>discal hernia</td>
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<tr>
<td>angiopathies</td>
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<td>2</td>
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<tr>
<td>polymialgia rheumatica</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>coxo-femoral arthritis</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:** 1) Zoster cases identified wrong initially were 48.02%.

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2) Incorrect nosologies conditioned by pain syndrome were 25.
3) Misdiagnoses depends on dermatome affected by zoster.
4) Neuritic pain that precedes zoster dermatomes needs to be considered in diagnosis of pathologies with pain syndrome.

Methods: A targeted small-interfering RNA (siRNA) screening platform assay was established and validated to identify and profile key cellular genes involved in processes of endocytosis, cytoskeletal dynamics and endosomal trafficking essential for HEV71 infection. Screen evaluation was conducted via the expression of well-characterised dominant-negative mutants, bioimaging studies (double-labeled immunofluorescence assays, transmission electron microscopy analysis), secondary siRNA-based dosage dependency studies and drug inhibition assays.

Results: The infectious entry of HEV71 into RD cells was shown to be significantly inhibited by siRNAs targeting genes associated with clathrin-mediated endocytosis (CME), such as AP2A1, ARRB1, CLTC, CLTCL1, SYNJ1, ARPC5, PAK1, ROCK1 and WASF1. The functional role of CME was verified by the observation of strong co-localisation between HEV71 particles and clathrin as well as dose-dependent inhibition of HEV71 infection upon siRNA knockdown of CME-associated genes. HEV71 entry by CME was further confirmed via inhibition by dominant-negative EPS15 mutants and treatment of CME drug inhibitors, with more than 80% inhibition observed at 20 μM chlorpromazine. The involvement of other entry pathways, such as caveolae-mediated endocytosis and macropinocytosis, was also found to be minimal, based on the failure of associated drug inhibitors in hampering HEV71 infection. Furthermore, HEV71 infection was shown to be sensitive to the disruption of human genes in regulating early to late endosomal trafficking as well as endosomal acidic pH. The importance and involvement of actin dynamics in mediating the infectious entry of HEV71 was also investigated.

Conclusion: The identification of clathrin-mediated endocytosis as the entry pathway for HEV71 infection of susceptible host cells contributes to a better understanding of HEV71 pathogenesis and enables future development of anti-viral strategies against HEV71 infection.

The clinical severity of Puumala hantavirus-induced nephropathia epidemica and partial complement protein C4 deficiencies

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Background: Hantaviruses are rodent-and insectivore-borne zoonotic viruses that are found worldwide. Hantaviruses cause two diseases: hemorrhagic fever with renal syndrome (HFRS) in Eurasia, and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. In Finland, Puumala hantavirus causes nephropathia epidemica (NE) that is referred to

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Deciphering the infectious entry process of human enterovirus 71

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Background: Enterovirus 71 (HEV71) is one of the most clinically significant enteroviruses known to cause severe morbidity and mortality and is most frequently presented as hand, foot and mouth disease (HFMD) in children, although infected individuals could also develop neurological complications. In the absence of anti-viral agents or vaccines, urgent emphasis is therefore being placed on developing anti-viral strategies against this viral pathogen. As yet, little is known of the initial interaction between HEV71 and host cells, which may represent potential anti-viral targeting sites.

Methods: A targeted small-interfering RNA (siRNA) screening platform assay was established and validated to identify and profile key cellular genes involved in processes of endocytosis, cytoskeletal dynamics and endosomal trafficking essential for HEV71 infection. Screen evaluation was conducted via the expression of well-characterised dominant-negative mutants, bioimaging studies (double-labeled immunofluorescence assays, transmission electron microscopy analysis), secondary siRNA-based dosage dependency studies and drug inhibition assays.

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