

1 Drug repurposing: omeprazole increases the efficacy of acyclovir
2 against herpes simplex virus type 1 and 2

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19 Abstract

20 **Objectives:** Omeprazole was shown to improve the anti-cancer effect of the
21 nucleoside-analogue 5-fluorouracil. Here, we investigated the effects of omeprazole
22 on the activities of the antiviral nucleoside analogues ribavirin and acyclovir.

23 **Methods:** West Nile virus-infected Vero cells and influenza A H1N1-infected MDCK
24 cells were treated with omeprazole and/ or ribavirin. Herpes simplex virus 1 (HSV-1)-
25 or HSV-2-infected Vero or HaCat cells were treated with omeprazole and/ or acyclovir.
26 Antiviral effects were determined by examination of cytopathogenic effects (CPE),
27 immune staining, and virus yield assay. Cell viability was investigated by MTT assay.

28 **Results:** Omeprazole concentrations up to 80µg/mL did not affect the antiviral effects
29 of ribavirin. In contrast, omeprazole increased the acyclovir-mediated effects on HSV-
30 1- and HSV-2-induced CPE formation in a dose-dependent manner in Vero and HaCat
31 cells. Addition of omeprazole 80µg/mL resulted in a 10.8-fold reduction of the acyclovir
32 concentration that reduces CPE formation by 50% (IC₅₀) in HSV-1-infected Vero cells
33 and in a 47.7-fold acyclovir IC₅₀ reduction in HSV-1-infected HaCat cells. In HSV-2-
34 infected cells, omeprazole reduced the acyclovir IC₅₀ by 7.3-fold (Vero cells) or by 12.9-
35 fold (HaCat cells). Omeprazole also enhanced the acyclovir-mediated effects on viral
36 antigen expression and virus replication in HSV-1- and HSV-2-infected cells. In HSV-
37 1-infected HaCat cells, omeprazole 80µg/mL reduced the virus titre in the presence of
38 acyclovir 1µg/mL by 1.6x10⁵-fold. In HSV-2-infected HaCat cells omeprazole 80µg/mL
39 reduced the virus titre in the presence of acyclovir 2µg/mL by 9.2x10³-fold. The
40 investigated drug concentrations did not affect cell viability, neither alone nor in
41 combination.

42 **Conclusions:** Omeprazole increases the anti-HSV activity of acyclovir. As clinically
43 well-established and tolerated drug, it is a candidate drug for antiviral therapies in
44 combination with acyclovir.

45

46 Introduction

47 Omeprazole and other proton pump inhibitors were found to increase the activity of
48 anti-cancer drugs including the nucleoside analogue 5-fluorouracil (Luciani et al., 2004;
49 Ikemura et al., 2017). Proton pump inhibitors are the most frequently prescribed drugs
50 for the treatment and prophylaxis of gastroesophageal reflux as well as of gastric and
51 duodenal ulcers that are associated with hyper-acidic states. Since they are known to
52 be well-tolerated, they were suggested as repositioning candidates for the use as part
53 of anti-cancer therapies (Ikemura et al., 2017).

54 Here, we investigated the effects of omeprazole on the efficacy of the antiviral
55 nucleoside analogues acyclovir and ribavirin. We found that omeprazole enhanced the
56 antiviral effects of acyclovir against herpes simplex virus type 1 (HSV-1) and HSV-2
57 but did not influence the activity of ribavirin against West-Nile viruses or influenza
58 viruses.

59

60 Materials and methods

61 Cell culture

62 Vero and MDCK cells were obtained from the American Type Culture Collection
63 (ATCC, Rockville, MD) and cultured at 37° C in minimum essential medium (MEM)
64 supplemented with 10% foetal bovine serum. HaCaT cells were purchased from CLS
65 Cell Line Services GmbH (Eppelheim, Germany) and cultivated in Iscove's modified
66 Dulbecco's medium (IMDM) supplemented with 10% foetal bovine serum.

67

68 Viruses

69 HSV-1 strain McIntyre and HSV-2 strain MS were both obtained from ATCC. West Nile
70 virus (WNV) strain NY385-99 was kindly provided by Dr J ter Meulen (Institut für
71 Virologie, Phillips-Universität, Marburg, Germany). Virus stocks were prepared in Vero
72 cells grown in MEM with 4% foetal bovine serum. The influenza virus strain Influenza
73 A/New Caledonia/20/99 (H1N1) was received from the WHO Influenza Centre
74 (National Institute for Medical Research, London, UK). Virus stocks were prepared in
75 MDCK cells grown in 4% foetal bovine serum. Infectious virus titres were determined
76 by titration on MDCK cell monolayer in 96-well plates as 50% tissue culture infectious
77 dose (TCID₅₀) by the method of Spearman and Kärber (Spearman, 1908; Kärber,
78 1931).

79

80 Drugs

81 Acyclovir was received from GlaxoSmithKline (Munich, Germany), omeprazole from
82 AstraZeneca (Wedel, Germany), and ribavirin from Valeant Pharmaceuticals Germany
83 GmbH (Eschborn, Germany).

84

85 **Cytopathogenic effect (CPE) reduction assay**

86 For the investigation of HSV-1- and HSV-2-induced cytopathogenic effects (CPEs),
87 confluent Vero or HaCaT cell monolayer in 96-well microtiter plates were inoculated
88 with HSV-1 or HSV-2 at MOI 1 or 0.1, respectively. Following a 1h incubation period,
89 the inoculum was removed and the drugs, either alone or in combination, were added.
90 The virus-induced CPE was recorded microscopically after 48h post infection.

91 For the investigation of WNV-induced CPEs, Vero cell monolayers were infected with
92 MOI 0.1. Following a 1h virus incubation period, the medium was removed and infected
93 cells were incubated in medium containing different concentrations of drugs at the
94 respective concentration. The CPE was recorded at 48h post infection.

95 Confluent MDCK cell monolayers were infected with Influenza H1N1 (MOI 0.01).
96 Following a 1h virus incubation period, the medium was removed and infected cells
97 were incubated in medium containing different concentrations of drugs at the
98 respective concentration. The CPE was recorded at 24h post infection.

99 CPEs were scored by two independent examiners and expressed in % of the untreated
100 virus control that was defined to be 100%.

101

102 **Immunostaining**

103 Intracellular HSV protein was evaluated by immunostaining. Cells were fixed with
104 60/40 ice cold methanol/acetone for 15 min. Staining was performed using a rabbit
105 polyclonal antibody directed against HSV-1 (ab9533) and a sheep polyclonal antibody
106 directed against HSV-2 (ab21112) in combination with biotin-conjugated secondary
107 goat anti-rabbit (ab6720) and rabbit anti-sheep (ab6746) antibodies (all antibodies
108 derived from Abcam, Cambridge, UK). Protein was visualised using streptavidin-
109 peroxidase complex with AEC as a substrate.

110

111 **Viability assay**

112 The cellular viability was assessed on confluent cell layers with the 3-(4,5-dimethyl-2-
113 thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay method as described
114 previously (Michaelis et al., 2007). The viability was expressed as percentage of non-
115 treated control.

116

117 Results

118 **Effects of omeprazole in combination with ribavirin on cytopathogenic effect** 119 **(CPE) formation in WNV- or influenza A H1N1-infected cells**

120 Omeprazole 80µg/mL did not affect the effects of ribavirin on CPE formation in WNV-
121 infected Vero cells or H1N1-infected MDCK cells (Figure 1A, Suppl. Table 1).

122

123 **Effects of omeprazole in combination with acyclovir on HSV-1 and HSV-2** 124 **replication**

125 In the presence of omeprazole 80µg/mL, acyclovir concentrations that reduced CPE
126 formation by 50% (IC₅₀) were reduced by 11-fold in HSV-1-infected Vero cells and by
127 7-fold in HSV-2-infected Vero cells. In addition, omeprazole 80µg/mL reduced the
128 acyclovir IC₅₀s by 48-fold in HSV-1-infected HaCat cells and by 13-fold in HSV-2-
129 infected HaCat cells (Figure 1A, Suppl. Table 1). Immune staining also indicated
130 reduced numbers of virus-infected cells after treatment with a combination of
131 omeprazole and acyclovir compared to either single treatment (Figure 1B). Further
132 experiments indicated that omeprazole reduced acyclovir IC₅₀s in HSV-1- and HSV-2-
133 infected HaCat cells in a dose-dependent fashion (Figure 1C, Suppl. Table 2).

134 The detection of virus titres revealed moderate effects of omeprazole on virus
135 replication. Again, omeprazole strongly increased the anti-HSV-1 and anti-HSV-2
136 effects of acyclovir (Figure 2, Suppl. Table 3). The investigated omeprazole and
137 acyclovir concentrations did not affect cell viability, neither alone nor in combination.

138

139 Discussion

140 Based on previous investigations that showed that omeprazole increases the anti-
141 cancer activity of the nucleoside analogue 5-fluorouracil (Luciani et al., 2004), we here
142 investigated the effects of omeprazole on the antiviral effects of ribavirin and acyclovir.
143 Ribavirin is a broad spectrum antiviral drug (Beaucourt & Vignuzzi, 2014). However,
144 omeprazole did neither modify ribavirin-mediated effects in H1N1 influenza A virus-
145 infected cell cultures nor in West Nile virus-infected cell cultures.

146 Acyclovir is a first line drug for HSV-1, HSV-2, and varicella zoster virus infection (Piret
147 & Boivin, 2016; Klysik et al., 2018). In contrast to the lack of effect of omeprazole on
148 ribavirin-mediated antiviral effects, omeprazole interfered with HSV-1 and HSV-2
149 replication in a dose-dependent fashion. Omeprazole increased the anti-HSV activity
150 of acyclovir in Vero cells and in the human keratinocyte cell line HaCat. Since
151 omeprazole and acyclovir were both added simultaneously to virus-infected cell
152 cultures after a 1h viral adsorption period, omeprazole increases the effects of
153 acyclovir during the viral replication cycle.

154 The mechanism by which omeprazole enhances the activity of acyclovir seems to differ
155 from the mechanism by which the compound increases 5-fluorouracil efficacy.
156 Omeprazole pre-treatment was necessary to increase 5-fluorouracil activity (Luciani et
157 al., 2004). In contrast, the combination of omeprazole and acyclovir exerted its
158 combined activity when added at the same time.

159 There is a need for improved therapies for HSV-1- and HSV-2-associated disease.
160 After primary infection, HSV-1 and HSV-2 establish life-long persistence which may
161 result in recurrent disease which typically manifests as herpes labialis or herpes
162 genitalis and which may be associated with significant morbidity (Gnann & Whitley,
163 2016; Heslop et al., 2016; Klysik et al., 2018). Even in the case of herpes labialis, which

164 is not commonly associated with complications, treatment success is not always
165 satisfactory as indicated by the introduction of topical acyclovir/ hydrocortisone
166 combinations (Nguyen et al., 2014). In immunodeficient individuals, HSV-1/-2 infection
167 is often associated with more severe disease, and resistance formation to acyclovir is
168 a severe problem (Piret & Boivin, 2016; Karrasch et al., 2018). Moreover, ocular HSV
169 infection is a major cause of blindness in industrialised countries (Klysik et al., 2018).
170 In conclusion, omeprazole substantially enhances the antiviral effects of acyclovir in
171 HSV-1- and HSV-2-infected cells. Improved therapies for HSV-1/2 infection are highly
172 desirable, in particular for immunocompromised individuals. Since omeprazole is a
173 clinically well-established drug with a preferable safety profile, it is an excellent
174 candidate for drug repositioning strategies (Ikemura et al., 2017).

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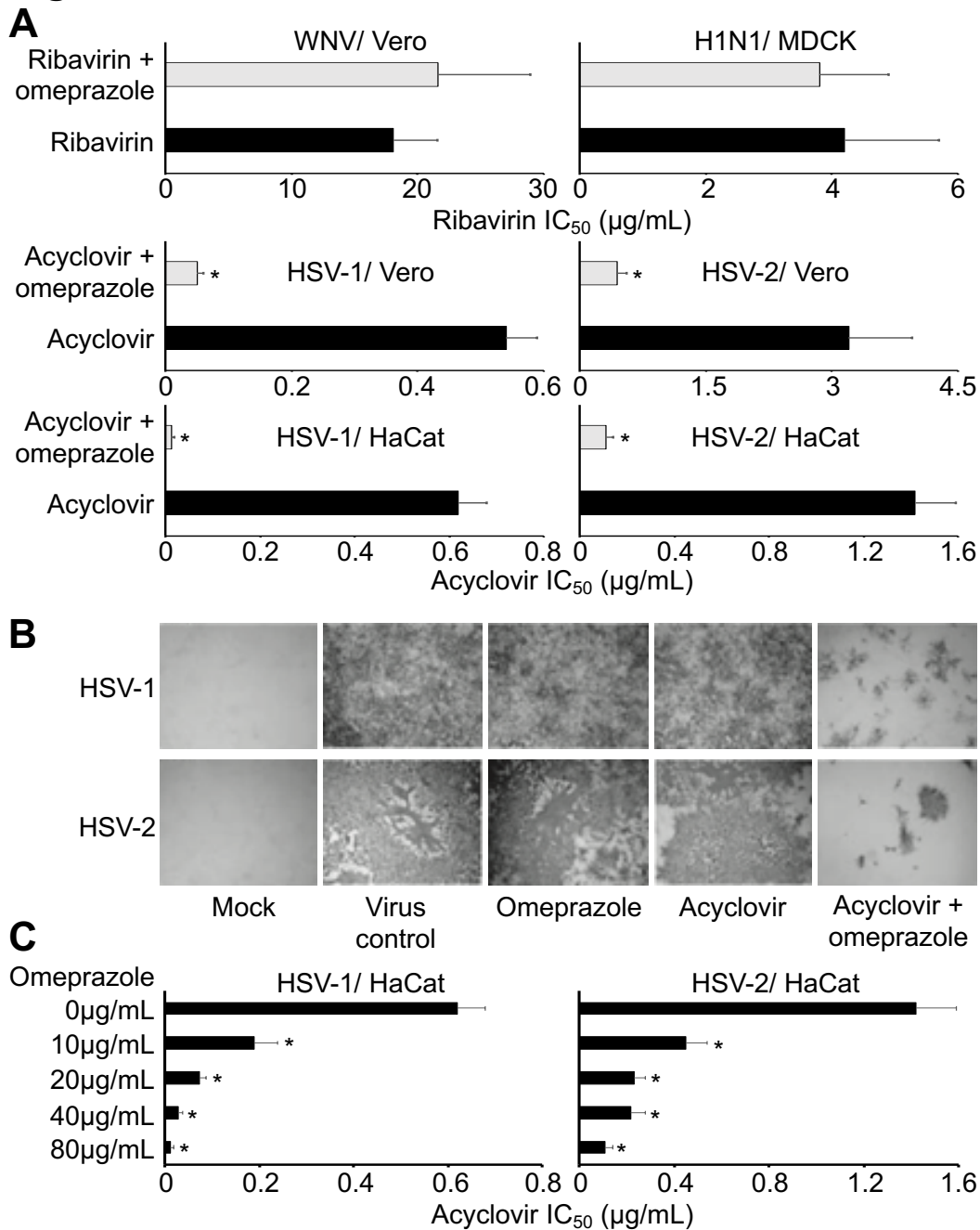
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213 **Figures**

Figure 1

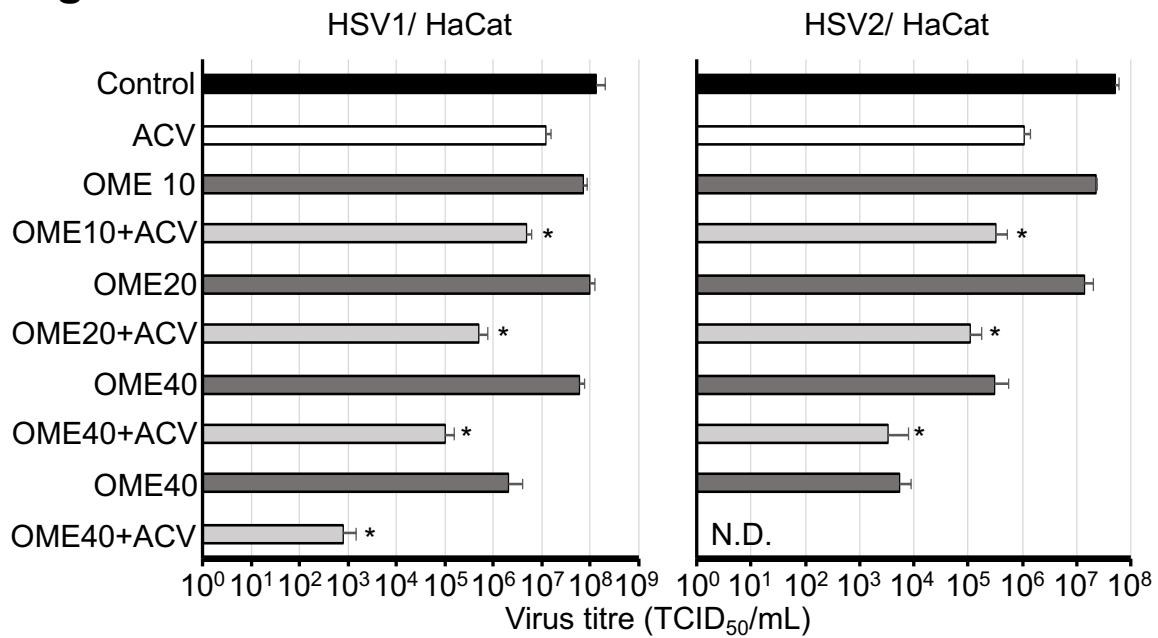


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215 **Figure 1.** Cytopathogenic effect (CPE) formation and viral gene expression in the
 216 presence of omeprazole antiviral nucleoside analogues. A) Effects of omeprazole
 217 (80µg/mL) on the concentrations of antiviral nucleoside analogues that reduce CPE
 218 formation by 50% (IC₅₀) using West Nile virus (WNV)-infected Vero cells, influenza A
 219 H1N1-infected MDCK cells, and HSV-1- or HSV-2-infected Vero or HaCat cells.

220 Omeprazole alone did not reduce CPE formation. Numerical values are presented in
221 Suppl. Table 1. B) Effects of omeprazole and acyclovir on the expression of virus
222 proteins in HSV-1- and HSV-2-infected Vero cells. HSV-1-infected cells were treated
223 with omeprazole 80µg/mL and/ or acyclovir 0.31µg/mL. HSV-2-infected cells were
224 treated with omeprazole 40µg/mL and/ or acyclovir 0.6µg/mL. C) Concentration-
225 dependent effects of omeprazole on the acyclovir IC₅₀ in HSV-1- or HSV-2-infected
226 HaCat cells as determined by CPE formation. Numerical values are presented in
227 Suppl. Table 2. The investigated drug concentrations did not affect cell viability, neither
228 alone nor in combination. * P < 0.05 relative to nucleoside analogue alone
229

Figure 2



230

231 **Figure 2.** Effect of acyclovir 1µg/mL (HSV-1) or 2µg/mL (HSV-2) alone or in
232 combination with varying omeprazole (OME) concentrations (µg/mL) on HSV-1 and
233 HSV-2 titres in HaCat cells. Numerical values are presented in Suppl. Table 3. * P <
234 0.05 relative to acyclovir alone; N.D. = no detectable virus titre

235