

in SARS patients, on infection of SARS virus to cell line cells.

**Methods:** The water extracts of seven medicinal herbs and five Kampo medicines (traditional Japanese herb medicines), which were reported to be used for the treatment of SARS patients, were studied using HIV/SARS-CoV S pseudotyped virus assay. The effects were also confirmed by wild type SARS-CoV infection assay. We also examined whether these herbs inhibit the bindings of anti-S or anti-ACE2 antibody to cells using FACS analysis.

**Results:** Cinnamomi Cortex extract (CCE) and Caryophylli Flos extract (CFE) showed inhibitory activities against HIV/SARS-CoV S pseudovirus. The 50% inhibitory concentration (IC<sub>50</sub>) of the former was lower than those of the latter, both of them were less than 60 mg/ml. We also confirmed that both extracts inhibited wild-type infection in the plaque reduction assay. The selective index (SI) of CCE was also higher than CFE. We further examined four fractionated samples from Cinnamomi Cortex (CC). The SI of the fraction 2 (Butanol fraction) in the plaque reduction assay was higher than those of CCE. To analyze the mechanism of inhibitory effects of herbs we examined whether the herbs inhibit the binding of anti-S protein and anti-ACE2 antibodies to cells. These extracts failed to inhibit the bindings of antibodies to S and ACE2 on cells surface.

**Conclusion:** These results strongly indicate that CC and Caryophylli Flos (CF) contain a potent inhibitor of SARS-CoV entry, and the inhibitor could be enriched in the Butanol fraction of CC. However they apparently did not interfere S/ACE2 interactions.

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#### **Uptake and Metabolism of Cidofovir and Oleyloxyethyl-cidofovir in Human Papillomavirus Positive ME-180 Human Cervical Cancer Cells**

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Nearly all cervical cancers are caused by the high-risk subtypes of human papillomavirus (HPV) which express the E6 and E7 oncoproteins. Several groups have shown previously that cidofovir (CDV) inhibits HPV<sup>+</sup> cervical cancer cell proliferation in vitro by lowering levels of E6, upregulating p53, and increasing susceptibility to apoptosis. CDV has been reported to have antiproliferative effects against HPV<sup>+</sup> cancers, both in animals and man. We found that the antiproliferative activity of alkoxyalkyl esters of CDV, such as oleyloxyethyl-cidofovir (OLE-CDV), is 1000–2700 times greater than CDV in CaSki, ME-180, HeLa, and SiHa human cervical cancer cell lines. To evaluate the mechanism involved, we studied the cellular uptake of <sup>14</sup>C-labeled CDV and OLE-CDV in the cervical cancer cell line, ME-180. ME-180 cells were exposed to 3 μM CDV or OLE-CDV for 24 h. Cellular uptake of OLE-CDV was 270-

fold greater than that of CDV in ME-180 cells. The cellular levels of CDV, CDVp and CDVpp were measured by Partisil SAX HPLC. Levels of the active metabolite, CDVpp, were 183 times greater with OLE-CDV than with CDV. These findings may explain, at least in part, the previously reported multi-log increase in the antiproliferative effects of OLE-CDV versus CDV in HPV<sup>+</sup> human cervical cancer cell lines. These results indicate that alkoxyalkyl esters of CDV, such as OLE-CDV, are of interest for further evaluation as agents for the treatment of cervical cancer.

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#### **Selection of Human Cytomegalovirus Resistant to a Second Generation Methylenecyclopropane Purine**

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We recently described a second generation of methylenecyclopropane purines (the bis-hydroxys) that have potent and selective activity against HCMV and MCMV (Zhou et al., 2004. J. Med. Chem. 47, 566). The guanine analog cyclopropavir was effective in vitro against HCMV and MCMV with IC<sub>50</sub>'s of 0.27–0.49 μM and no cytotoxicity at 100 μM. It was active when administered orally in animal models for CMV infections (Kern et al., 2004. Antimicrob. Agent. Chemother. 48, 4745). To investigate its mechanism of action, HCMV resistant to this compound was selected by passage of Towne strain HCMV in the presence of 0.625 μM cyclopropavir until 50% CPE was evident (2 weeks). Cells were harvested, recombined with supernatant, and used to infect 25 cm<sup>2</sup> flasks of HFF cells. Cultures were maintained with periodic replacement of drug-containing media until CPE was >50% (6 weeks) or nearly 100% (10 weeks). Medium from 10-week cultures was used to infect fresh HFF cells. After 4 weeks in the presence of 2.5 μM cyclopropavir, supernatant virus was plaque purified in the presence of 2.5 μM drug. An initial survey of drug resistance showed that IC<sub>50</sub>'s for virus from five plaque isolates were approximately 10-fold greater than for wild-type (wt) HCMV. More extensive dose-response experiments gave IC<sub>50</sub>'s of 22 and 42 μM for cyclopropavir and ganciclovir compared to IC<sub>50</sub>'s of 0.9 and 1.5 μM, respectively, for wt virus. DNA sequencing and marker transfer studies with HCMV resistant to a first generation analog (synadenol) have now shown that mutations M460I and C603Y in gene UL97 are necessary and sufficient to give resistance to both synadenol and cyclopropavir. Sequencing UL97 and UL54 from cyclopropavir-resistant HCMV is being used to determine if the cause of resistance to this second generation drug is similar to that responsible for resistance to the first generation compound.