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The Development of the Antiviral Drug RC 28 from *Rozites caperata* (Pers.:Fr.) P.Karst. (Agaricomycetideae)

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Many mushrooms are reported to have antiviral activity against viruses that cause human disease. The active substances described include polysaccharides, conjugated polysaccharides, proteins, peptides, lignins, triterpenes, phenoloic derivates, and nucleic acid bases. RC 28 is an antiviral protein of MW 28 kD prepared from aqueous extracts of the mushroom *Rozites caperata* with novel antiviral activity. It is active exclusively against enveloped viruses including human Herpes viruses HSV-1 and HSV-2, *Cytomegalovirus, Varicella zoster,* Respiratory syncytial virus, and Influenza Virus type A. It is not active against the non-enveloped viruses Adenovirus type 6, Coxsackie viruses B5 and B6, and several strains of ECHO viruses. Our present objectives are to obtain the complete amino acid sequence of RC 28, determine its antiviral mechanism, and identify its cellular targets. I will present some initial studies with these objectives in mind.

The antiviral effects of RC 28 were studied in Buffalo Green Monkey kidney cells (BGMK) and Hep-2 cells infected with the KOS strain of HSV-1 virus. The synthesis of viral proteins in BGMK cells was studied using Western blots of infected cell extracts from 4–12% PAGE gels stained with antibodies to HSV-1. Effects of RC 28 on the early functions of the HSV-1 immediate early protein, ICPO, and disaggregation of PML nuclear bodies was studied in Hep-2 cells stained with anti-ICPO antibodies and anti-PML antibodies. Localization and colocalization studies of RC 28 with cellular organelles were studied in Hep-2 cells stained with antibodies to RC 28, lysosomes, proteasomes, and ICPO. Cellular targets were studied using the Invitrogen Yeast ProtoArray Slide Kit.

RC 28 interfered with the synthesis of viral structural proteins even when the drug was added as late as 13 hours after infection. RC 28 prevented the translocation of the HSV-1 immediate early protein, ICPO from the nucleus to the cytoplasm, and ICPO directed disaggregation of PML nuclear bodies. RC 28 localized exclusively in the cytoplasm but not in the nucleus of Hep-2 cells, and RC 28 did not colocalize with nuclear ICPO, cytoplasmic proteasomes, or lysosomes.

Presently, the drugs of choice for the treatment of Herpes virus infections are those that interfere with viral DNA replication—i.e., Acyclovir and second generation derivatives. RC 28 has unique and novel antiviral activities because it interferes with both early and late viral functions. It interferes with the functional activities of the immediate early protein ICPO and prevents the synthesis of late structural proteins even when RC 28 is added after viral DNA replication. Because of its unique antiviral activity, RC 28 may prevent infection by HSV-1 and HSV-2 Herpes viruses if the drug is applied within the first few hours following contact. It is to be hoped that the elucidation of the antiviral mechanism of RC 28 and the identification of its cellular targets will lead to the discovery of second generation RC 28-like drugs with even greater effectiveness, specificity, and usefulness for the treatment of Herpes virus infections.