125

In vitro and in vivo evaluation of the antiherpetic activity of a partially cyclized mu/nu-carrageenan. C. A. Pujol*, M. J. Carlucci*, L. A. Scolaro*, M. Ciancia**, M. C. Matulewicz**, A. S. Cerezo**, E. B. Damonte*. *Laboratorio de Virologia and **Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

In a screening for antiherpes agents with a number of marine natural substances we found that a partially cyclized mu/nu-carrageenan named 1C3, isolated from the red seaweed *Gigartina skottsbergii*, had a potent inhibitory effect against herpes simplex virus type 1 and 2 (HSV-1 and HSV-2). In plaque reduction assays in Vero cells, the effective dose 50% (ED50) against HSV-1 (strain F) and HSV-2 (strain G) were 1.0 and 1.2 µg/ml respectively. The anti HSV activity of 1C3 was also assessed in vitro in neural cells of OF1 mice as well as against clinical isolates of HSV-1 and HSV-2 and TK^- acyclovir-resistant variants. The compound was found to be highly selective, causing no impairment of cell growth at concentrations that were at least 1000 fold in excess over the ED50. Furthermore, 1C3 had no direct inactivating effect on virions by in vitro incubation in a virucidal assay. As for other polyanionic substances, the antiherpetic activity was mainly due to inhibition of virus adsorption. The protective effect of 1C3 was also evaluated in vivo against HSV-2 infection in mice. The administration of 1C3 intraperitoneally (five dosis of 1 mg each, two previous and three after virus inoculation) to OF1 mice challenged with 10^5 PFU of HSV-2 by the same route, reduced significantly the mortality when compared with a control group of mice where 1C3 was replaced by saline.

126

The Inhibitory effect of nonionic surfactants on herpes simplex Type 2 replication *in vitro*. SF Reising*, KZ Bourne and L R Stanberry. Children's Hospital Research Foundation, Cincinnati, OH.

The development of safe and effective vaginal microbicides designed to prevent acquisition of sexually transmitted diseases is a pubic health priority. Much of the current work has focused on the antimicrobial activity of spermicides containing nonionic detergents. However, clinical reports indicate that the repetitive use of these products may cause injury to cervicovaginal epithelium. Rather than focusing on nonionic surfactants present in commercially available preparations, a more rationale approach may be to identify agents with a high selectivity index (ratio of CD_{50} to the ED_{50}) for further *in vivo* evaluation. Several non-formulated polyoxyethelene ethers were evaluated *in vitro* for there ability to inactivate HSV-2 and for their cellular cytotoxicity. HSV-2 was mixed with concentrations of the different surfactants ranging from 0.005 - 2.5%. All of the nonionic surfactants demonstrated anti-HSV-2 activity, however some were more cytotoxic than others. The ED_{50}'s ranged from 0.11µM to 0.63µM and the CD_{50}'s from 0.35µM to 0.59µM. Triton X-165 was the most selective with a SI of 5.36. In order for these types of chemicals to be used as intra-vaginal microbicides, they must have selective virucidal activity against sexually transmitted pathogens and non-toxic to the vaginal mucosa. Selecting nonionic surfactants with high selective indices can be used for later formulation and in vivo studies.

127

Potent and selective inhibitors of Herpes simplex virus (HSV) thymidine kinase (TK) reduce reactivation of virus from latency in explanted ganglionic tissue *in vitro*

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HSV establishes a life-long latent infection in sensory neuronal ganglia. In a proportion of individuals, the latent virus can reactivate sporadically, producing lesions at oral (for HSV-1) or genital (for HSV-2) mucosal surfaces. This reactivation is thought to involve an initial round of replication of the virus in the neuronal tissue, for which virally-encoded TK is essential. In contrast, subsequent replication of the virus at the mucosal surface following reactivation does not require viral TK, since cellular TK can substitute. Inhibition of viral TK should reduce replication of the virus in neuronal tissue, and so prevent emergence of virus from latency. We have designed a series of potent inhibitors of viral TK, based on the natural substrate thymidine. In particular, compounds Ro 32-1520 and Ro 32-2313 are highly selective for viral TK over cellular TK, and show similar inhibition of HSV-1 and HSV-2 TK. Using a modified plaque assay, the compounds were shown to be capable of inhibiting virus replication in cell cultures when cellular TK activity was blocked. In addition, the compounds prevented the reactivation of HSV-2 in explanted dorsal root ganglia taken from mice harbouring a latent virus infection. The latter inhibition was shown to be reversed by the addition of excess thymidine but not cytidine, suggesting that the compounds acted as competitive substrate inhibitors of viral TK. These compounds have potential as therapeutic agents for the treatment of HSV reactivation in the clinic.

128

Rapid Method for Plaque Enumeration in Herpes Plaque Reduction Assays. C.A. Hodges-Savola, L.M. Werness, S.L. Henry, C.L. Walker, and N.T. Wetherall, ViroMed Laboratories, Inc., Minneapolis, MN USA

Plaque reduction assays are considered by many to be the "gold standard" for evaluating viral sensitivity to proven and putative antiviral compounds. Indeed, we are using plaque assays as part of an extensive surveillance program that was implemented to identify acyclovir-resistant strains of HSV-1 or HSV-2 isolated from up to 5000 specimens taken from genital Herpes lesions. To sidestep some of the chief disadvantages of plaque assays (i.e., the tedium in counting plaques and variability of resulting data), we have adapted a computerized image analysis system to aid in the rapid quantitation of numerous plaques in large numbers of test plates. For the Herpes resistance surveillance program, six-well cell culture plates are seeded with Vero cells, grown to confluency and inoculated with approximately 100 pfu virus (clinical isolate) per well. Cells are then overlayed with serial dilutions of acyclovir prepared in culture media containing human immune serum globulin. After a 72 h incubation (37°C, 5-7% CO_2), cells are fixed, stained with crystal violet and air-dried. Once plates are thoroughly dried, plaques are counted using a Domino Image Analysis system comprising a high resolution CCD camera coupled to a Macroviewer Lightbox and PC. Plates are positioned on the lightbox and the camera adjusted so that the complete area of one well can be analyzed. Contrast and focus are manually adjusted on the camera to provide optimum plaque delineation. "Matrix" and "threshold" settings, which influence detection of plaques based on size and relative intensity, respectively, are set using software menus. Once plaques are counted, the data may be saved into Excel spreadsheets for additional analyses. Cumulative data indicate that there are no significant differences in plaque counts obtained "by hand" and those obtained using the image analysis system. Moreover, the slight differences that may occur do not significantly influence the resulting IC_{50} values. Accordingly, we propose that this method offers a means for rapid and accurate plaque enumeration in situations requiring high through-put and involving large numbers of clinical Herpes plaque reduction assays.

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