11

RESISTANCE OF HSV-1 TO PEPTIDOMIMETIC RIBONUCLEOTIDE REDUCTASE INHIBITORS: SELECTION AND CHARACTERIZATION OF MUTANT ISOLATES. A.M. Bonneau*, P. Kibler, P. White, C. Bousquet, N. Dansereau and M. Cordingley. Bio-Méga Boehringer Ingelheim Research Inc., Canada.

We previously reported the antiviral activity of the ribonucleotide reductase (RR) inhibitor BILD 733 against herpes simplex virus (HSV) *in vitro*. This peptidomimetic inhibitor specifically prevents association of the two viral RR subunits by mimicking the C-terminal end of the small subunit (RR2). In order to determine if viral mutants with a drug resistance phenotype could be selected, HSV-1 KOS was serially passaged in BHK-21 cells in the presence of increasing concentrations of inhibitor. Plaque purified isolates from passage 5 (K-733^r p5) and passage 8 (K-733^r p8) were characterized with regard to drug resistance phenotype and growth properties in culture. Both K-733^r p5 and p8 exhibited significantly increased EC50s for the RR inhibitor. The K-733^r p5 isolate exhibited moderate (3-fold) resistance to BILD 733 while higher levels of resistance (9-fold) were seen for K-733^r p8. Their sensitivity remained indistinguishable from wild type KOS against a thiosemicarbazone which inhibits RR by a different mode of action. Infection of BHK-21 cells with K-733^r p5 or p8 isolates showed virus yields, kinetics and levels of DNA replication similar to wild type HSV-1 KOS. Together these results demonstrate that the mutation(s) in KOS-733^r p5 and K-733^r p8 isolates do not significantly impair their growth in cell culture. Marker rescue was carried out to locate the mutation(s) associated with the drug resistance phenotype. DNA encoding the HSV-1 KOS RR large subunit (RR1) was capable of rescuing wild type BILD 733-sensitivity into KOS-733^r p5, while no rescue could be achieved with DNA encoding RR2. DNA analysis identified nucleotide changes in the C-terminal end of RR1. These data suggest that mutation(s) within the gene encoding RR1 can confer BILD 733-resistance to HSV-1 KOS. This is consistent with the demonstrated mode of action of this class of inhibitor which targets the RR1 molecule.

12

ANTIVIRAL ACTIVITY OF 2-PHOSPHONOMETHOXYALKYL DERIVATIVES OF N6-SUBSTITUTED 6-AMINOPURINES

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The title compounds were prepared by treatment of 9-[2-phosphonomethoxyalkyl]-6-chloropurine diesters (I) with primary or secondary amines followed by bromotrimethylsilane treatment and hydrolysis. 2-Phosphonomethoxyethyl derivatives II bearing one or two aliphatic substituents, benzyl, aryl or alicyclic group at the position N6 exhibit antiviral activity against herpesviruses (HSV-1,HSV-2, CMV, VZV) and vaccinia which is comparable or exceeds the effect of the parent 6-amino derivative. The N6-substitution in the series of antiretroviral (R)-(2-phosphonomethoxypropyl derivatives (II) causes a loss or a substantial decrease of the antiviral effect.