A SEARCH FOR NEW ANTI-HERPES VIRUS COMPOUNDS

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Summary: This study reports on the synthesis and the activity of a series of new compounds of nucleoside-type structure. They are characterized for being differently substituted in the aromatic ring of the base (deazoadenosine derivatives) or by bearing a dansyl group in the sugar moiety (dansylthymidine). One molecule belonging to this latter class of compounds (the 3'-0-dansylthymidine) is showing an anti-herpesvirus potential while being active in inhibiting the virus-encoded enzyme thymidine kinase. This finding may represent an important step for the synthesis of new enzyme inhibitors and it is discussed in terms of future developments of more active congeners.

INTRODUCTION

Members of the family Herpesviridae are the causative agents of a number of infections which are often characterized by severe morbidity and mortality, especially in immunosuppressed patients. They are also involved in several pathological conditions of obstetrical and gynaecological relevance ([1]). In recent years much attention has been paid to these viruses as the obvious candidates for a chemotherapy program. The possibility of developing effective anti-herpes drugs rests primarily on the relative complexity of the herpesvirus genome encoding for a series of enzymes which are peculiar to the physiology of virus replication ([2]). Some of these enzymes are in fact quite distinct from the homologous cellular ones, mainly for their substrate specificity and for their susceptibility to certain inhibitors.

Although the number of new antiviral agents has been rapidly increasing and antiviral chemotherapy has come of age, the number of licensed drugs is still very limited. It is also noteworthy that the only selective drug so far available for general clinical use, the 9-(2-hydroxyethoxymethyl)guanine or acyclovir (ACV), is endowed with quite a narrow spectrum of activity for being effective mainly against infections sustained by herpes simplex virus type 1 and 2 (HSV-1, HSV-2) and varicella zoster virus (VZV).

Trends in antiviral drug design have been focused on the development of derivatives of already existing substances with the aim of increasing their potency and improving their pharmacokinetic profile. Another approach has consisted in the synthesis of broad-spectrum antivirals, potentially active against viral agents such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), rhinoviruses, hepatitis viruses, adenoviruses, papovaviruses, retroviruses, reoviruses, togaviruses, among others.

In this paper we report on the research and development of a series of new compounds some of which are targeted at the HSV-1-encoded thymidine kinase (TK).

Their structural formulae are depicted in fig. 1.

MATERIAL AND METHODS

Synthesis of deaza analogues of adenosine: The synthesis of these compounds has been reported in detail elsewhere ([3]).

Synthesis of dansylthymidine derivatives: Synthesis of 5'-0-dansylthymidine and 3'0-dansylthymidine was achieved by reaction of thymidine with dansyl chloride in pyridine, in the presence of dimethylaminopyridine as catalyst ([4]).

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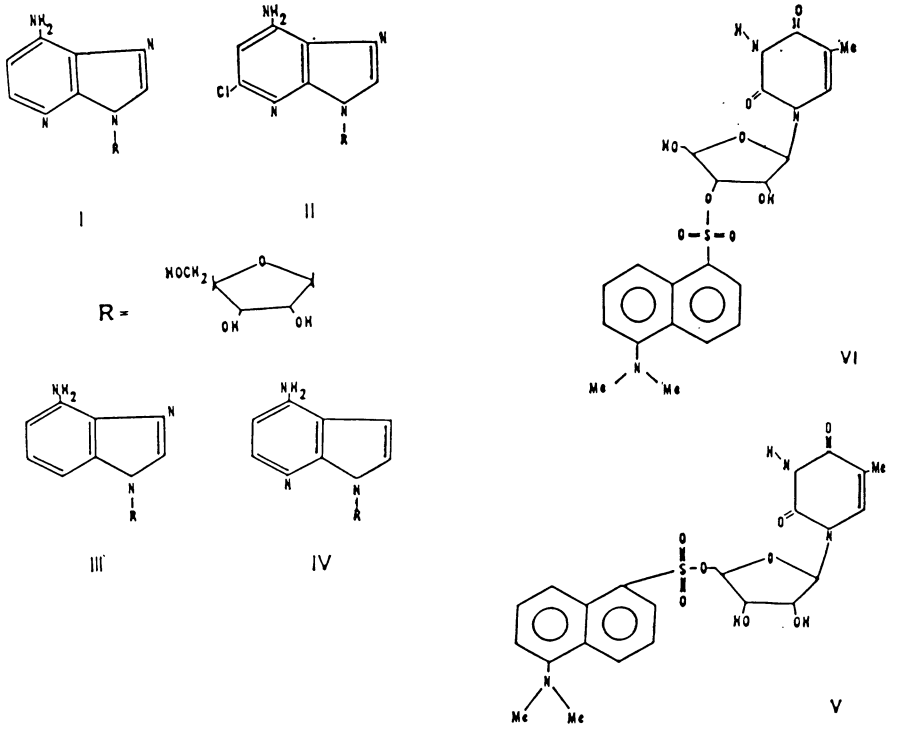

Fig. 1. — Structural formulae of four deaza analogues of adenosine (compound I to IV), 5'-O-dansylthymidine (V) and 3'-O-dansylthymidine (VI).

Spectrophotometric measurements: Absorption spectra of the dansyl-derivatives were performed in a Perkin Elmer Lamda 5 instrument, equipped with a Haake F3C thermostat, using a quartz curvette of 1 cm lightpath.

Cells and viruses: Vero cells and L TK$^-$, HPRGT$^-$ cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% (v/v) fetal calf serum. A wild type HSV-1 virus originally isolated from a skin vescicle of an immunosuppressed patient and previously characterized [5], was used in the chemosensitivity assays and as a source of viral TK.

Drug sensitivity testing in vitro: Susceptibility of HSV-1 wt to the nucleoside analogues was assayed on Vero cells by a plaque reduction method. The amount of substance required to reduce plaque formation by 50% (ED_{50}) was obtained as previously reported [6].

Cell growth inhibition: The cytotoxic potential of the analogues was measured on exponentially growing Vero cells treated with increasing drug amounts for a period of 24 hr. ED_{50} values were obtained from the best plot of a least square fit relating cell survival to the log of drug concentration [7].

Inhibition of HSV-1 thymidine kinase: The enzyme preparation was obtained from cells infected with a multiplicity of 20 and maintained in culture for 16-18 hrs after infection. At the end of this period cells were disrupted by sonication and the enzyme recovered in the high speed supernatant fraction [8]. The TK assay was carried out following a procedure previously reported [8], by measuring initial rate kinetics.

For determination of apparent Ki three different concentrations of thymidine (dThd), ranging from half to three fold the apparent Km of our viral enzyme (0.8-1uM), and at least four different molar concentrations of the inhibitors were employed. Values were derived from Dixon's plots [9].

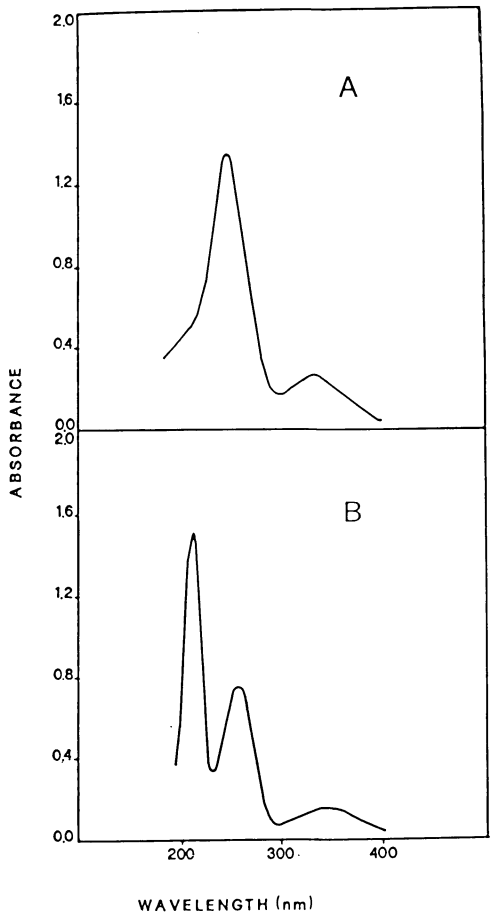

Fig. 2. — Absorption spectra of 3'-O-dansylthymidine in 5% DMSO in water (A) an in 100% methanol (B).

RESULTS

Spectrophotometric measurements: fig. 2 shows the UV spectra of 3'-0-dansylthymidine (compound VI) in a mixture of 5% DMSO in water (A) and in (100%) methanol (B).

The compound exhibits three principal peaks in methanol: the first centered at 215 nm, the second at 255 nm, and the third, (corresponding to the dansyl group absorption), at 347 mm.

A different spectral profile is shown in water/DMSO solution: the two major peaks are superimposed, with an absorption maximum centered at 250 nm (ε = 20,200), while the dansyl peak is slightly blue shifted. The absorption spectrum of compound V (5'-0-dansylthymidine) is virtually identical to that of its congener in either solvent (not shown).

Antiviral and cytotoxic activity: The effects produced by the studied compounds on the growth of HSV-1 and Vero cells *in vitro* are reported in table 1 as the relative ED_{50} values. It can be noticed that all deaza analogues are quite poor inhibitors of HSV-1 replication and rather cytotoxic. Their therapeutic index, with the exception of compound IV, is virtually one.

On the contrary, a certain degree of selectivity is shown by the two dansylthymidine derivatives. Both are in fact capable of reducing viral multiplication (VI is slightly more active) at concentrations at which they are not cytotoxic. Moreover, they inhibit the HSV-1-encoded TK (see apparent Ki in Table 1), and this could be in relation with their efficacy as antiviral agents. As for the deazaderivatives, compound IV was the only one to be assayed for its ability to interfere with the viral enzyme, since being characterized by a therapeutic index higher than

Table 1. – *Some parameters of the biological activity of nucleoside analogues.*

Compounds	Viral ED_{50} μM	Cell ED_{50} μM	Ki of HSV-1 TK μM
I	30	40	nd *
II	35	40	nd
III	40	35	nd
IV	15	35	>500 **
V	12	50	70
VI	5	70	25

* not done

** Ki for compound IV could not be determined due to solubility limit.

one. Its Ki, however, could not be determined, for the low water solubility of this substance.

DISCUSSION

The search for new anti-herpesvirus agents is justified by a number of reasons. These include: a) the high pleiomorphism of herpesvirus infections; b) the widespread diffusion of these viruses; c) their high frequency of recurrence after primary infection, due to reactivation from the latent state; d) the increasing prevalence of herpesviruses in immunosuppressed patients; e) the possibility of sexual transmission; f) the relatively low number of selective drugs currently licensed for clinical use; g) the emergence of drug resistant mutants.

Although one can foresee the rapid development of new antivirals through the mass screening of synthetic and natural substances, the final availability of a «safe» drug requires, in addition to the precise definition of its mechanism of action, the assessment of the pharmacokinetic profile and a thorough toxicological evaluation.

It is therefore warrantable, in the design of new molecules, to undertake directly the synthesis of structures most likely recognized by specific viral enzymes.

With this in mind we have studied six new compounds which are reported in fig. 1. The deazaderivatives of adenosine were supposedly aimed to the viral DNA polymerase besides being potential inhibitors of S-adenosyl-homocysteine hydrolase (SAH hydrolase), an ubiquitous cellular enzyme involved in transmethylation reactions ([10]). On the other hand, the dansylthymidine analogues were thought to be competitors of the natural substrate thymidine for the viral TK.

The results obtained in the anti-HSV-1 assay indicate quite clearly that the deaza analogues are almost devoid of any therapeutic potential. Such observation would suggest that these substances, once converted into triphosphate nucleosides inside the cells, do not discriminate between viral and cellular DNA polymerase. Their moderate degree of cytotoxicity may also account, by analogy with other molecules of similar structure, for an interference with different cellular targets such as, for instance, some of the enzymes involved in purine metabolism ([11]). For this reason derivatives I to IV could be better evaluated as potential antiproliferative agents. However, we cannot rule out the possibility that these drugs may affect the growth of other viruses such as riboviruses, which are better known to depend on SAH hydrolase activity for their replication ([10]). This possibility is currently being investigated.

The activity exhibited by the dansyl compounds both in reducing HSV-1 growth and competing with dThd for the viral TK is, instead, significant, (see table 1).

These drugs are the first dansyl derivatives of thymidine to have been synthesized so far and the presence of the fluorescent chromophore may render them particularly useful as non radioactive probes for interaction studies with the purified target enzyme.

The fact that the 3'-0-dansyl compound is more effective than its 5'-0-dansyl congener, both in plaque reduction and TK inhibition assays, can likely be explained in terms of the different metabolic fate that the two drugs may follow.

The 3' derivative has in fact a free 5' hydroxyl group susceptible of phosphorylation whereas it is blocked by the aromatic substituent at the 3' position of the sugar moiety.

Thus, as a dansyl nucleotide, it could be incorporated into viral DNA and function as a chain terminator in blocking on a synthesis.

Quite differently, the 5' derivative can compete with dThd for the viral TK but

cannot be phosphorylated in this position (dead end product). Its anti-herpesvirus activity may rest primarily on TK inhibition, although an interference with cellular functions can also play a role. This aspect can be better elucidated by a comparative analysis of the relative affinities of these two molecules for the viral and cellular TKs. Moreover, by virtue of the compound fluorescence, the differential incorporation of the 3'-0-dansyl dThd into host and viral DNA could be followed.

It seems worth mentioning that compound VI is still inhibiting HSV-1 growth at concentrations as low as 0.1 uM (about 20% reduction of plaque formation). This finding can only be explained by postulating that the molecule is undergoing a partial degradation in the cell environment. Its weakest portion, which may be cleaved by cellular esterases, is represented by the sulphonyl esteric bond, linking the dansyl group to the 3' hydroxyl of the sugar.

Modifications occuring in the absorption spectra of compound VI after incubation with Vero cell extracts, and a changing pattern of drug migration in thin layer chromatography, would suggest that this cleavage is indeed taking place.

Albeit still preliminary, the results obtained with this substance are prompting us to pursue further investigations, encouraged by the ascertainment that, to our knowledge, few thymidine analogues are effective inhibitors of HSV-1 TK.

In this regard, it is our intention to accomplish the synthesis of 3' and 5'-amino-dansyl-dThd, which, by effect of the suphonylamido bond, could be more resistant to esterase degradation.

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