

Review

Genetic risks of antiviral nucleoside analogues – a survey

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Abstract

The available informations on the genotoxic effects in experimental systems of the antiherpesvirus nucleosides aciclovir, penciclovir, ganciclovir, brivudine and cidofovir as well as of the antiretrovirals zidovudine (AZT), lamivudine, zalcitabine (ddC), didanosine and stavudine are reviewed. Furthermore, data on carcinogenic activity of these drugs in laboratory rodents are compiled. Most nucleoside analogue antivirals induce chromosomal aberrations but are inactive in gene mutation assays. Carcinogenicity findings in mice and rats are variable but clearly positive for AZT and ddC. The possible mechanisms by which these agents may cause damage in the genetic information are still largely hypothetical, and experimental findings do not permit relevant extrapolations to the situation in man. There is no conclusive evidence that any of the drugs caused tumours in humans. The use of nucleoside analogues in antiviral therapy remains a pragmatic option that seems justified by risk/benefit assessment. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Vaccination is the most efficient way for the control and therapy of virus diseases. But for many viral infections vaccines are not available so far, and the only hitherto existing alternative are drugs that inhibit virus replication without extensively impairing host cell physiology by taking advantage of subtle differences between viral and

cellular metabolism. Nucleoside analogues belong to this class of drugs and play a prominent role in the therapy of herpesvirus and HIV infections. The ultimately active metabolites of most nucleoside analogues are their triphosphates. In case of antiherpes nucleosides virus-encoded nucleoside kinases, e.g. thymidine kinase of HSV or VZV, or a protein kinase of CMV, accomplish the formation of the analogue monophosphates that are metabolised to the respective triphosphates by cellular kinases, whereby the monophosphorylation step appears to be rate-limiting, i.e. a major factor of antiviral selectivity of antiherpes drugs

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(for reviews see Cameron (1993), Kulikowski (1994), Darby (1995) and De Clercq (1995)). On the other hand, retroviruses do not encode their own nucleoside kinases and antiretrovirals are phosphorylated to triphosphates by cellular enzymes. In both cases, the triphosphates formed interact with either the virus-encoded DNA polymerase of herpesviruses or with the reverse transcriptase of retroviruses by competition with natural nucleoside triphosphates and/or substrate inhibition. The much lower K_i values of analogue triphosphates for viral polymerases as compared to cellular DNA polymerases are another component of the antiviral selectivity of these drugs (Wright and Brown, 1990; Coen, 1992).

Antiviral nucleoside analogues, similar to other drugs, may cause a plethora of acute side effects which are mostly controllable or may lead to discontinuance of the therapy and replacement by other drugs. Chronic toxicity, above all potential carcinogenicity, is of more concern. Toxicity of antiretrovirals recently even became a political issue in South Africa with respect to zidovudine (AZT) treatment of pregnant women in order to prevent vertical HIV transmission (Birmingham, 2000).

Although any conclusive evidence for human carcinogenicity of antiviral nucleosides is lacking, they are reputed to be carcinogens. Presumably, this assumption came from the fact that the mode of action of these drugs relies on interference with nucleic acid metabolism and, thus, hereditary changes in the genetic information of the host organism might be suspected. In this survey, we compile what is known so far on this topic with regard to the majority of licensed antiherpes and antiretroviral nucleosides (Fig. 1). Unfortunately, many of the findings that were acquired for drug approval by the manufacturers have not been published as original data. In these cases, we had to rely on informations given in the Physicians' Desk Reference (2000).

Altogether, the overview shows that great gaps still exist in our knowledge of possible genetic risks of antiviral nucleoside analogues and, if genotoxic effects have been observed in diverse systems, how to explain them in mechanistic terms.

2. Bioassays for the detection of genetic damage

About a hundred tests for genotoxic activity have been described during the last three or four decades. The primary aim of these assays was to replace the expensive, laborious and time-consuming animal experiments, especially those for carcinogenicity. As outlined below, these expectations were not fulfilled, nevertheless a battery of screening assays for genotoxicity became an established constituent of preclinical toxicol-

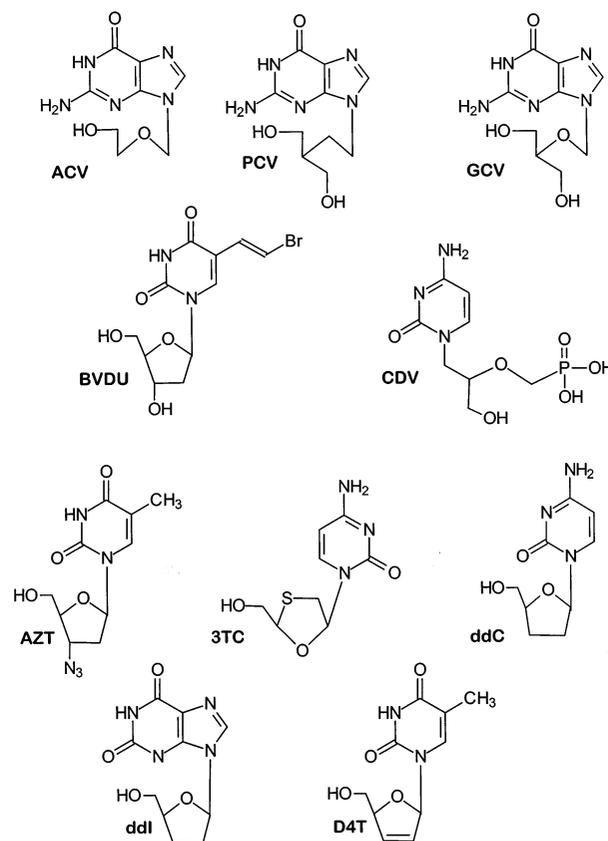


Fig. 1. Chemical structure of the nucleoside analogues. ACV, aciclovir (9-[(2-hydroxyethoxy)methyl]guanine); PCV, penciclovir (9-(4-hydroxymethylbut-1-yl)guanine); GCV, ganciclovir (9-[1.3-dihydroxy-2-propoxy)methyl]guanine); BVDU, brivudine ((E)-5-(2-bromovinyl)-2'-deoxyuridine); CDV, cidofovir (1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine); AZT, zidovudine (3'-azido-2',3'-dideoxythymidine); 3TC, lamivudine (2',3'-dideoxy-3'-thiacytidine); ddC, zalcitabine (2',3'-dideoxycytidine); ddI, didanosine (2',3'-dideoxyinosine); D4T, stavudine (2',3'-didehydro-3'-deoxythymidine).

ogy without which novel drugs cannot be approved nowadays. In the following, important test systems, in which antiviral nucleosides have been checked, are shortly described because this appears essential for the interpretation of findings.

2.1. Bacterial gene mutation

The most important gene mutation assay uses *Salmonella typhimurium* as the target organism and was developed by the group of Bruce Ames, Berkeley, therefore termed the Ames test. Endpoint is the reversion from histidine auxotrophy to histidine prototrophy caused by mutations in the histidine operon. By means of a large panel of *S. typhimurium* strains with different constitution at critical sites and with enhanced sensitivity due to repair deficiency the test can distinguish between base substitution- and frameshift-inducing mutagens. This is essential in studies on the mode of action of chemical compounds. Another achievement of the Ames test was the addition of liver microsomal fractions, which can activate premutagens, mainly by monooxygenation, but this does not play a role in case of nucleoside analogues (for a review on this test see Maron and Ames, 1983).

2.2. Mammalian gene mutation

In contrast to bacteria, the organization of genetic information in eukaryotes is much more complex and, therefore, a variety of genotoxic endpoints can be shown in higher organisms. For detection of single gene mutations the HPRT assay in cell cultures of the Chinese hamster (V79 and CHO cells) is most popular. It reveals a loss of function of the hemizygous, X-linked hypoxanthine phosphoribosyltransferase (*hprt*) gene. Mutant cells acquire resistance to 6-thioguanine (6-TG) as a marker and can be quantified in colony assays in the presence of 6-TG.

2.3. L5178YTK^{+/-} assay

This assay is conducted in mouse lymphoma L5178Y cells. The autosomal, heterozygous thymidine kinase gene of this cell line is the target,

and loss of function mutants are detected by culture in the presence of trifluorothymidine (TFT) which serves as a selection marker. But cytogenetic investigations in TFT-resistant L5178Y cells have shown large deletions of the long arm of chromosome 11 where the *tk* gene is located. So this endpoint is more akin to 'chromosome mutations' and not to point mutations in the strict sense (Glatt, 1994). The close congruence of L5178YTK^{+/-} and clastogenicity findings in antiviral drugs (Table 1) reflects this assumption.

2.4. Clastogenicity assay

The induction of structural chromosome aberrations (clastogenicity) belongs to the oldest methods for the detection of genetic damage by chemical and physical noxes in eukaryotic cells in vitro and in vivo. The process of aberration formation is only partially understood so far but appears to rely mainly on un- or misrepaired DNA double strand breaks. Clastogenicity tests are usually conducted in cell lines of the Chinese hamster that have a low chromosome number and rather large and easily distinguishable chromosomes. In addition, some of the clastogenicity data mentioned in Table 1 came from studies in human peripheral blood lymphocytes in vitro. Most of the aberrations visible by light microscopy, e.g. chromatid and chromosome breaks, exchange figures etc., are lethal to the affected cells and, thus, not heritable, i.e. not mutations in the proper meaning.

2.5. Micronucleus assay

Micronuclei are chromatin structures distinctly smaller than the normal cell nucleus and are enclosed by a nuclear membrane. They consist of either acentric chromosome fragments due to clastogenic events or of whole chromosomes, which were abnormally segregated during anaphase and telophase in consequence of disturbed function of the spindle apparatus. Thus this assay detects clastogenic and aneugenic properties of genotoxins as well. Mostly it is made in vivo, in bone marrow preparations from laboratory rodents,

Table 1
Genotoxicity and carcinogenicity data of antiviral nucleoside analogues^a

Drug	Bacterial gene mutation	Mammalian gene mutation	L5178YTK ^{+/-} assay	Clastogenicity assay	Micronucleus assay	SCE assay	Cell transformation in vitro	Carcinogenicity
ACV	neg. (Clive et al., 1983)	neg. (Clive et al., 1983; Pizer et al., 1987)	pos. (Clive et al., 1983; ≥ 2 mM)	pos. (Clive et al., 1983; ≥ 1 mM) pos. (Thust et al., 1996; ≥ 0.6 mM)	pos. (Haynes et al., 1996; ≥ 225 mg/kg/day)	(Thust et al., 1996; ≥ 0.4 mM) pos. (Thust et al., 2000b; 10 μ M)	neg. (Clive et al., 1983)	neg. (Tucker et al., 1983)
PCV	neg. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)	pos. (Physicians' Desk Reference, 2000; ≥ 2.5 mM)	pos. (Physicians' Desk Reference, 2000; ≥ 1 mM) neg. (Thust et al., 1996) pos. (Thust et al., 2000b; ≥ 1 μ M)	pos. (Physicians' Desk Reference, 2000; ≥ 500 mg/kg)	pos. (Thust et al., 1996; ≥ 2 mM) pos. (Thust et al., 2000b; ≥ 0.04 μ M)	?	pos. (Physicians' Desk Reference, 2000; 600 mg/kg/day)
GCV	neg. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)	pos. (Physicians' Desk Reference, 2000; ≥ 0.2 mM)	pos. (Physicians' Desk Reference, 2000; ≥ 1 mM) pos. (Thust et al., 1996; ≥ 0.1 mM) pos. (Thust et al., 2000b; ≥ 0.1 μ M)	pos. (Physicians' Desk Reference, 2000; ≥ 150 mg/kg)	pos. (De Clercq and Cassiman, 1986; 35 μ M) pos. (Thust et al., 1996; ≥ 1 μ M) pos. (Thust et al., 2000b; ≥ 1 nM)	?	pos. (Physicians' Desk Reference, 2000; ≥ 20 mg/kg/day)
BVDU	neg. (Marquardt et al., 1985, 1988; Oshiro et al., 1992)	neg. (Marquardt et al., 1988; Oshiro et al., 1992)	pos. (Oshiro et al., 1992; ≥ 1.5 mM)	pos. (Thust et al., unpublished; ≥ 0.03 μ M)	pos. (Oshiro et al., 1992; ≥ 1.5 μ M)	pos. (Thust et al., unpublished; ≥ 0.25 mM) pos. (Cassiman et al., 1981; 0.15 mM)	?	?
CDV	neg. (Physicians' Desk Reference, 2000)	?	?	pos. (Thust et al., unpublished; ≥ 0.1 mM)	pos. (Physicians' Desk Reference, 2000; ≥ 2000 mg/kg)	neg. (Thust et al., unpublished)	?	pos. (Physicians' Desk Reference, 2000; ≥ 0.6 mg/kg/week)

Table 1 (Continued)

Drug	Bacterial gene mutation	Mammalian gene mutation	L5178YTK ^{+/-} assay	Clastogenicity assay	Micronucleus assay	SCE assay	Cell transformation in vitro	Carcinogenicity
AZT	neg. (Physicians' Desk Reference, 2000)	pos. (Sussman et al., 1999; ≥ 0.3 mM) pos. (Ayers, 1988; ≥ 4 mM)	pos. (Physicians' Desk Reference, 2000)	pos. (Gonzalez Cid and Larripa, 1994; ≥ 0.4 mM)	pos. (Phillips et al., 1991; ≥ 100 mg/kg/day) neg. (Motimaya et al., 1994; < 28 mg/kg)	pos. (Gonzalez Cid and Larripa, 1994; ≥ 1.9 mM)	pos. (Ayers et al., 1996; ≥ 2 μ M)	pos. (Ayers et al., 1996; mice: > 30 mg/kg/day; rats: 300 mg/kg/day)
3TC	neg. (Physicians' Desk Reference, 2000)	?	pos. (Physicians' Desk Reference, 2000)	pos. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)	?	neg. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)
ddC	neg. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)	pos. (Physicians' Desk Reference, 2000; ≥ 7 μ M)	pos. (Physicians' Desk Reference, 2000; > 2500 mg/kg)	?	pos. (Physicians' Desk Reference, 2000; ≥ 2.4 mM)	pos. (Rao et al., 1996; ≥ 500 mg/kg/day)
ddI	neg. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)	pos. (Physicians' Desk Reference, 2000; ≥ 8 mM)	pos. (Physicians' Desk Reference, 2000; ≥ 2 mM)	pos. (Phillips et al., 1991; ≥ 200 mg/kg/day)	?	pos. (Physicians' Desk Reference, 2000; ≥ 12 mM)	neg. (Physicians' Desk Reference, 2000)
D4T	neg. (Physicians' Desk Reference, 2000)	neg. (Physicians' Desk Reference, 2000)	?	pos. (Schilling et al., 1995; > 0.1 mM)	pos. (Physicians' Desk Reference, 2000; ≥ 600 mg/kg/day)	?	pos. (Schilling et al., 1995; ≥ 0.1 mM)	neg. (Physicians' Desk Reference, 2000)

^a Abbreviations: neg. – negative; pos. – positive. References are given in parentheses, with minimal effective doses in case of positive results. Data from herpesvirus thymidine kinase gene transfected cells are given in italics. (?) unknown/not tested.



Fig. 2. SCEs in an untreated CHO-HSV tk^+ mitosis (left) and in a mitosis from a culture of the same cell line but treated with 0.1 μ M GCV for the duration of one cell cycle (14 h) and prepared 28 h thereafter (right). The untreated cell shows seven SCEs recognizable by the change of the staining pattern to the opposite sister chromatid, while the GCV-treated mitosis contains 146 SCEs (fluorescence-plus-Giemsa staining).

where the frequency of micronucleus-containing poly- and normochromatic erythrocytes is evaluated after administration of the test agent to the animals. It is one of the few screening tests, which are accessible to automated image analysis.

2.6. Sister chromatid exchange assay

Sister chromatid exchanges (SCEs) are exchanges of DNA molecules at homologous sites of replicating chromosomes and reflect recombinogenic activity of genotoxins. SCEs are detectable by incorporation of 5-bromodeoxyuridine for one or two cell cycles and special staining procedures of cytogenetic preparations (Fig. 2). This assay is one of the most sensitive tests for many chemical mutagens and carcinogens, but the biological consequences of SCEs are still largely unclear. The SCE test is conducted with the same target cells as the clastogenicity assay and, because of many technical similarities, both can be performed concomitantly.

2.7. Cell transformation *in vitro*

The above mentioned screening tests, which detect genotoxic activity, have a certain predictive

value for carcinogenicity of chemical agents because a correlation exists between mutagenic/genotoxic events and tumour induction in animals and humans. But this correlation is not absolute and there is no doubt that many mutagens are non-carcinogenic and, on the other hand, certain carcinogens appear to exert their oncogenic properties via non-mutagenic pathways, e.g. by epigenetic alterations that change the regulation of genes in a hereditary manner without changing the genetic code. These latter agents, the so-called non-genotoxic carcinogens, are not detectable by genotoxicity screening. One approach to circumvent this crucial problem was the development of *in vitro* cell transformation assays. Normal cells from solid tissues show a characteristic behaviour *in vitro*: they grow as adherent monolayers (anchorage dependence) in an ordered pattern and cease proliferating upon confluence (contact inhibition). Moreover, they have a limited life-span and do not form tumours when transplanted into syngeneic animals. On the other hand, transformed cells show a criss-cross pattern of growth, are not contact-inhibited and form colonies in soft agar and tumours in animals. Most *in vitro* transformation assays are conducted in either mouse fibroblasts (C3H 10T1/2 or Balbc/3T3 cells) which, although being immortal and aneuploid, have a normal growth pattern and an extremely low background of spontaneous transformation, or in primary Syrian hamster embryonic fibroblasts. Indicators of transforming activity of a chemical agent are the induction of transformed piling-up foci, growth in soft agar, and transplantability. Transformation tests sometimes pose problems with reproducibility and require much experimental experience (for reviews see Thust, 1976; Heidelberger et al., 1983; Combes et al., 1999).

2.8. Carcinogenicity assays

Since cancer formation is the result of a multi-step process comprising several genetic and epigenetic alterations (Fearon and Vogelstein, 1990), neither of the short-term screening assays so far known is able to replace long-term animal experiments. These are conducted in laboratory rodents,

mice and rats, to which the test agents are usually administered up to maximally tolerated doses for the duration of the experiment, and the animals are observed over lifetime. Occurrence of tumours in comparison with those observed in untreated, parallel controls reflects carcinogenic activity.

All the screening assays mentioned have been validated mainly under the framework of the US Environmental Protection Agencies' Gene-Tox Program where respective rodent carcinogenicity data served as the standard. In general, this assessment gave average predictive values between 60% and 80% as to sensitivity (carcinogens tested positive in screening assays) and specificity (negative findings with non-carcinogens).

3. Data assessment

Fig. 1 shows the structure of licensed nucleoside analogue drugs with antiherpes (aciclovir (ACV), penciclovir, ganciclovir, brivudine and cidofovir) or antiHIV (AZT, lamivudine, zalcitabine, didanosine, and stavudine) activity for which findings presented in original publications or reviewed in the Physicians' Desk Reference (2000) seemed sufficient for an assessment of genotoxic and/or carcinogenic activity. These data are summarized in Table 1. Where positive results have been reported, the lowest effective concentrations are given and, in case of *in vitro* studies, the doses were converted to molar concentrations because this yields a more realistic picture of potency than weight per volume.

A salient property of these drugs is that neither of them is active in the microbial gene mutation assay and, with exception of AZT, this also applies to induction of point mutations in mammalian cells, while 'typical' mutagens, e.g. alkylating agents, are highly potent in these assays. Presumably, this crucial difference has mechanistic reasons. The mode of action of the vast majority of single gene mutagens is characterized by formation of DNA adducts. However, as discussed below, this mechanism is unlikely with respect to nucleoside analogues. Another reason of the inactivity of these drugs as to induction of point mutations might be an insufficient activa-

tion (phosphorylation) by the target organisms. Whether this applies to *S. typhimurium* remains unclear. However, the fact that all nucleoside antivirals tested so far cause chromosomal damage makes this assumption rather unlikely in case of the mammalian cell gene mutation assay.

Data from studies in the L5178YTK^{+/-} test, the clastogenicity and the micronucleus assay show that all antiherpes and antiHIV nucleosides cause structural chromosome damage, although in most cases at doses that are considerably higher than maximum blood plasma concentrations attainable in the clinical setting. As incorporation into the genomic DNA of the target cells and subsequent processing of the analogue-substituted DNA presumably is a precondition of genotoxic activity of these agents (Section 4), the extent of damage depends on the experimental design, e.g. on the cell type used, its proliferative activity, the duration of exposure and post-exposure periods etc. This may explain the variability of lowest effective doses reported by different authors. Most of the antiherpes nucleosides meanwhile also have been examined for genotoxic activity in 'metabolically competent' target cells, i.e. in cell lines transfected with *tk* genes of HSV or VZV, respectively. As expected, this brought dramatic increases in genotoxic effects induced by the agents per dose level (Table 1, data printed in italics). After these general comments the findings shall be shortly discussed for each drug separately because it is well known that the biological properties of nucleoside analogues do not show 'class effects' (Darby, 1994).

3.1. Aciclovir

This drug has the best safety profile of all antivirals licensed so far. If positive results were observed in screening assays, the threshold concentrations were several orders of magnitude higher than those attainable in therapeutic regimens. ACV was inactive in the *in vitro* cell transformation assay in C3H/10T1/2 cells but weakly positive in BALB/c 3T3 cells (Clive et al., 1983).

More importantly, no drug-related increase of neoplasias has been observed in rats and mice following chronic ACV administration up to

doses of 450 mg/kg/day (Tucker et al., 1983). Observations in patients corroborate the experimental data. According to Darby (1995) more than 30 million individuals were exposed to oral ACV but no serious side effects of the drug were identified. Also, cytogenetic monitoring of patients with recurrent genital herpes medicated with ACV did not reveal an elevation of chromosomal aberrations in peripheral blood lymphocytes in comparison with lifestyle and pre-treatment controls, or placebo treatment (Clive et al., 1991).

Two factors are thought to be responsible for this outstanding safety: (i) the extraordinary stringent dependence of ACV on monophosphorylation by the herpesvirus-encoded thymidine kinase and (ii) due to the lack of a second hydroxyl group in the acyclic moiety ACV cannot form phosphodiester linkages and, thus, incorporation of ACV-MP into DNA causes obligate chain termination (Darby, 1995). The latter property distinguishes ACV from the other antiherpes nucleoside analogues reviewed here, which have two hydroxyl groups in their acyclic side chain and, therefore, might be able to form phosphodiester linkages in the DNA backbone. However, as discussed below, whether this happens or not is still a matter of debate.

3.2. Penciclovir

Penciclovir (PCV) is clinically used in the form of its oral prodrug famciclovir (FCV). As FCV is rapidly and extensively metabolized in vivo to PCV by an aldehyde oxidase and esterases, with a total systemic availability of 77% (Pue and Benet, 1993), the experimental data obtained with PCV should be representative for FCV as well. PCV was found positive in all assays detecting clastogenicity, but at doses that are irrelevant as to the clinical setting (≥ 1 mM; Table 1). On the other hand, in HSV tk -expressing Chinese hamster ovary cells PCV caused chromosomal damage and SCE induction in the submicromolar dose range (Thust et al., 2000a,b). These are concentrations well below those observed in human blood plasma under FCV standard therapy (≥ 10 μ M; Vere Hodge, 1993). However, it is noteworthy that the

PCV concentrations, which caused genotoxicity in CHO-HSV tk^+ cells, were cytotoxic to these cells by triggering the apoptotic pathway (Thust et al., 2000a). Moreover, the chromosomal changes were mainly premature chromosome condensations that presumably resulted from an uncoupling of karyokinesis and cytokinesis, while typical aberrations barely increased in frequency (Thust et al., 2000b).

Administration of FCV to rats and mice induced a significant increase of mammary adenocarcinomas in female rats at a dose that is equivalent to 1.5 times the human systemic exposure at the recommended oral dose of 500 mg t.i.d. based on AUC plasma concentrations for PCV. Furthermore, marginal increases in the incidence of fibrosarcomas or squamous cell carcinomas of the skin were observed in female rats and male mice (Physicians' Desk Reference, 2000). As these tumour types occur rather frequently in laboratory rodents, the relevance of these findings with respect to man remains unclear.

3.3. Ganciclovir

Although with respect to molecular structure ganciclovir (GCV) differs from PCV only by a 'small' modification, the methylene group of the aliphatic side chain is replaced by an ether oxygen, this has profound consequences not only with regard to antiviral specificity but also to genotoxic activity. GCV is a drug that causes cytogenetic damage (aberrations and SCEs) in genetically unmodified cells, i.e. in cells not expressing a herpesvirus nucleoside kinase, even in the dose range of the IC_{50} of CMV, ~ 10 μ M (Thust et al., 1996). This suggests that the drug can be monophosphorylated to a certain extent by normal, uninfected or non-transfected cells and possibly explains its cytotoxic side effects (myelosuppression) in human patients. Because GCV gained considerable interest in the context of a novel gene therapeutic strategy for the treatment of malignant diseases (Section 4), we studied the interaction between cell killing and genotoxicity by this agent in more detail. In CHO-HSV tk^+ cells GCV is an extraordinarily potent genotoxin

that induces chromosome breaks and translocations in a dose range not influencing the proliferative activity of the target cells ($\sim 0.1 \mu\text{M}$). Furthermore, in the same cells the drug is one of the strongest recombinogenic agents so far known (Fig. 2). The lowest concentration tested, 1 nM, still provoked a statistically significant increase of the SCE rate (Thust et al., 2000a,b). In this respect GCV even surpasses the activity of certain alkylating 'supermutagens' such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Genotoxicity of GCV is of the delayed type, i.e. no adverse effects are seen in the target cells immediately after drug exposure but they rise dramatically during a drug-free post-exposure interval one or more cell cycles thereafter. It has been shown by several groups (St. Clair et al., 1987; Rubsam et al., 1998; Thust et al., 2000a) that radiolabelled GCV is extensively and internally incorporated into the genomic DNA of the exposed cells. At present the most likely hypothesis to explain the genotoxic effects of this drug is to assume a disturbance of replication at the sites of incorporated GCV, which may lead to recombination and DNA strand breaks. GCV incorporated into DNA may also be subject to DNA repair processes which is supported by findings in cell lines with impaired DNA repair functions that are highly tolerant to GCV (Tomicic et al., unpublished observations). This topic deserves further examination, which is under way in our groups. We should note that GCV is a highly potent apoptosis-inducing agent. Apoptosis by GCV in HSV tk -expressing Chinese hamster cells is triggered via the mitochondrial damage pathway and is independent of p53. Crucial events involved are Bcl-2 decline and caspase-9/caspase-3 activation. We hypothesize that DNA damage due to incorporated GCV either in form of DNA breaks or aberrations are the primary trigger of cytotoxicity and apoptosis (Tomicic et al., 2000).

The extreme cytogenetic genotoxicity of GCV *in vitro* is not reflected by carcinogenicity data. Mice were given oral doses of 1, 20 and 1000 mg/kg/day, respectively. At the highest dose, which is about 1.4 times the mean drug exposure in humans following the recommended *i.v.* dose of 5 mg/kg, GCV caused a significant increase in

incidence of tumours of the preputial gland in males, forestomach tumours in both sexes, and of the ovaries, uterus, mammary gland, clitoral gland and vagina in females, while the lower dose gave borderline findings, and 1 mg/kg/day was negative. It was concluded that this drug should be considered a potential carcinogen in humans (Physicians' Desk Reference, 2000).

3.4. Brivudine

Brivudine (BVDU) is a weak clastogen at millimolar concentrations in genetically unmodified V79 cells (Thust, unpublished) and in the micronucleus assay in CHO cells (Oshiro et al., 1992). Similar observations were made in the SCE assay in human lymphocytes (Cassiman et al., 1981) and in V79 cells (unpublished). Surprisingly, treatment with BVDU of CHO cells transfected with the *tk* gene of HSV-1 did not enhance genotoxicity or cytotoxicity of the drug in comparison to syngeneic non-transfected cells (unpublished). This contradicts the clinical experience in the therapy of HSV-1 infections where BVDU is highly efficient as well as opposes the investigations of Balzarini et al. (1994) and Balzarini (1996) who demonstrated a very high cytostatic/cytotoxic activity of this pyrimidine analogue in HSV tk -transfected mouse mammary carcinoma cells. One reason for this discrepancy might be that the murine cell line was negative for TK1 (cytosolic thymidine kinase) while the CHO cells we used are TK2-proficient (Bublely et al., 1983; Balzarini et al., 1986). This topic clearly deserves further studies, however. On the other hand, BVDU was found to be highly cytotoxic and a strong clastogen but almost inactive in the SCE assay at submicromolar concentrations in CHO cells transfected with the *tk* gene of VZV (Tomicic, Kaina and Thust, unpublished). Similar findings with respect to cytotoxicity have been reported by Grignet-Debrus and Calberg-Bacq (1997), Grignet-Debrus et al. (2000) and Degreve et al. (1997).

It seems that BVDU exerts its antiviral and cytotoxic/genotoxic activity, respectively, via two different mechanisms. In quantitative terms, the major intracellular metabolite of BVDU is the

monophosphate, which is a potent inhibitor of thymidylate synthase (TS) (Balzarini et al., 1987). TS is an essential enzyme of the *de novo* pathway of deoxythymidine monophosphate synthesis and, thus, its inhibition may cause nucleotide pool imbalance. It is well known that nucleotide pool imbalances can provoke, by impairing the fidelity of DNA replication, a variety of types of genotoxic damage as well as cell killing (for review see Kunz et al., 1994). According to Hirota et al. (1995), BVDU metabolism differs in HSV-infected versus HSV tk -transfected cells. While in infected cells BVDU triphosphate is formed that inhibits the virus-encoded DNA polymerase or, upon incorporation, destabilizes the viral DNA (Mancini et al., 1983; Ciucci et al., 1997), in HSV tk transfectants only the monophosphate should be formed which is cytotoxic due to the above mentioned TS inhibition.

According to an abstract report quoted by Oshiro et al. (1992) BVDU induced tumours in the liver and testes of rats treated with the agent for 1 yr. But as no details have been published so far, the carcinogenic activity of BVDU is essentially unknown.

3.5. Cidofovir

Cidofovir (CDV), a phosphonate, is a 'monophosphate nucleotide analogue' and therefore the herpesvirus TK-based antiviral selectivity is lacking in this drug. The active intracellular metabolite is CDV diphosphate that competitively inhibits viral DNA polymerases owing to K_i values several orders of magnitude lower for viral polymerases than for cellular DNA polymerases (Ho et al., 1992; De Clercq, 1996; Hitchcock et al., 1996; Naesens et al., 1997). Original genotoxicity data have not been published so far. According to the Physicians' Desk Reference (2000) the drug was clastogenic in human peripheral blood lymphocytes *in vitro* (no details given) and induced micronucleated polychromatic erythrocytes in mice receiving a dosage that was about 65 times higher than the maximum recommended clinical *i.v.* dose based on body surface area estimations. We checked CDV for clastogenic and SCE-inducing capacity in CHO-9 cells and ob-

served a dose- and time-dependent induction of chromosomal aberrations at CDV concentrations > 0.1 mM. Concomitant survival assays showed that the clastogenic doses abolished the colony-forming ability of the target cells, *i.e.* were cytotoxic. On the other hand, CDV was totally inactive for SCE induction in the same dose range (unpublished). From these observations we conclude that the drug is not a typical DNA reactive agent and the chromosomal aberrations are probably an indirect consequence of cytotoxicity.

In a 26-week *i.v.* toxicology study in rats, up to a maximum dose equivalent to 1.1 times the human systemic exposure at the recommended dose of CDV, based on comparisons of AUC measurements, a significant increase in mammary adenocarcinomas in female animals as well as of Zymbal's gland carcinomas in both sexes was observed. It was concluded that CDV should be considered carcinogenic in rats and a potential human carcinogen (Physicians' Desk Reference, 2000).

3.6. Zidovudine

Zidovudine is, amongst the drugs reviewed here, the only nucleoside analogue for which induction of gene mutations in the HPRT assay has been reported. According to Grdina et al. (1992), it was mutagenic in HepG2 cells at a concentration that was severely cytotoxic (~ 20 mM), but no HPRT mutations were induced in CHO cells at similar or even higher concentrations. A moderate, 1.8-fold, increase of mutations at the same locus was observed in a human lymphoblastoid cell line, TK6 (Sussman et al., 1999). The administered dose of 300 μ M is 35–70 times higher than those measured in the blood plasma of AIDS patients under AZT standard therapy. However, with respect to AZT incorporation into the genomic DNA of the TK6 cells, the mutagenic concentration was similar to that found in cord blood lymphocytes under conditions of perinatal AZT therapy, 500 mg/day (Sussman et al., 1999). Molecular analysis of the HPRT mutants revealed large deletions, thus resembling the 'mutations' detected in the L5178YTK $^{+/-}$ assay (Section 2). It was concluded that the deletions are the result

of AZT incorporation into the cellular DNA and subsequent chain termination. Presumably, this mechanism also causes chromosomal damage. AZT was clastogenic and induced micronuclei in human peripheral blood lymphocytes but at fairly high doses (Gonzalez Cid and Larripa, 1994). Similar observations were made in the clastogenicity assay in CHO cells and in the micronucleus test in the mouse bone marrow, but only a weak recombinogenic effect of AZT was reported in the SCE assay in CHO cells (Phillips et al., 1991; Gonzalez Cid and Larripa, 1994). Contrary to the weak to moderate cytogenetic genotoxicity, AZT induced transformed foci in the cell transformation assay using BALB/c 3T3 cells at a concentration as low as $\sim 2 \mu\text{M}$ (Ayers et al., 1996). The latter finding appears to correlate with the carcinogenicity data.

Lifetime oral administration of AZT doses $> 30 \text{ mg/kg/day}$ to mice caused vaginal squamous cell papillomas and carcinomas in about 10% of the females. Such tumours were absent in control animals, and no evidence of carcinogenicity was detected in male mice and rats. Female rats appeared to be less sensitive than mice, vaginal epithelial neoplasias were seen only at a dosage of 300 mg/kg/day (Ayers et al., 1996). Measurements of excreted AZT per day in the urine revealed concentrations in mice and rats that were 26- and 136-fold, respectively, higher than the human urine concentration at the recommended daily dose. In a subsequent lifetime carcinogenicity study in mice in which AZT was given intravaginally at concentrations of 5 or 20 mg/ml in saline, a high incidence of vaginal carcinomas was observed. It was concluded that the neoplasias result from chronic local exposure of the vaginal epithelium due to retrograde flow of urine containing high concentrations of the drug (Ayers et al., 1996). Incorporation of AZT into the DNA of the vaginal epithelium has been shown upon oral administration to female mice. Presumably, the incorporation plays an essential role in the ability of AZT to induce abnormal differentiation and tumorigenesis at this anatomical site (Olivero et al., 1994a,b). Perhaps of more practical relevance are studies showing the transplacental carcinogenic activity of AZT. When daily doses of either

12.5 or 25 mg were given to pregnant mice on days 12 through 18 of gestation, a distinct increase in tumour rates was observed, preferentially in female offspring. The dosage was assessed to be about 5 times higher than the equivalent daily dose received by pregnant women. Clearly AZT-related malignancies occurred in lung, liver, ovary and mammary gland. Also, the sensitivity of neonatal mice was examined by administration of $25\text{--}200 \text{ mg/kg}$ to neonates during postnatal days 1–8. The effects were similar to those seen after transplacental exposure. Altogether the findings suggest that AZT is a moderately effective perinatal carcinogen in mice and shows some organotropic properties (Olivero et al., 1997; Diwan et al., 1999).

3.7. *Lamivudine*

The drug was negative in most screening assays for genotoxic activity as well as for *in vitro* cell transformation. According to the Physicians' Desk Reference (2000), a weak clastogenic response was observed in human peripheral lymphocytes *in vitro* and in L5178Y cells (details not given). Also, no induction of micronuclei has been detected in rat bone marrow up to 65 times the recommended human dose.

Lifetime carcinogenicity studies showed no evidence of carcinogenic potential in mice and rats at exposures 10 or 58 times, respectively, the recommended dose in humans (Physicians' Desk Reference, 2000).

3.8. *Zalcitabine*

Data on the cytogenetic genotoxicity of zalcitabine (ddC) are inconsistent. While the drug was reported to be clastogenic in human peripheral lymphocytes *in vitro* at a concentration $\geq 7 \mu\text{M}$ (Physicians' Desk Reference, 2000), oral doses between 500 and 2000 mg/kg/day induced micronuclei in the bone marrow of mice but without a clear dose response (Phillips et al., 1991). Moreover, the *in vitro* cell transformation assay was positive at $\geq 2.4 \text{ mM}$ (Physicians' Desk Reference, 2000).

Gavage of ddC, 500 and 1000 mg/kg/day, for up to 6 months induced very high rates of thymic lymphomas with multiple organ involvement in two different mouse strains, especially in female mice (Rao et al., 1996). The tumour induction was not due to activation of an endogenous ecotropic retrovirus. The dosage as well as maximum ddC plasma concentrations in mice were > 1000 times higher than in human patients at the recommended therapeutic dose of 2.25 mg/day. However, because of the much lower bioavailability and phosphorylation of the drug in mice, it is not appropriate to extrapolate the findings to man. Nevertheless, ddC should be considered potentially carcinogenic in humans, especially in children and adolescent patients (Rao et al., 1996).

3.9. *Didanosine*

This drug was assessed to be non-genotoxic at biologically and pharmacologically relevant doses and, at significantly elevated concentrations in vitro, the genotoxic effects were similar in magnitude to those seen with natural nucleosides (Phillips et al., 1991; Physicians' Desk Reference, 2000).

In lifetime carcinogenicity studies in mice (up to 1.7 times the human maximum exposure) or rats (up to 3 times the human maximum exposure) no drug-related increase of tumour incidence was observed (Physicians' Desk Reference, 2000).

3.10. *Stavudine*

Stavudine (D4T) was weakly clastogenic in human peripheral lymphocytes and mouse fibroblasts in vitro and induced transformed foci in BALB/c 3T3 fibroblasts. However, similar effects were also observed with thymidine (Schilling et al., 1995; Physicians' Desk Reference, 2000).

No carcinogenic activity of D4T was detected in mice and rats in 2-yr carcinogenicity studies at dosages which produced exposures equivalent to 39 times in mice or 168 times in rats, respectively, the human exposure at the recommended clinical dose of ~ 80 mg/day (Physicians' Desk Reference, 2000).

4. Possible mechanisms of induction of genetic damage by nucleoside antivirals

The mechanisms causing genetic alterations in mammalian cells by antiviral nucleoside analogues are still essentially unknown. While most chemical mutagens are converted, via metabolism or spontaneous hydrolysis, to highly reactive electrophilic intermediates that bind to nucleophilic sites in DNA, thus forming adducts which impair ordinary base pairing and, in consequence, lead to alterations of base sequences, such a mode of action is irrelevant for nucleoside analogues. The antiviral drugs reviewed here may induce genetic damage in at least two different ways.

(i) By altering intracellular nucleotide pools. It is well known that faithful DNA replication and repair requires a subtle balance of intracellular nucleotide levels, and disturbance of such a balance may cause all forms of genetic alteration, from point mutations to oncogenic transformation, although the proper mechanisms are far from being clear (Kunz et al., 1994). It has been shown that AZT dramatically decreases intracellular dTTP concentrations (for review see Balzarini, 1994) and it is conceivable that other nucleoside analogues, which are metabolised by the same nucleoside kinases as the natural substrates, may competitively inhibit the formation of natural nucleoside triphosphates as well. Another drug that probably causes nucleotide pool imbalance is BVDU whose monophosphate is a potent inhibitor of TS (Section 3).

(ii) Obviously more important with regard to genetic alterations is the incorporation of nucleoside analogue phosphates into the genomic DNA of mammalian cells. The majority of antiviral nucleosides have an unchanged base moiety. The only exception among the compounds reviewed here is BVDU in which the 5-methyl group of thymidine is replaced by a bromovinyl substituent. However, this group is not involved in hydrogen bond formation in the DNA double helix and it can be assumed, in general, that base pairing should remain unaffected upon incorporation of all these drugs. However, profound differences exist in the structure of the 'sugar' moieties. ACV and the anti-HIV drugs have only one hy-

droxyl group in the aliphatic side chain that is functionally similar to the 5'-hydroxyl group of natural nucleosides. Incorporation of these nucleotides into the growing DNA chain thus leads to obligate chain termination. When the triphosphates of these chain terminators are accepted by eukaryotic DNA polymerases, maximally one nucleotide analogue might be added per replicon followed by stalling of DNA synthesis at the replication forks. Whether this happens in the case of ACV is not yet proven. According to Rubsam et al. (1998) no significant incorporation of radiolabelled ACV in the genomic DNA of HSV thymidine kinase-expressing human glioma cells has been detected. On the other hand, we observed a distinct clastogenic and SCE-inducing capacity as well as triggering of apoptosis by this drug in CHO-HSV tk^+ cells, although at concentrations that are not relevant to the clinical setting (Thust et al., 2000a,b). It is noteworthy that the genotoxic effects occurred immediately after exposure and increased during the next two cell cycles in the absence of ACV.

Incorporation of AZT into the eukaryotic DNA has been demonstrated in various cell culture and animal models (Sommadossi et al., 1989; Vazquez-Padua et al., 1990; Olivero et al., 1994a,b; Sussman et al., 1999). Furthermore, rapid incorporation was detected in human placentas perfused ex vivo (Olivero et al., 1999a) and in leukocyte DNA from AZT-treated patients as well as from infants exposed in utero (Olivero et al., 1999b). As shown in human leukemia cells in vitro, incorporated AZT is removed by an exonuclease function that is not associated with any of the DNA polymerases (Vazquez-Padua et al., 1990). Olivero et al. (1999a,b) and Meng et al. (2000) hypothesize that the exonuclease-mediated removal of this chain terminator might trigger recombinational repair and gap-filling processes. Both mechanisms could be error-prone and may lead to the inactivation of tumour suppressor genes due to loss of heterozygosity. This hypothesis is highly attractive and, in view of the clinical importance of AZT, further detailed investigations are needed urgently.

Much less is known whether other antiretroviral nucleosides are incorporated into eukaryotic

DNA. It was mentioned (Physicians' Desk Reference, 2000) that ddC is incorporated, but to our knowledge no details have been published.

In vitro experiments on the incorporation of GCV and PCV into synthetic primer templates in the presence of different purified DNA polymerases revealed that the nucleotides are incorporated and that at least one further nucleotide is added, followed by chain termination (Reid et al., 1988; Reardon, 1989; Vere Hodge, 1993; Vere Hodge and Cheng, 1993; Ilsley et al., 1995). It has been questioned, however, whether the artificial conditions used in these experiments are relevant to the intracellular situation considering the complexity of the replication machinery (Vere Hodge, 1993; Martin et al., 1994). Extensive and internal incorporation of GCV into the genomic DNA of mammalian cells has been mentioned above (Section 3). We assume that the dramatic genotoxic effects caused by this drug in HSV tk -transfected cells are due to impaired function of the replication complex that is induced by structural alterations occurring in the sugar-phosphate backbone of GCV-substituted DNA (Marshalko et al., 1995; Foti et al., 1997; Thust et al., 2000a,b). Furthermore, we speculate that, if the repair capacity of the affected cells is exhausted, the apoptotic pathway is triggered. Induction of apoptosis is the rationale of the gene therapy concept for the treatment of malignant diseases using a combination of intratumoral transduction of the HSV thymidine kinase gene, as a suicide gene, with systemic administration of GCV (Moolten, 1986). Hitherto published experiences with this therapy schedule in clinical trials, however, are rather disappointing and do not promise significant benefits to the patients, mainly due to insufficient transduction of the suicide gene into cancer cells (Izquierdo et al., 1996; Ram et al., 1997; Klatzmann et al., 1998; Shand et al., 1999). The HSV tk /GCV combination, however, seems to offer a realistic chance for the treatment of graft-versus-host reactions occurring in consequence of allogeneic bone marrow transplantation. In this setting, transduction is performed ex vivo and, by highly sophisticated techniques, up to 100% pure lymphocyte populations expressing the transgene are selectable. First reports from clinical trials are

very encouraging and promise long-term curative responses (Bonini et al., 1997; Cohen et al., 1999). The clinical indication of GCV so far is mainly restricted to severe and life- or sight-threatening CMV diseases/reactivations, pneumonia in transplant recipients and retinitis in AIDS patients. The potential genetic risk of GCV, however, should be considered when the drug is given to patients with curable diseases such as, e.g., the graft-versus-host disease, if the above described gene therapy schedule should succeed.

We have checked the incorporation of PCV into the genomic DNA of CHO-HSV t_k^+ cells and the genotoxic activity of this drug in the same cell line. Incorporation of PVC was about 500 times lower than that of GCV (Thust et al., 2000a). Moreover, cytogenetic genotoxicity was observed only at cytotoxic drug concentrations, but PCV was highly active in inducing apoptosis at clinically relevant doses. From this we proposed the use of PCV instead of GCV as a safer alternative drug in the suicide gene therapy schedule (Thust et al., 2000a,b). Of course, these in vitro findings must still be confirmed in in vivo models. The mechanism of apoptosis induction by PCV is not yet known. The cytogenetic phenomena that accompany cell killing by this drug, polyploidization and premature chromosome condensation, suggest that PCV disturbs the coordination of cell cycle checkpoints, thus leading to an uncoupling of karyokinesis and cytokinesis (Thust et al., 2000b). Probably, a similar mechanism might also explain the antiviral activity of PCV, which is still poorly understood. Previous investigations did not demonstrate incorporation of this antiviral drug into viral or cellular DNA. Neither the different monophosphorylating capacities of herpesvirus-encoded nucleoside kinases nor the interaction of PCV triphosphate with viral DNA polymerases can explain the antiviral activity and specificity of PCV (Earnshaw et al., 1992; Vere Hodge and Cheng, 1993; Darby, 1995). From our findings we suggest that PCV triphosphate formed in herpesvirus-infected cells triggers host cell apoptosis and thereby leads to an interruption of virus infection and production. It is clear that this important topic still needs detailed investigations.

Taken together, the preceding discussion has shown that the hitherto existing knowledge about the possible mechanisms of genotoxicity and carcinogenicity and, in some cases, even of antiviral action of antiviral nucleoside drugs is rather fragmentary and largely hypothetical. Nevertheless, based on the data it can be concluded that incorporation of the analogue phosphates into the genomic DNA of mammalian cells is critical in causing adverse effects. Presumably neither of the drugs directly provokes base mispairing because there is, at least in theory, no structural hindrance for the formation of regular Watson–Crick base pairs. Obviously, the decisive steps occur after incorporation, when error-prone replication processes are triggered that alter the genetic information with all the known consequences, from gene mutation to cancer induction.

5. Iatrogenic carcinogenicity of antiviral drugs

The findings listed in Section 3 of this survey suggest that, perhaps with a few exceptions, neither of the drugs showed dramatic yields in the induced genotoxic effects in diverse bioassays or in cancer induction in animals. The crucial question is how to extrapolate experimental findings to the situation in patients. In quantitative and even in qualitative terms, neither genotoxicity nor carcinogenicity are intrinsic and independent properties of chemical compounds. They depend on species-specific pathways of activation and deactivation as well as are strongly modulated by different repair mechanisms and capacities for lesions formed in the genetic material. It is well known that neither of the so far existing animal models can faithfully predict carcinogenic activity in man. A positive result from long-term carcinogenicity experiments may yield the information that the agent under study is a potential human carcinogen but does not prove this.

To our knowledge, hitherto no unequivocal evidence has been presented that antiviral nucleoside analogues caused tumours in human patients. Since these drugs are relatively novel and there is no long-term practice with them, this is not surprising. Only extensive epidemiological in-

vestigations in exposed populations may give a conclusive answer to this question.

For several reasons, epidemiological research in patient groups suffering from viral diseases and treated with antiviral drugs poses particular problems.

(i) Certain viruses per se may be an etiological factor of cancer induction in man and, thus, at least may superimpose eventual iatrogenic consequences of drug therapy.

(ii) It is well known that patients with impaired immune function are especially prone to develop 'spontaneous' tumours, and certain herpesvirus diseases/reactivations with life-threatening complications mainly occur in immunocompromised patients (patients under antineoplastic chemo- or radiotherapy, transplant recipients and, most importantly, in AIDS patients). Therefore, it would be very difficult to clarify whether increased tumour rates in these groups are iatrogenic, i.e. due to antiherpes drugs, caused by insufficient immunocompetence, or by the basic disease. The problem whether antiretrovirals cause cancer is almost unsolvable in AIDS patients, which frequently show 'opportunistic' tumours such as lymphomas and Kaposi's sarcomas that belong to the AIDS-related complex. Furthermore, mostly this population is treated with a variety of drugs for the therapy of accompanying complaints. This makes any elucidation of causal relationships between drug administration and iatrogenic tumour formation highly problematic.

(iii) The time period between cancer induction and manifestation in man may take many years up to decades, which also complicates epidemiological research. An event like the Chernobyl disaster where a dramatic increase of thyroid cancer due to the release of radioactive iodine isotopes occurred within a few years in Belarus, Ukraine and Russia (Balter, 1996) will, hopefully, remain a rare exception.

(iv) Last but not least, the chance to find out whether a drug is carcinogenic or not in humans also depends on the type of tumours induced. Perhaps the 'best' example is diethylstilbestrol. This drug was administered to several million women for the prevention of miscarriages. That diethylstilbestrol very soon was discovered to be a

human transplacental carcinogen was essentially influenced by the facts that the drug caused malignancies with an uncommon morphology (clear cell adenocarcinoma), at an uncommon site (vagina and vulva), and after a fairly short latency period (the first peak of incidence occurred in the late teens and early twenties of the daughters; for review see Herbst (2000)). A similar situation could hardly be expected if any of the antiviral drugs should be carcinogenic in humans.

We think that human carcinogenicity of drugs needed in the therapy of otherwise fatal diseases and for which so far no definitely safe alternatives exist, is not the proper problem (Section 6). Much more pressing is the question whether AZT is a human transplacental carcinogen. According to the ACTG 076 clinical trial (Connor et al., 1994) maternal–infant transmission of HIV in pregnant HIV-infected women was decreased from about 25% in the placebo group to about 8% in the treatment group by a therapy regimen comprising antepartum AZT administration in the second and third trimester, intrapartum AZT and then treatment of the newborns with the same drug for six weeks. The outcome was further improved by delivery through Caesarean section and preventing breastfeeding from the HIV-positive mothers. Altogether this brought a reduction of vertical HIV transmission up to approximately 2% or even less. This was a real achievement, and the therapy schedule is now the standard option for the prevention of maternal–infant transmission of HIV infection. The resulting population of HIV-free children who were transplacentally, peri- and post-natally exposed to AZT is a suitable group for epidemiological investigations whether this drug is carcinogenic or not in man. A first study report (Hanson et al., 1999) from 727 children with a known perinatal AZT exposure did not reveal evidence of malignancies. The mean follow-up was just 38.3 months. In a similar study in 234 children with a follow-up between 3.2 and 5.6 yr also, no adverse effects have been observed (Culnane et al., 1999). These observation periods are much too short and, therefore, it would be premature to draw any conclusions as to the carcinogenic potential of AZT in man.

6. Conclusions

As discussed above, hitherto existing data of genotoxic and/or carcinogenic properties of antiviral nucleoside analogues do not allow a reliable assessment of the long-term genetic risk posed by these drugs in man. Whereas ACV, PCV/FCV, lamivudine (3TC), didanosine (ddI) and D4T were inactive or gave borderline effects in most experimental systems, AZT and ddC were clearly carcinogenic in mice and, partially, in rats. Furthermore, it has been shown that GCV is an extremely potent cytogenetic genotoxin in cells that are able to monophosphorylate this drug. However, it remains an important scientific and practical challenge to clarify what the experimental findings mean with regard to the clinical setting.

The use of nucleoside analogues in antiviral therapy is essentially a strategic decision requiring careful weighing of risks and benefits. In case of AIDS, which is a lethal disease if untreated, the benefit of potentially carcinogenic drugs clearly outweighs possible risks. In our view, this also applies to the perinatal administration of AZT in order to prevent vertical HIV transmission. In certain aspects, the situation resembles that of cytostatic drugs, which are irreplaceable in anti-cancer chemotherapy. However, while conclusive evidence exists that widely used cytostatic drugs such as cyclophosphamide, melphalan, chloroethylnitrosoureas and others cause neoplasia in man (for discussion see Sorsa and Anderson (1996)), human carcinogenicity of antiviral nucleosides is at present a purely speculative assumption. It is noteworthy that neither of the agents addressed in this review showed a carcinogenic potency comparable to strong animal carcinogens such as *N*-nitroso compounds, polycyclic hydrocarbons or aromatic amines, e.g., which, depending on the animal species or strain, dosage and administration mode, may cause tumour incidences up to nearly 100% in extreme cases. Furthermore, it should be considered that cancer induction is a stochastic process that usually affects a small proportion of the exposed population.

Future research in this field should be focused on the following topics:

(i) Elucidation of the mechanisms of damage induction in the genetic information which, in the case of nucleoside analogues, are still largely hypothetical, particularly complex and appear to vary in a drug-specific manner because, with respect to biological activities, among nucleoside analogues there is no such thing as a close congener.

(ii) Genetic monitoring of exposed individuals, e.g. by scoring of peripheral lymphocytes for chromosomal aberrations, SCEs, and gene mutation. To our knowledge, only one such study has been published with respect to AZT. In a group of seven AIDS patients that received 1200 mg/day AZT as their sole medication for 4 weeks to 7 months, a mean rate of 8% lymphocytes with chromosomal aberrations was observed, while in four untreated AIDS patients, who served as the control population, a mean rate of 0.5% aberrant metaphases was detected (Shafik et al., 1991). This is a very distinct clastogenic effect, but much larger patient groups must be checked before any conclusions can be drawn as to genotoxic activity of the drug in man.

(iii) Only extensive epidemiological investigations in large populations of exposed individuals may yield definitive informations on cancer induction in man, as discussed in Section 5.

The use of non-nucleoside antivirals instead of nucleoside analogues, in principle, would not alter the situation because neither drug is a priori safe, until the proof of the contrary. In the meantime and until efficient antiviral vaccines become available, potential genetic risks of nucleoside analogues must be accepted in the therapy of life-threatening virus diseases.

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References

- Ayers, K.M., 1988. Preclinical toxicology of zidovudine. An overview. *Am. J. Med.* 85, 186–188.
- Ayers, K.M., Clive, D., Tucker, W.E., Hajian, G., de Miranda, P., 1996. Nonclinical studies with zidovudine: genetic toxicity tests and carcinogenicity bioassays in mice and rats. *Fund. Appl. Toxicol.* 32, 148–158.
- Balter, M., 1996. Children become the first victims of fallout. *Science* 272, 357–360.
- Balzarini, J., 1994. Metabolism and mechanism of antiretroviral action of purine and pyrimidine derivatives. *Pharmacy World Sci.* 16, 113–126.
- Balzarini, J., 1996. Herpes simplex virus thymidine kinase gene-transfected tumor cells: sensitivity to antiherpetic drugs. *Nucleosides Nucleotides* 15, 821–831.
- Balzarini, J., de Clercq, E., Verbruggen, A., Crumpacker, C., Ayusawa, D., Seno, T., 1986. Increased sensitivity of thymidine kinase-deficient (TK⁻) tumor cell lines to the cell growth inhibitory effects of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) and related compounds. *Anticancer Res.* 6, 1077–1084.
- Balzarini, J., de Clercq, E., Verbruggen, A., Ayusawa, D., Shimizu, K., Seno, T., 1987. Thymidylate synthase is the principal target enzyme for the cytostatic activity of (E)-5-(2-bromovinyl)-2'-deoxyuridine against murine mammary carcinoma (FM3A) cells transformed with the herpes simplex virus type 1 or type 2 thymidine kinase gene. *Molec. Pharmacol.* 32, 410–416.
- Balzarini, J., Bohman, C., Walker, R.T., de Clercq, E., 1994. Comparative cytostatic activity of different antiherpetic drugs against herpes simplex virus thymidine kinase gene-transfected tumor cells. *Molec. Pharmacol.* 45, 1253–1258.
- Birmingham, K., 2000. UN acknowledges HIV/AIDS as a threat to world peace. *Nat. Med.* 6, 117.
- Bonini, C., Ferrari, G., Verzeletti, S., Servida, P., Zappone, E., Ruggieri, L., Ponzoni, M., Rossini, S., Mavilio, F., Traversari, C., Bordignon, C., 1997. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 276, 1719–1724.
- Buble, G., Crumpacker, C., de Clercq, E., Schnipper, L., 1983. Effects of (E)-5-(2-bromovinyl)-2'-deoxyuridine on the proliferation of herpes simplex virus type 1-transformed and thymidine kinase deficient mouse cells. *Virology* 129, 490–492.
- Cameron, J.M., 1993. New antiherpes drugs in development. *Rev. Med. Virol.* 3, 225–236.
- Cassiman, J.J., de Clercq, E., Jones, A.S., Walker, R.T., van den Berghe, H., 1981. Sister chromatid exchange induced by anti-herpes drugs. *Br. Med. J.* 283, 817–818.
- Ciucci, A., Iafate, E.M., Manzini, S., Giachetti, A., 1997. Mechanism of antiviral action of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU): direct evidence with [¹⁴C]BVDU in herpes simplex virus-infected cells. *Antivir. Chem. Chemother.* 8, 565–571.
- Clive, D., Turner, N.T., Hozier, J., Batson, A.G., Tucker, W.E., 1983. Preclinical toxicology studies with acyclovir: genetic toxicity tests. *Fund. Appl. Toxicol.* 3, 587–602.
- Clive, D., Corey, L., Reichman, R.C., Davis, L.G., Hozier, J.C., 1991. A double-blind, placebo-controlled cytogenetic study of oral acyclovir in patients with recurrent genital herpes. *J. Infect. Dis.* 164, 753–757.
- Coen, D.M., 1992. Molecular aspects of anti-herpesvirus drugs. *Virology* 3, 3–12.
- Cohen, J.L., Boyer, O., Thomas-Vaslin, V., Klatzmann, D., 1999. Suicide gene-mediated modulation of graft-versus-host diseases. *Leuk. Lymphoma* 34, 473–480.
- Combes, R., Balls, M., Curren, R., et al., 1999. Cell transformation assays as predictors of human carcinogenicity. The report and recommendations of ECVAM workshop 39. *ATLA* 27, 745–767.
- Connor, E.M., Sperling, R.S., Gelber, R., Kiselev, P., Scott, G., O'Sullivan, M.J., van Dyke, R., Bey, M., Shearer, W., Jacobson, R.L., et al., 1994. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N. Engl. J. Med.* 331, 1173–1180.
- Culnane, M., Fowler, M., Lee, S.S., McSherry, G., Brady, M., O'Donnell, K., Mofenson, L., Gortmaker, S.L., Shapiro, D.E., Scott, G., Jimenez, E., Moore, E.C., Diaz, C., Flynn, P.M., Cunningham, B., Oleske, J., 1999. Lack of long-term effects of in utero exposure to zidovudine among children born to HIV-infected women. Pediatric AIDS Clinical Trials Group Protocol 219/076 Teams. *J. Am. Med. Assoc.* 281, 151–157.
- Darby, G., 1994. A history of antiherpes research. *Antivir. Chem. Chemother.* 5 (Suppl. 1), 3–9.
- Darby, G., 1995. In search of the perfect antiviral. *Antivir. Chem. Chemother.* 6 (Suppl. 1), 54–63.
- De Clercq, E., 1995. Trend in the development of new antiviral agents for the chemotherapy of infections caused by herpesviruses and retroviruses. *Rev. Med. Virol.* 5, 149–164.
- De Clercq, E., 1996. Therapeutic potential of cidofovir (HPMPC, Vistide) for the treatment of DNA virus (i.e. herpes-, papova-, pox- and adenovirus). *Verh. Koninkl. Acad. Geneesk. Belgie* 58, 19–49.
- De Clercq, E., Cassiman, J.-J., 1986. Mutagenic potential of anti-herpes agents. *Life Sci.* 38, 281–289.
- Degreve, B., Andrei, G., Izquierdo, M., Piette, J., Morin, K., Knaus, E.E., Wiebe, L.I., Basrah, I., Walker, R.T., de Clercq, E., Balzarini, J., 1997. Varicella-zoster virus thymidine kinase gene and antiherpetic pyrimidine nucleoside analogues in a combined gene/chemotherapy treatment for cancer. *Gene Ther.* 4, 1107–1114.
- Diwan, B.A., Riggs, C.W., Logsdon, D., Haines, D.C., Olivero, O.A., Rice, J.M., Yuspa, S.H., Poirier, M.C., Anderson, L.M., 1999. Multiorgan transplacental and neonatal carcinogenicity of 3'-azido-3'-deoxythymidine in mice. *Toxicol. Appl. Pharmacol.* 161, 82–99.
- Earnshaw, D.L., Bacon, T.H., Darlison, S.J., Edmonds, K., Perkins, R.M., Vere Hodge, R.A., 1992. Mode of antiviral action of penciclovir in MRC-5 cells infected with herpes simplex virus (HSV-1), HSV-2, and varicella-zoster virus. *Antimicrob. Agents Chemother.* 36, 2747–2757.

- Fearon, E.R., Vogelstein, B., 1990. A genetic model for colorectal tumorigenesis. *Cell* 61, 759–767.
- Foti, M., Marshalko, S., Schurter, E., Kumar, S., Beardsley, G.P., Schweitzer, B.I., 1997. Solution structure of a DNA decamer containing the antiviral drug ganciclovir: combined use of NMR, restrained molecular dynamics, and full relaxation matrix refinement. *Biochemistry* 36, 5336–5345.
- Glatt, H.R., 1994. Comparison of common gene mutation tests in mammalian cells in culture: a position paper of the GUM commission for the development of guidelines for genotoxicity testing. *Mutat. Res.* 313, 7–20.
- Gonzalez Cid, M., Larripa, I., 1994. Genotoxic activity of azidothymidine (AZT) in in vitro systems. *Mutat. Res.* 321, 113–118.
- Grdina, D.J., Dale, P., Weichselbaum, R., 1992. Protection against A2T-induced mutagenesis at the HGPRT locus in a human cell line by WR-151326. *Int. J. Radiat. Oncol. Biol. Phys.* 22, 813–815.
- Grignet-Debrus, C., Calberg-Bacq, C.-M., 1997. Potential of Varicella zoster virus thymidine kinase as a suicide gene in breast cancer cells. *Gene Ther.* 4, 560–569.
- Grignet-Debrus, C., Cool, V., Baudson, N., Degreve, B., Balzarini, J., de Leval, L., Debrus, S., Velu, T., Calberg-Bacq, C.-M., 2000. Comparative in vitro and in vivo cytotoxic activity of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) and its arabinosyl derivative, (E)-5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil (BVaraU), against tumor cells expressing either the Varicella zoster or the Herpes simplex virus thymidine kinase. *Cancer Gene Ther.* 7, 215–223.
- Hanson, I.C., Antonelli, T.A., Sperling, R.S., Oleske, J.M., Cooper, E., Culnane, M., Fowler, M.G., Kalish, L.A., Lee, S.S., McSherry, G., Mofenson, L., Shapiro, D.E., 1999. Lack of tumors in infants with perinatal HIV-1 exposure and fetal/neonatal exposure to zidovudine. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 20, 463–467.
- Haynes, P., Lambert, T.R., Mitchell, I., de, G., 1996. Comparative in-vivo genotoxicity of antiviral nucleoside analogues; penciclovir, acyclovir, ganciclovir and the xanthine analogue, caffeine, in the mouse bone marrow micronucleus assay. *Mutat. Res.* 369, 65–74.
- Heidelberger, C., Freeman, A.E., Pienta, R.J., Sivak, A., Bertram, J.S., Casto, B.C., Dunkel, V.C., Francis, M.W., Kakunaga, T., Little, J.B., Schechtman, L.M., 1983. Cell transformation by chemical agents – a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 114, 283–385.
- Herbst, A.L., 2000. Behavior of estrogen-associated female genital tract cancer and its relation to neoplasia following intrauterine exposure to diethylstilbestrol (DES). *Gynecol. Oncol.* 76, 147–156.
- Hirota, Y., Arai, Y., Matsumoto, A., Nii, S., Wataya, Y., 1995. Different mechanisms of inhibition of DNA synthesis by of (E)-5-(2-bromovinyl)-2'-deoxyuridine in cells transfected with gene for thymidine kinase of herpes simplex virus type 1 and in cells infected with the virus. *Nucleosides Nucleotides* 14, 2079–2087.
- Hitchcock, M.J.M., Jaffe, H.S., Martin, J.C., Stagg, R.J., 1996. Cidofovir, a new agent with potent anti-herpesvirus activity. *Antivir. Chem. Chemother.* 7, 115–127.
- Ho, H.-T., Woods, K.L., Bronson, J.J., de Boeck, H., Martin, J.C., Hitchcock, M.J.M., 1992. Intracellular metabolism of the antiherpes agent (S)-1-[3-hydroxy-2-(phosphonyl-methoxy)propyl]cytosine. *Molec. Pharmacol.* 41, 197–202.
- Ilsley, D.D., Lee, S.-H., Miller, W.H., Kuchta, R.D., 1995. Acyclic guanosine analogs inhibit DNA polymerases α , δ , and ϵ with very different potencies and have unique mechanisms of action. *Biochemistry* 34, 2504–2510.
- Izquierdo, M., Martin, V., de Felipe, P., Izquierdo, J.M., Perez-Higueras, A., Cortes, M.L., Paz, J.F., Isla, A., Blazquez, M.G., 1996. Human malignant brain tumor response to herpes simplex thymidine kinase (HSVtk)/ganciclovir gene therapy. *Gene Ther.* 3, 491–495.
- Klatzmann, D., Valery, C.A., Bensimon, G., Marro, B., Boyer, O., Mokhtari, K., Diquet, B., Salzmann, J.L., Philippon, J., 1998. A phase I/II study of herpes simplex virus type 1 thymidine kinase “suicide” gene therapy for recurrent glioblastoma. Study group on gene therapy for glioblastoma. *Hum. Gene Ther.* 9, 2595–2604.
- Kulikowski, T., 1994. Structure-activity relationships and conformational features of antiherpetic pyrimidine and purine nucleoside analogues. A review. *Pharm. World Sci.* 16, 127–138.
- Kunz, B.A., Kohalmi, S.E., Kunkel, T.A., Mathews, C.K., McIntosh, E.M., Reidy, J.A., 1994. Deoxyribonucleoside triphosphate levels: a critical factor in the maintenance of genetic stability. *Mutat. Res.* 318, 1–64.
- Mancini, W.R., de Clercq, E., Prusoff, W.H., 1983. The relationship between incorporation of (E)-5-(2-bromovinyl)-2'-deoxyuridine into herpes simplex virus type 1 DNA with virus infectivity and DNA integrity. *J. Biol. Chem.* 258, 792–795.
- Maron, D.M., Ames, B.N., 1983. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* 113, 173–215.
- Marquardt, H., Westendorf, J., de Clercq, E., Marquardt, H., 1985. Potent antiviral 5-(2-bromovinyl)uracil nucleosides are inactive at inducing gene mutations in *Salmonella typhimurium* and V79 Chinese hamster cells and unscheduled DNA synthesis in primary rat hepatocytes. *Carcinogenesis* 6, 1207–1209.
- Marquardt, H., Westendorf, J., Schaefer, A., Boldt, J., de Clercq, E., Marquardt, H., 1988. Mutagenic and antimutagenic effects of 5-substituted 2'-deoxyuridines depending on the nature of the 5-substituent. *Drug Res.* 38, 1820–1824.
- Marshalko, S.J., Schweitzer, B.I., Beardsley, G.P., 1995. Chiral chemical synthesis of DNA containing (S)-9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) and effects on thermal stability, duplex structure, and thermodynamics of duplex formation. *Biochemistry* 34, 9235–9248.
- Martin, J.L., Brown, C.E., Matthews-Davis, N., Reardon, J.E., 1994. Effects of antiviral nucleoside analogs on hu-

- man DNA polymerases and mitochondrial DNA synthesis. *Antimicrob. Agents Chemother.* 38, 2743–2749.
- Meng, Q., Olivero, O.A., Poirier, M.C., Shi, X., Ding, X., Walker, V.E., 2000. Relationships between DNA incorporation, mutant frequencies, and loss of heterozygosity at the TK locus in human lymphoblastoid cells exposed to 3'-azido-3'-deoxythymidine. *Toxicol. Sci.* 54, 322–329.
- Moolten, F.L., 1986. Tumor chemosensitivity conferred by inserted thymidine kinase genes: paradigm for prospective cancer control strategy. *Cancer Res.* 46, 5276–5281.
- Motimaya, A.M., Subramanya, K.S., Curry, P.T., Kitchin, R.M., 1994. Lack of induction of micronuclei by azidothymidine (AZT) in vivo in mouse bone marrow cells. *Environ. Mol. Mutagen.* 23, 74–76.
- Naesens, L., Snoeck, R., Andrei, G., Balzarini, J., Neyts, J., de Clercq, E., 1997. HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogues: a review of their pharmacology and clinical potential in the treatment of viral infections. *Antivir. Chem. Chemother.* 8, 1–23.
- Olivero, O.A., Beland, F.A., Fullerton, N.F., Poirier, M.C., 1994a. Vaginal epithelial DNA damage and expression of preneoplastic markers in mice during chronic dosing with tumorigenic levels of 3'-azido-2',3'-dideoxythymidine. *Cancer Res.* 54, 6235–6242.
- Olivero, O.A., Beland, F.A., Poirier, M.C., 1994b. Immunofluorescent localization and quantitation of 3'-azido-2',3'-dideoxythymidine (AZT) incorporated into chromosomal DNA of human, hamster and mouse cell lines. *Int. J. Oncol.* 4, 49–54.
- Olivero, O.A., Anderson, L.M., Diwan, B.A., Haines, D.C., Harbaugh, S.W., Moskal, T.J., Jones, A.B., Rice, J.M., Riggs, C.W., Logsdon, D., Yuspa, S.H., Poirier, M.C., 1997. Transplacental effects of 3'-azido-2',3'-dideoxythymidine (AZT): tumorigenicity in mice and genotoxicity in mice and monkeys. *J. Natl. Cancer Inst.* 89, 1602–1608.
- Olivero, O.A., Parikka, R., Poirier, M.C., Vähäkangas, K., 1999a. 3'-azido-3'-deoxythymidine (AZT) transplacental perfusion kinetics and DNA incorporation in normal human placentas perfused with AZT. *Mutat. Res.* 428, 41–47.
- Olivero, O.A., Shearer, G.M., Chougnat, C.A., Kovacs, A.A., Landay, A.L., Baker, R., Stek, A.M., Khoury, M.M., Proia, L.A., Kessler, H.A., Sha, B.E., Tarone, R.E., Poirier, M.C., 1999b. Incorporation of zidovudine into leukocyte DNA from HIV-1-positive adults and pregnant woman, and cord blood from infants exposed in utero. *AIDS* 13, 819–825.
- Oshiro, Y., Piper, C.E., Soelster, S.G., Balwierz, P.S., Garriott, M.L., 1992. Genotoxic properties of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU). *Fund. Appl. Toxicol.* 18, 491–498.
- Phillips, M.D., Nascimbeni, B., Tice, R.R., Shelby, M.D., 1991. Induction of micronuclei in mouse bone marrow cells: an evaluation of nucleoside analogues used in the treatment of AIDS. *Environ. Mol. Mutagen.* 18, 168–183.
- Physicians' Desk Reference, 2000. Healthcare Ser. 103 (online version). Micromedex Inc.
- Pizer, L.I., Mitchell, D.H., Bentele, B., Betz, J.L., 1987. A mammalian cell line designed to test the mutagenic activity of anti-herpes nucleosides. *Int. J. Cancer* 40, 114–121.
- Pue, M.A., Benet, L.Z., 1993. Pharmacokinetics of famciclovir in man. *Antivir. Chem. Chemother.* 4 (Suppl. 1), 47–55.
- Ram, Z., Culver, K.W., Oshiro, E.M., Viola, J.J., de Vroom, H.L., Otto, E., Long, Z., Chiang, Y., McGarrity, G.J., Muul, L.M., Katz, D., Blaese, R.M., Oldfield, E.H., 1997. Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat. Med.* 3, 1354–1361.
- Rao, G.N., Collins, B.J., Giles, H.D., Heath, J.E., Foley, J.F., May, R.D., Buckley, L.A., 1996. Carcinogenicity of 2',3'-dideoxycytidine in mice. *Cancer Res.* 56, 4666–4672.
- Reardon, J.E., 1989. Herpes simplex virus type 1 and human DNA polymerase interactions with 2'-deoxyguanosine 5'-triphosphate analogues. *J. Biol. Chem.* 264, 19039–19044.
- Reid, R., Mar, E.-C., Huang, E.-S., Topal, M.D., 1988. Insertion and extension of acyclic, dideoxy, and ara nucleotides by herpesviridae, human α and human β polymerases. *J. Biol. Chem.* 263, 3898–3904.
- Rubsam, L.Z., Davidson, B.L., Shewach, D.S., 1998. Superior cytotoxicity with ganciclovir compared to aciclovir and araT in HSV-TK-expressing cells: a novel paradigm for cell killing. *Cancer Res.* 58, 3873–3882.
- Schilling, B.E., Nelson, D.R., Proctor, J.E., Diamond, S.S., Kaul, S., Hawkins, H.C., 1995. The nonclinical toxicologic profile of stavudine. *Curr. Therapeut. Res.* 56, 201–218.
- Shafik, H.M., Nokta, M.A., Pollard, R.B., 1991. Recombinant human interferon beta ser protects against zidovudine-induced genetic damage in AIDS patients. *Antiviral Res.* 16, 205–212.
- Shand, N., Weber, F., Mariani, L., Bernstein, M., Gianella-Borradori, A., Long, Z., Sorensen, A.G., Barbier, N., 1999. A phase 1–2 clinical trial of gene therapy for recurrent glioblastoma multiforme by tumor transduction with the herpes simplex thymidine kinase gene followed by ganciclovir. GLI328 European-Canadian study group. *Hum. Gene Ther.* 10, 2325–2335.
- Sorsa, M., Anderson, D., 1996. Monitoring of occupational exposure to cytostatic anticancer agents. *Mutat. Res.* 355, 253–261.
- St. Clair, M.H., Lambe, C.U., Furman, P.A., 1987. Inhibition by ganciclovir of cell growth and DNA synthesis of cells biochemically transformed with herpesvirus genetic information. *Antimicrob. Agents Chemother.* 31, 844–849.
- Sussman, H.E., Olivero, O.A., Meng, Q., Pietras, S.M., Poirier, M.C., O'Neill, J.P., Finette, B.A., Bauer, M.J., Walker, V.E., 1999. Genotoxicity of 3'-azido-3'-deoxythymidine in the human lymphoblastoid cell line, TK6: relationships between DNA incorporation, mutant frequency, and spectrum of deletion mutations in HPRT. *Mutat. Res.* 429, 249–259.
- Thust, R., 1976. Screening of potential chemical carcinogens by means of mammalian cells in vitro. *Arch. Geschwulstforsch.* 46, 538–548.

- Thust, R., Schacke, M., Wutzler, P., 1996. Cytogenetic genotoxicity of antiherpes virostatics in Chinese hamster V79-E cells. I. Purine nucleoside analogues. *Antiviral Res.* 31, 105–113.
- Thust, R., Tomicic, M., Klöcking, R., Voutilainen, N., Wutzler, P., Kaina, B., 2000a. Comparison of the genotoxic and apoptosis-inducing properties of ganciclovir and penciclovir in CHO cells transfected with the thymidine kinase gene of HSV-1: implications for gene therapeutic approaches. *Cancer Gene Ther.* 7, 107–117.
- Thust, R., Tomicic, M., Klöcking, R., Wutzler, P., Kaina, B., 2000b. Cytogenetic genotoxicity of anti-herpes purine nucleoside analogues in CHO cells expressing the thymidine kinase gene of herpes simplex virus type 1: comparison of ganciclovir, penciclovir and aciclovir. *Mutagenesis* 15, 177–184.
- Tomicic, M., Thust, R., Kaina, B. 2000. Caspase-driven cleavage of Bcl-2 is a major pro-apoptotic event induced by the antiviral drug ganciclovir, submitted for publication.
- Tucker, W.E., Krasny, H.C., de Miranda, P., Goldenthal, E.I., Elion, G.B., Hajian, G., Szczech, G.M., 1983. Preclinical studies with acyclovir: carcinogenicity bioassays and chronic toxicity tests. *Fund. Appl. Toxicol.* 3, 579–586.
- Vazquez-Padua, M.A., Starnes, M.C., Cheng, Y., 1990. Incorporation of 3'-azido-3'-deoxythymidine into cellular DNA and its removal in a human leukemic cell line. *Cancer Comm.* 2, 55–62.
- Vere Hodge, R.A., 1993. Famciclovir and penciclovir. The mode of action of famciclovir including its conversion to penciclovir. *Antivir. Chem. Chemother.* 4, 67–84.
- Vere Hodge, R.A., Cheng, Y.-C., 1993. The mode of action of penciclovir. *Antivir. Chem. Chemother.* 4 (Suppl. 1), 13–24.
- Wright, G.E., Brown, N.C., 1990. Deoxyribonucleotide analogs as inhibitors of DNA polymerases. *Pharmacol. Ther.* 47, 447–497.