Short Conceptual Overview

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Deoxyadenosine family: improved synthesis, DNA damage and repair, analogs as drugs

Abstract: Improved synthesis of 2′-deoxyadenosine using *Escherichia coli* overexpressing some enzymes and gram-scale chemical synthesis of 2′-deoxynucleoside 5′-triphosphates reported recently are described in this review. Other topics include DNA damage induced by chromium(VI), Fenton chemistry, photoinduction with lumazine, or by ultrasound in neutral solution; 8,5′-cyclo-2′-deoxyadenosine isomers as potential biomarkers; and a recapitulation of purine 5′,8-cyclonucleoside studies. The mutagenicities of some products generated by oxidizing 2′-deoxyadenosine 5′-triphosphate, nucleotide pool sanitization, and translesion synthesis are also reviewed. Characterizing cross-linking between nucleosides in opposite strands of DNA and endonuclease V-mediated deoxyinosine excision repair are discussed. The use of purine nucleoside analogs in the treatment of rarer chronic lymphoid leukemias is reviewed. Some analogs at the C8 position induced delayed polymerization arrest during HIV-1 reverse transcription. The susceptibility of clinically metronidazole-resistant *Trichomonas vaginalis* to two analogs, toyocamycin and 2-fluoro-2′-deoxyadenosine, were tested *in vitro*. GS-9148, a dAMP analog, was translocated to the priming site in a complex with reverse transcriptase and double-stranded DNA to gain insight into the mechanism of reverse transcriptase inhibition.

Keywords: 2′-deoxyadenosine analogs as drugs; DNA damage; DNA repair; improved synthesis.

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Introduction

Long known as one of the four building blocks of all genetic material or DNA, research continues on various aspects of 2′-deoxyadenosine and its possible uses. The present review aims to summarize the significant advances in 18 recent articles on 2′-deoxyadenosine covering its synthesis, studies in DNA damage and DNA repair, and its analogs being used as drugs for various diseases (1–18). The articles were selected on the basis of their novelty, importance in advancing fundamental knowledge or applications to human health, or both.

The development of novel processes that are convenient and environment friendly has been accelerated since the discovery of nucleoside phosphorylases, as an alternative to *de novo* pathways of purine and pyrimidine biosynthesis. Xiong et al. (1) have cloned three different nucleoside phosphorylases into a recombinant *Escherichia coli* strain and used thymidine and adenine as the more inexpensive starting materials to make 2′-deoxyadenosine without using isopropyl β-D-thiogalactoside (IPTG). Horinouchi et al. (2) used glucose, acetaldehyde, and nucleobase to produce the four deoxynucleosides using the coexpression of three enzymes in one *E. coli* strain. Kore et al. (3) provided a general improved procedure for the synthesis of deoxynucleoside triphosphates by organic chemistry.

Arakawa et al. (4) studied the effect of chromium in three valence states regarding DNA adduct formation and oxidative damage, both in lung cells and *in vitro*, focusing on exons 5 and 7 of the *p53* gene, suggesting their role in carcinogenesis. Bhattacharjee et al. (5) reported the detection and imaging of free radical DNA in cells induced by Fenton chemistry as seen by various methods. Chattopadhyaya and Goswami (6) studied in detail the effect of exposing various 2′-deoxyadenosine family substrates to iron-mediated Fenton reactions, and how their outcomes depend on the nature of the substrate, polymeric state, and reaction conditions. Jaruga et al. (7) used urine samples from atherosclerosis patients and healthy individuals to show that the two groups exhibit different levels of 8,5′-cyclo-2′-deoxyadenosine (*R* & *S*) as a possible marker of disease. Purine 5′,8-cyclonucleoside lesions have been reviewed by Chatgilialoglu et al. (8). Karwowski et al.'s (9) theoretical quantum mechanical study explains the small frequency of the 8,5′-cyclo-2′-deoxyadenosine lesion. Denofrio et al. (10) studied lumazine-sensitized

oxidation of 2′-deoxyadenosine 5′-monophosphate important for human skin cancers. Suzuki et al. (11) studied how prolonged exposure to 42-kHz ultrasound can damage deoxynucleosides and how the presence of various halide ions influence the extent of the nucleoside damage.

The mutagenicities of DNA damage products existing within the dNTP pool in living cells, the roles of pool sanitization, and DNA repair enzymes were reviewed by Kamiya (12). Peng et al. (13) studied nucleotide excision repair of a DNA interstrand cross-link involving an A-T base pair. Lee et al. (14) studied endonuclease V-mediated deoxyinosine excision repair *in vitro*.

Robak and Robak (15) reviewed purine nucleoside analogs for the treatment of rarer indolent lymphoid leukemias. Vivet-Boudou et al. (16) studied how C8-modified 2′-deoxyadenosine analogs induce delayed polymerization arrest in HIV-1 reverse transcriptase. Wright et al. (17) studied some metronidazole-resistant isolates of *Trichomonas vaginalis*, finding that some such strains were susceptible to toyocamycin and 2-fluoro-2′-deoxyadenosine, two 2′-deoxyadenosine analogs, whereas some are moderately susceptible. GS-9148 is a dAMP analog having antiviral activity against drug-resistant HIV. Lansdon et al. (18) obtained crystal structures of HIV-1 reverse transcriptase bound to double-stranded DNA, ternary complexes with either GS-9148-diphosphate or dATP, and a post-incorporation structure with GS-9148 translocated to the priming site.

Improved synthesis of 2′-deoxyadenosine and its derivatives

Nucleoside analogs are used for the treatment of viral diseases and cancer. The preparation of such analogs often uses 2′-deoxyadenosine as an intermediate. Hence, the development of convenient and environmentally friendly methods for 2′-deoxyadenosine synthesis is important. Xiong et al. (1) described the improved synthesis of 2′-deoxyadenosine and 5-methyluridine by cloning three different nucleoside phosphorylases – purine nucleoside phosphorylase, uridine phosphorylase, and thymidine phosphorylase – into a recombinant *E. coli* strain, one at a time, using whole cells, thus avoiding isolation and purification of those enzymes. Instead of the more expensive IPTG method of induction, they have employed the auto-induction ZYM Fe 5052 medium successfully. The ZYM Fe 5052 medium contained 100 μM $FeCl_3$, 2 mM $MgSO_4$, 25 mM Na_2HPO_4, 25 mM KH_2PO_4, and 5 mM Na_2SO_4. Genes encoding the three aforementioned phosphorylases were cloned from *E. coli* K12 genomic DNA and separately recombined into the plasmid pET-28a(+), and the resulting plasmids were then transformed into *E. coli* BL21 (DE3). Cells producing thymidine phosphorylase and purine nucleoside phosphorylase, as culture mixtures, would be needed for the reaction

β-Thymidine+adenine→thymine+2′-deoxyadenosine,

whereas cells producing uridine phosphorylase and thymidine phosphorylase, as culture mixtures, would be needed for the reaction

Uridine+thymine→uracil+5-methyluridine.

In detail, a 0.6-ml culture mixture would contain 0.3 ml each of the two appropriate *E. coli* strains, grown in the ZYM Fe 5052 medium for 8 h, then added to 9.4 ml of the appropriate nucleoside-base reaction mixture (mixture 1 contained 60 mM β-thymidine and 30 mM adenine, whereas mixture 2 contained 60 mM uridine and 60 mM thymine, both also containing 50 mM potassium phosphate buffer, pH 8.0). Only 60 min of incubation was required to reach a 96% conversion yield of 2′-deoxyadenosine. The yield obtained for 5-methyluridine, 54%, was not as high, as the thermodynamic equilibrium of thymidine phosphorylase is shifted toward nucleoside cleavage. Being simpler than pre-existing methods, the work (1) has a potential for the industrial production of nucleoside analogs.

Horinouchi et al. (2) add that the need for deoxynucleosides will increase due to the rising popularity of polymerase chain reaction. Although, currently, hydrolyzed salmon and herring sperm DNA are the major sources of deoxynucleosides and deoxynucleoside 5′-phosphates, they will not be able to meet future requirements. Microbial processes could remove this deficiency in deoxynucleoside supply, with inexpensive starting materials. Horinouchi et al. (2) focused on the enzymatic production of deoxynucleosides through the reversible deoxynucleoside degradation pathway catalyzed by deoxyriboaldolase and phosphopentomutase coexpressed by one *E. coli* strain and commercial nucleoside phosphorylase. Their one-pot synthesis of deoxynucleosides uses commercially available baker's yeast to generate energy-rich molecules from inexpensive glucose, acetaldehyde, and a nucleobase, along with the three aforementioned enzymes. To determine the optimal conditions, the reaction mixture was varied as follows: volume 1.5–10.5 ml and including 600 mM glucose, 0–900 mM acetaldehyde, 0–100 mM adenine, 26 mM $MgSO_4.7H_2O$, 0–80 mM potassium phosphate buffer (pH 7.0), 1.0 mM $MnCl_2.4H_2O$, 0.1 mM glucose

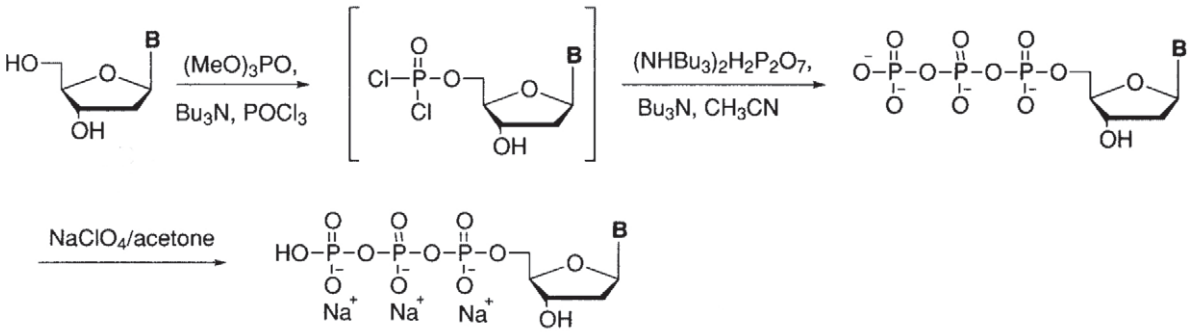

Figure 1 Preparation of purine-specific dNTPs, where B represents adenine or guanine.The procedures for preparing the pyrimidine dNTPs are very similar to the above, except that Bu_3N added in the first reaction for the purine nucleosides is not required for dCTP and replaced by proton sponge for TTP and dUTP, with the corresponding 2′-deoxynucleosides as starting materials. Reproduced from Kore et al. (3), copyright 2012, with permission of Current Protocols & John Wiley & Sons, Inc.

1,6-diphosphate, 0.4% (v/v) polyoxyethylenelaurylamine, 1.0% (v/v) xylene, 10 mM adenosine, 2–30% (w/v) acetone-dried yeast, 2.5–22.8% (w/v) wet *E. coli* cells coexpressing the two enzymes, and 5–60 U/ml commercial nucleoside phosphorylase. With adenine as the nucleobase, not 2′-deoxyadenosine, but its deaminated product, 2′-deoxyinosine was produced. The production increased with increasing concentrations of the yeast cells up to 4% (w/v) and that of wet *E. coli* cells up to 15% (w/v), then decreased. Similarly, the optimal concentration of the nucleoside phosphorylase was found to be 30 U/ml. It was also found that baker's yeast should be fed into the reaction vessel after 20 h, although the other two catalysts need not be so added during reactions lasting 30 h. It was also possible to produce all four deoxynucleosides starting from 2′-deoxyinosine using the deoxyribosyltransferase activity of *Lactobacillus helveticus* JCM 1008. The study shows that an energy-requiring bioprocess can be combined with an energy-generating one for the production of 2′-deoxynucleosides.

A reliable, simple gram-scale protocol for the synthesis of 2′-deoxynucleoside 5′-*O*-triphosphates is challenging because of the multiple functional groups within nucleosides, requiring protection and deprotection. Kore et al. (3) have provided a general improved procedure for the synthesis of deoxynucleoside triphosphates such as dGTP, dATP, dCTP, TTP, and dUTP in yields between 65% and 70% and high purity exceeding 99%. Among older alternative approaches, there was one chemoenzymatic method and a second using solid support, but both of these work at the milligram scale (3). A third one-pot method using a phosphitylating reagent results in lower yields (19–46%). The one-pot synthetic procedures of Kore et al. have been optimized with regard to solvent, reaction times, temperature, mode of addition, and ratio of reagents for each of the dNTPs and are likely to be accepted as a general protocol that could be slightly modified for other sugar and base-modified nucleotide triphosphates (Figure 1).

DNA damage involving 2′-deoxyadenosine

Exposure to chromium, a ubiquitous contaminant, causes lung cancer. Before Arakawa et al.'s study (4), it was known that DNA damage affects p53 mutational patterns in lung cancer for >50% patients who were smokers. In particular, the *p53* mutations in cigarette smoke-related lung cancers occur primarily at guanine residues, whereas for chromium exposure, it was distributed at both adenine and guanine residues. To further understand how chromium damages DNA, Arakawa et al. (4) treated DNA fragments with Cr(III), Cr(V), and Cr(VI), and found that (i) Cr(VI) and Cr(V) can form adducts with both adenines and guanines and that Cr(III) forms adducts with guanines only, and (ii) Cr(VI) and Cr(V) can induce oxidative DNA damage. Using UvrABC, formamidopyrimidine glycosylase, and the ligation-induced PCR method, the bulky DNA adducts and oxidative DNA damage were mapped in their study. What may be most important is that many of these adducts or oxidative damage products were not repaired after 24 h of post-treatment incubation, whereas normal oxidative damage induced by hydrogen peroxide could be repaired. In addition to lung cells, the authors experimented with exons 5 and 7 of *p53 in vitro* and found the same results. Interestingly, Cr(V) produces more bands compared with Cr(VI) and Cr(III) (Figure 2). Taken together, their results suggest that inside of cells, Cr(VI) and Cr(V) interact with adenines and guanines of genomic

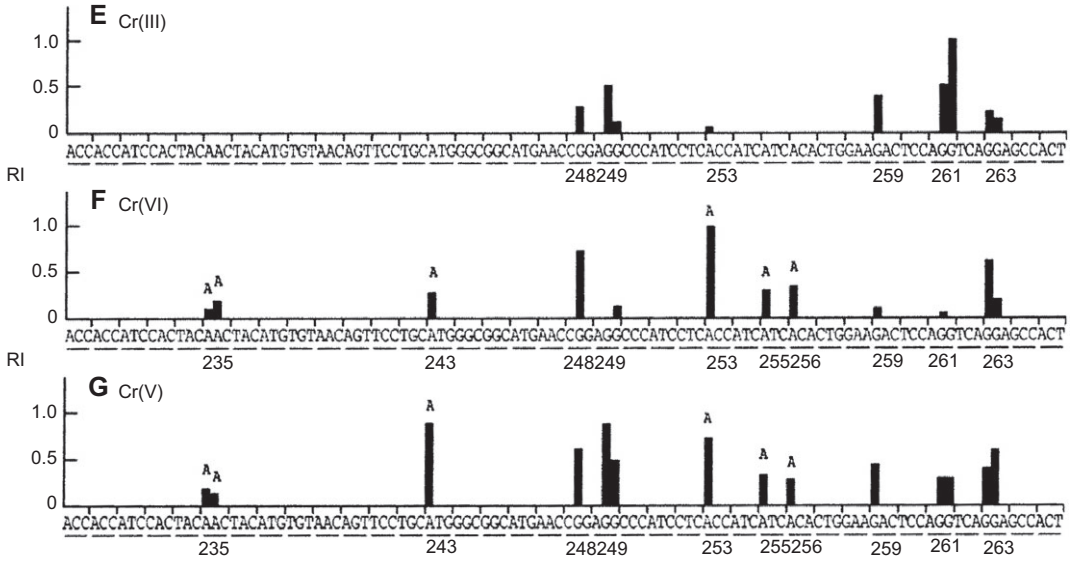

Figure 2 Reprinted with permission from Arakawa et al. (4), copyright 2012 Oxford University Press. The figure depicts the distribution of Cr(III), Cr(VI), and Cr(V) adducts induced in exon 7 of the *p53* gene in human lung cells. It is found that Cr(III) forms adducts at guanines only. The patterns of Cr(VI) and Cr(V) are almost similar with the latter showing a bit more adduct formation; the adducts are formed both at guanines and adenines, particularly at -CA- or -CAA- sequences.

DNA, cause oxidative and bulky damage that are poorly repaired, and trigger lung carcinogenesis (4).

DNA-centered radicals are implicated as a root cause of carcinogenesis and aging through DNA damage. The spin-trapping technique reacts the DNA-centered radical with the double bond of the trap molecule, producing a relatively stable adduct that may be converted to a nitrone, a more stable product. Bhattacharjee et al. (5) used a method that detects the stable nitrone coupled to an antibody-based assay following damage produced by the Fenton chemistry. Electron spin resonance studies were carried out for an equimolar mixture of all four deoxynucleosides by treatment with 300 μM $CuCl_2$, 100 μM H_2O_2, and 100 mM 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO); however, radicals were detected apparently only for 2′-deoxyadenosine (5). RAW 264.7 macrophage cells were treated with the same Fenton chemistry and DNA extracted. To determine the localization of the nitrone adducts, the cytosolic and nuclear fractions were separated, and clearly the latter had a higher level of DMPO nitrone adducts. No DMPO-protein adducts were noticed in Western blots. In parallel, confocal microscopy was also used to confirm the nuclear localization of the adducts. Repair of DMPO nitrone adducts also occurs predominantly through the base excision repair pathway (5).

Hydroxyl radicals generated by *in vivo* Fenton reactions can cause oxidative DNA damage. Chattopadhyaya and Goswami (6) studied the effect of exposing 2′-deoxyadenosine, 5′-dAMP, 3′-dAMP, dApA, $dA(pdA)_{19}$, and poly(dA):oligo(dT) to iron/H_2O_2. The substrate nucleoside concentration of 1 mM, 2 mM H_2O_2, 1 mM Fe^{2+}, or 1 mM Fe^{3+}/2 mM NADH was used, so that an equal number of moles of ·OH was produced. Post-reaction analysis was done by reverse-phase high-performance liquid chromatography (HPLC) after appropriate enzyme treatments as needed, and the eluates were characterized by their HPLC retention times, UV absorption spectra, and/or the use of specifically radiolabeled substrates and fast atom bombardment mass spectrometry. Out of the 20 products arising from the reaction, 16 have been identified and four anomers proposed by comparison with earlier gamma radiation studies. Two new dimeric adducts arising from the generation of hydroxylamine at N7 and its subsequent condensation with two known sugar damage products containing aldehydes, dR-adenine-N1-oxide, and two isomers of 2′-deoxyribose formamidopyrimidine (dR-FAPy) arising from radical attacks at C4 and C5, may be considered novel in their study. Ethanol could efficiently scavenge Fenton reaction-induced damage in case of 2′-deoxyadenosine, which has no phosphate to bind the iron. Here, damage is reduced by a factor of 27 by using 100 mM ethanol. However, in case of substrates containing terminal phosphate groups or phosphodiester linkages, the scavenging effect of ethanol is much smaller or nil. It is proposed that the iron binds to the phosphate group and generates the radical in its vicinity, making it difficult to eliminate. The total damage produced with Fe^{3+}/NADH/H_2O_2 is slightly less compared with that with Fe^{2+}/H_2O_2, for 2′-deoxyadenosine, 5′-dAMP, 3′-dAMP,

dApA, and $dA(pdA)_{19}$. dR-FAPy is the major product from poly(dA):oligo(dT) using $Fe^{2}+/H_2O_2$ constituting 30% of the damage, whereas in 2′-deoxyadenosine, it constituted only 16% of the damage. N_2 flushing before the Fenton reaction had little or no effect on the extent or nature of the products formed (6).

Over the past two decades, 8-hydroxy-2′-deoxyguanosine and 8-hydroxy-guanine have been monitored as potential biomarkers for disease risk assessment, disease progression, and efficacy of therapeutic drugs, as following DNA repair, lesions may be excreted into blood and then to urine. Most of the DNA damage products are repaired through base excision repair; however, some, such as 8,5′-cyclopurine 2′-deoxynucleosides, are repaired through nucleotide excision repair. Jaruga et al. (7) used urine samples from 22 patients who underwent carotid endarterectomy and 22 healthy individuals without any symptoms of atherosclerosis, measuring the concentration of the two isomers of 8,5′-cyclo-2′-deoxyadenosine (*R* & *S*) and 8-hydroxy-2′-deoxyguanosine by liquid chromatography-tandem mass spectrometry with isotope dilution, and 8-hydroxy-guanine by liquid chromatography coupled with gas chromatography and mass spectrometry. They also measured the creatinine levels and plotted the ratio of these damage products to creatinine. They found that the 8,5′-cyclo-2′-deoxyadenosine isomers were present in the patients at significantly higher levels compared with the healthy individuals. Although the ratio of 8-hydroxy-2′-deoxyguanosine was also higher with the patients, and almost equal for 8-hydroxy-guanine, the data were more significant in the case of the 8,5′-cyclo-2′-deoxyadenosine isomers. The authors took this higher level as indicative of excessive oxidative stress and suggested that they may even play a causative role in atherosclerosis (7).

The chemistry and biology, especially the former, of purine 5′,8-cyclonucleoside lesions have been reviewed by Chatgilialoglu et al. (8). Although most DNA damage occurs through hydroxyl radical attack on the bases, a minority of these radicals attack the deoxyribose and are thought to abstract a hydrogen atom in the process. When this occurs at the C5′ atom, the purine 5′,8-cyclonucleosides are formed. Among chemistry topics, the authors discuss the structural features and stabilities of the diastereomers, reaction rate constants, synthesis methods, selective generation of the C5′ radicals and cyclization, and synthesis of oligomers containing cyclonucleoside lesions. Among biological features, the review describes two earlier studies that showed that these lesions cannot be repaired by base excision repair but can be repaired by nucleotide excision repair. How certain DNA polymerase activities and the binding of certain transcription factors are affected by the presence of these cyclonucleoside lesions was also recapitulated (8). At the end, there is a discussion on quantitation of purine 5′,8-cyclonucleoside lesions, regarding the amount of damage, the degree of prevalence of the R isomer over the S isomer, and dissolved gases. Increasing concentrations of O_2 reduced the amount of the purine cyclic damage products (8).

An elaborate quantum chemical study about the possible reaction modes of radicals generated from 2′-deoxyadenosine, leading to the formation of the 8,5′-cyclo-2′-deoxyadenosine isomers, was carried out by Karwowski et al. (9). They found that the process of intermolecular cyclization requires the proximity of C5′ and C8, which is only afforded by the C4′-exo conformation of the sugar moiety. This sugar conformation, however, has high energy and therefore a low population among the molecules; hence, the amount of formation of the 8,5′-cyclo-2′-deoxyadenosine isomers are expected to be equally small. The experimental results of Chattopadhyaya and Goswami (6) using Fenton reaction-mediated damage verify this expectation as they found that the amount of the 8,5′-cyclo-2′-deoxyadenosine (*R*) went down from ~0.02 to ~0.0006 in changing the substrate from 2′-deoxyadenosine to poly(dA) in duplex form. In 2′-deoxyadenosine, it may still be possible to sample the high energy C4′-exo form, but it is even more unlikely in the B-form duplex, where the C2′-endo form is known to predominate.

Lumazine derivatives are present in cells, as 6,7-dimethyl-8-ribityllumazine is the biosynthetic precursor of riboflavin (vitamin B2). Riboflavin is further the precursor of flavin mononucleotide and flavin adenine dinucleotide. Lumazines are those derived from pteridine-2,4(1,3*H*)-dione and are members of the pteridine family. As photosensitized reactions are implicated in human skin cancers, Denofrio et al. (10) have studied the oxidation of 2′-deoxyadenosine 5′-monophosphate photosensitized by lumazine in aqueous solution under UV-A (320–400 nm) and UV-B (280–300 nm) radiations. The experiments were carried out in two pH ranges, 5.0–5.8, where lumazine is predominantly in the acidic form, and 10.2–10.7, where it is predominantly in the basic form. In the absence of radiation, it was verified that simple incubation in the dark produced no changes. Little spectral changes occurred with the basic form of lumazine. Significant changes in the absorption spectra occurred with UV exposure in acidic pH. The amount of 2′-deoxyadenosine 5′-monophosphate was reduced with irradiation time, chromatography showed the appearance of mostly polar products, and the dissolved O_2 concentration decreased and H_2O_2 was generated (10). Their mechanistic analysis indicates that lumazine-sensitized oxidation of 2′-deoxyadenosine

5′-monophosphate does not involve $^1O_2$ as an intermediate. Rather, the excitation of lumazine is followed by an electron transfer from dAMP to the lumazine triplet excited state, leading to the formation of the corresponding ion radicals. Negatively charged lumazine donates this charge to oxygen, converting it to superoxide anion, which can give rise to H_2O_2. It was previously known that adenine does not react with singlet oxygen, but the authors undertook a detailed kinetic analysis to rule it out (10).

Ultrasound can penetrate deep into tissues and produce physical and chemical effects, including homolysis of water molecules, thus generating radicals. Suzuki et al. (11) studied the effects of chloride, bromide, and iodide upon the decomposition of all four deoxynucleosides in solution induced by 42-kHz ultrasound in neutral solution. Their starting mixture was 100 μM in each of the four deoxynucleosides in 100 mM phosphate buffer, pH 7.4. After irradiation of a small volume in an ultrasound bath at 37°C, reverse-phase HPLC showed the gradual disappearance of the nucleosides with irradiation time, and appearance of products including free bases. The effect of adding 1 μM to 100 mM of NaCl, NaBr, and NaI to the system was investigated. Whereas NaCl had no effect on the decomposition, NaBr at concentrations above 1 mM suppressed it, although with 100 mM some damage still occurred. NaI was slightly better than NaBr in suppressing nucleoside damage. The nucleoside damage was also studied using a Fenton reaction adding 5 mM $FeSO_4$ and 10 mM H_2O_2. Here, the addition of NaCl had no effect, whereas that of NaBr showed some suppression and NaI showed complete suppression. These data were then rationalized in terms of standard oxidation potentials of the deoxynucleosides and those of the halides. Essentially, the hydroxyl radicals produced reacted with the halide anions giving halide radicals, which in turn resulted in dihalide radical anions (11). Addition of bromide through certain food additives can increase its plasma concentration to check the nucleoside decomposition, but not completely.

DNA repair involving 2′-deoxyadenosine

DNA precursors such as the deoxynucleoside triphosphates are also subject to oxidative damage and could lead to mutagenesis (12). Once a damaged nucleotide becomes incorporated into DNA, it could lead to an incorporation of a different nucleoside in the opposing strand compared with the original one. Some damage products such as 8-hydroxy-2′-deoxyguanosine and 2-hydroxy-2′-deoxyadenosine (also called 2′-deoxyisoguanosine, arising from 2′-deoxyadenosine) are known to cause transversion mutations frequently in bacterial cells; however, the mutagenic potential of the latter was limited in mammalian cells, as reported by Kamiya (12). Other damage products appear not to be mutagenic. *Escherichia coli* DNA polymerase IV (DinB) seems to facilitate the incorporation of 2-hydroxy-2′-deoxyadenosine and the *UmuDC* gene products play suppressive roles, whereas Pol V suppresses the mutagenesis with this product and 8-hydroxy-2′-deoxyguanosine. In humans, the translesion synthesis DNA polymerases σ and ζ and Rev I, but not DNA polymerase ι, are involved in the misincorporation of 8-hydroxy-2′-deoxyguanosine opposite 2′-deoxyadenosine. *Escherichia coli* MutT, Orf17 (NudB), and Orf135 (NudG) proteins degrade the nucleoside diphosphates and triphosphates to the corresponding monophosphates; the human NUDT5 also degrades nucleoside diphosphates to their monophosphates. It was found that these act on the damaged nucleoside triphosphates or diphosphates, thus reducing their mutagenicity. *Escherichia coli* MutY protein and endonucleases III and VIII do not seem to defend against 2-hydroxy-2′-deoxyadenosine. The enzymes that thus prevent or reduce mutagenesis are termed nucleotide pool sanitization enzymes, as a first defense against damaged precursors and most of the DNA repair enzymes do not prevent the induced mutations (12).

Peng et al. (13) studied nucleotide excision repair of a DNA interstrand cross-link that produces single- and double-strand breaks. The radical resulting from a hydrogen abstraction from the thymidine methyl group forms interstrand cross-links with the opposing 2′-deoxyadenosine. The nucleotide excision repair pathway participates in correcting the interstrand cross-links. In bacteria, this is carried out by the three-component UvrABC system, whose detailed mechanism is as follows. The UvrA dimer recruits UvrB to the lesion, which in turn induces a conformational change in the duplex and binds the actual endonuclease, UvrC. Hydrolysis of the cross-linked substrates typically occur at the eighth or ninth phosphodiester on the 5′ side of the lesion and at the third to fifth phosphodiester on the 3′-side of the lesion (Figure 3). Oligonucleotides containing the cross-link precursor were synthesized by Peng et al. (13) and the cross-link formed with 45–65% yield using $NaIO_4$ as an oxidant. The cross-linked DNA was purified by denaturing PAGE, desalted, and rehybridized before UvrABC experiments. The preference for UvrABC incision was on the strand containing the 2′-deoxyadenosine of the interstrand cross-link, although

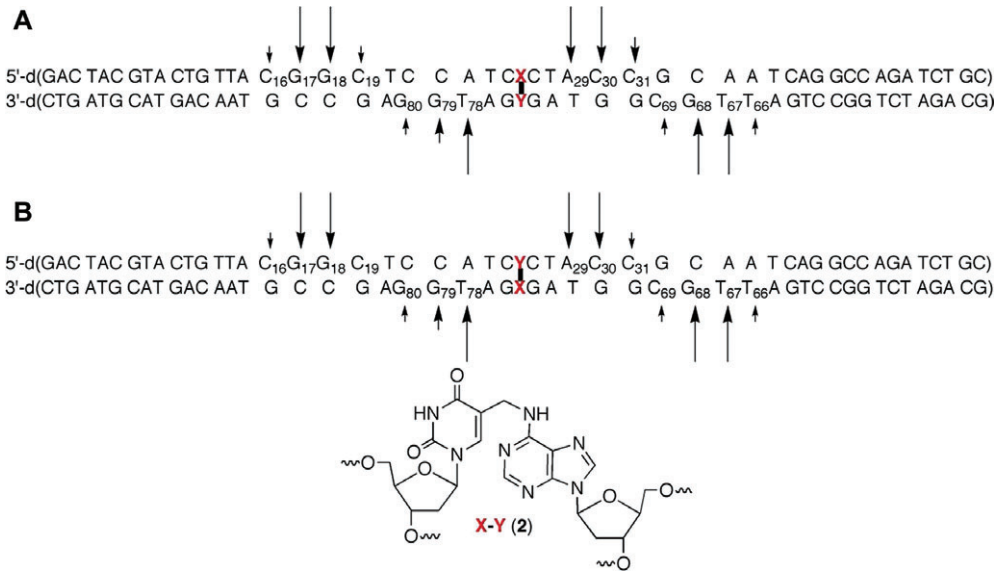

Figure 3 Histograms of UvrABC incision data for the strand containing modified thymidine (X) and the strand containing cross-linked 2′-deoxyadenosine (Y) both labeled at their 3′ termini.The histograms compare relative cleavage intensities within a strand but not relative to the complementary strand – the Y-containing strand was generally cleaved faster. In part A, X is flanked by two 2′-deoxycytidines in the sequence, whereas in part B, X is flanked by two 2′-deoxyguanosines. Reprinted with permission from Peng et al. (13), copyright 2010 American Chemical Society.

the other strand was also cleaved. Double-strand breaks were also observed for about a quarter of the substrate molecules. The authors claim uniqueness for their results for two reasons – the cross-link formed within a Watson-Crick A-T base pair, and the production of the double-strand breaks produced by the UvrABC system.

Lee et al. (14) studied endonuclease V-mediated deoxyinosine excision repair *in vitro*. Deoxyinosine can arise from deamination of deoxyadenosine. It can be repaired by endonuclease V in *E. coli*, a well-studied enzyme. Lee et al. (14) developed a functional assay with a deoxyinosine embedded in a restriction endonuclease recognition site paired with 2′-deoxyguanosine in the opposing strand. When such a substrate is exposed to bacterial cell-free extracts, restriction endonuclease cleavage is used as a diagnostic tool for the excision repair. The authors found that endonuclease V, DNA polymerase I, and DNA ligase, along with the four deoxynucleoside triphosphates, are sufficient to correct the designed substrate. The authors used a soluble endonuclease V fusion protein instead of simple endonuclease V, which is less soluble. Site-specific incision of the deoxyinosine-containing heteroduplex was observed to occur at the second phosphodiester bond 3′ to the deoxyinosine. The subsequent repair is carried out by the 3′–5′ exonuclease activity of DNA polymerase I, which generates a small gap of at least two nucleotides. Later, the gap is filled by DNA polymerase I and sealed by DNA ligase.

2′-Deoxyadenosine analogs as drugs

A recent review article by Robak and Robak (15) summarizes the mechanism of action, pharmacokinetics, pharmacological properties and clinical activity, and toxicity of purine nucleoside analogs for the treatment of rarer indolent lymphoid leukemias, scrutinizing original articles from 1980 to December 2011. The purine nucleoside analogs compete with natural nucleosides during DNA and RNA synthesis and as inhibitors of key cell enzymes. The pharmacology and pharmacokinetics of several purine analogs such as cladribine, fludarabine, and clofarabine related to 2′-deoxyadenosine, whereas others such as nelarabine and forodesine related to 2′-deoxyguanosine are presented (15). Fludarabine can be administered as an infusion, injection, or orally; it is effective for chronic lymphocytic leukemias and low-grade B- and T-cell non-Hodgkin's lymphomas. Cladribine suppresses DNA synthesis and induces DNA damage, which eventually leads to apoptosis; administered intravenously, orally, or as injections, it is effective against a wide range of leukemias. Clofarabine has the same modified adenine base chlorinated at the 2-position as in cladribine, but has an additional fluorine atom bound to C2′ of the sugar; it is a third-line treatment for refractory leukemias, acute lymphoblastic leukemia, and hematopoietic stem cell

transplantation. Pentostatin has a seven-membered ring instead of the six-membered ring of a purine base and is the drug of choice currently for hairy cell leukemias. Nelarabine has a methoxy group at the O6 position of deoxyguanosine and an additional OH group at C2′; it is recommended for T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma or in case of relapse after 2 chemotherapy treatments. Forodesine has modifications both in the base and the sugar relative to deoxyguanosine; it is used for relapsed and refractory B- and T-cell leukemias and some others (15). The later part of the review deals with hairy cell leukemia, hairy cell leukemia variant, prolymphocytic leukemia, large granular lymphocyte leukemia, adult T-cell leukemia/lymphoma, and how each of the seven aforementioned purine nucleoside analogs fared as reported in previous studies.

Drugs for treating HIV-1, generally reverse transcriptase inhibitors, may be nucleoside/nucleotide based or of the non-nucleoside variety. All inhibitors of the first category mimic deoxynucleoside triphosphates, and once incorporated through the action of the reverse transcriptase in the growing DNA chain, act as chain terminators. Whereas all HIV-1 nucleoside analogs in clinical use or clinical trial involve ribose modifications, Vivet-Boudou et al. (16) designed and studied nucleosides with a natural deoxyribose and adenine modified at C8. Resistance to these inhibitors occurs through decreased incorporation efficiency of the drug analog, or excision of the chain terminator at the end of the DNA chain by a reverse activity of the transcriptase (phosphorolysis); hence, the search for new analogs that may act in alternate modes. One class of inhibitors would get incorporated by reverse transcriptase but will not block DNA synthesis until a few more nucleotides have been added to the chain, and resist excision. These are called ‘delayed chain terminators’. Vivet-Boudou et al. (16) studied 11 such modifications at C8, most of them inducing delayed chain termination *in vitro* and one has moderate anti-HIV-1 activity in cell culture. They describe the syntheses of the phosphoramidite derivatives of these analogs, later included near the 3′ end of DNA oligomers to be used as primers in *in vitro* reverse transcription assays. Except for one analog at C8, all others showed a pause in DNA synthesis at position +3 (16). The potency of the various analogs in terms of percentage of inhibition of the (-) strong-stop synthesis were tabulated and varied between 45% and 90%. It was also found that the (+) strand DNA synthesis was inhibited by the C8 modifications studied. One of the compounds studied, with an isopropyl group at C8, had relatively low cytotoxicity (16).

The protozoan parasite *Trichomonas vaginalis* causes trichomoniasis in the female urogenital tract. The infection is generally treated by the approved drugs metronidazole and tinidazole, but clinical resistance has been reported. Wright et al. (17) has studied some metronidazole-resistant isolates, having a microaerobic minimal inhibitory concentration (MIC) of 50–100 μM, instead of susceptible isolates characterized by 3.2 μM, of metronidazole. Nitazoxamide, toyocamycin, and 2-fluoro-2′-deoxyadenosine were used by Wright et al. (17) to test susceptible and resistant *T. vaginalis* isolates. Under 6% O_2 assay conditions, the susceptible isolates were also susceptible to the three new drugs tested, and had MICs of 1.6–6 μM. One metronidazole-resistant isolate was susceptible to toyocamycin (0.8 μM), whereas another resistant isolate was susceptible to 2-fluoro-2′-deoxyadenosine (0.8 μM). Similarly, one toyocamycin-resistant isolate was somewhat susceptible to 2-fluoro-2′-deoxyadenosine (6.3–12.5 μM). However, the highly resistant isolate B7268 also appears to be cross-resistant to the deoxyadenosine analogs toyocamycin (moderately, 12.5 μM) and 2-fluoro-2′-deoxyadenosine (25–100 μM). The authors conclude that newer drugs are needed because of the cross-resistance observed.

For anti-HIV therapy, successful therapy has to avoid unwanted side effects and be effective against drug-resistant viral variants. GS-9148 ([5-(6-amino-purin-9-yl)-4-fluoro-2,5-dihydro-furan-2-yloxy-methyl]-phosphonic acid) is a dAMP analog having antiviral activity against drug-resistant HIV. Fluorine was added at the 2′ position of the furan ring to increase the selectivity for the transcriptase over DNA polymerase γ. Crystal structures of HIV-1 reverse transcriptase bound to double-stranded DNA, ternary complexes with either GS-9148-diphosphate or dATP, and a post-incorporation structure with GS-9148 translocated to the priming site were obtained by Lansdon et al. (18). The binding of either the drug or dATP to the binary reverse transcriptase-DNA complex resulted in the fingers subdomain closing around the nucleotide/analog, producing up to a 9 Å shift in the former toward the palm and thumb subdomains. The reverse transcriptase and the DNA were covalently tethered for the purpose of these crystallographic studies through a Q258C mutation introduced for this purpose in the transcriptase and an N2-modified guanosine into either the primer or the template six bases upstream from the priming site (18). One of the crystal structures was for the transcriptase attached to the primer terminating with dideoxycytidine monophosphate, which was found to exist in the priming site as expected. Ternary complexes were formed either with the drug or dATP by soaking the crystal obtained in the first study with these, and the incoming nucleotides were found in the N site with their adenines making a

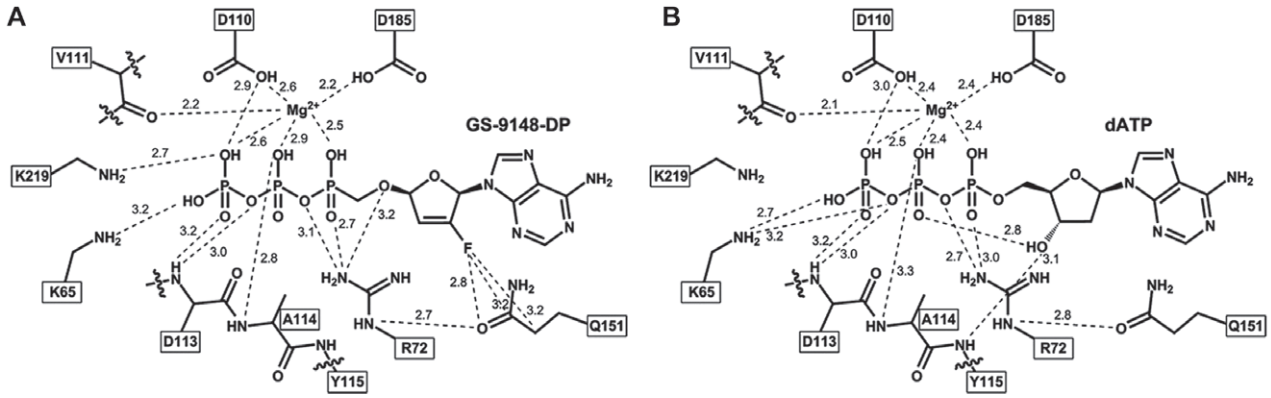

Figure 4 Schematic drawing of (A) GS-9148-DP and (B) dATP occupying the N site of the reverse transcriptase-DNA complex.Hydrogen bonds are shown as dashed lines and corresponding distances in angstroms. Reprinted from Lansdon et al. (18), copyright 2010, with permission from Elsevier.

Watson-Crick base pair with a thymine from the template. Both GS-9148-diphosphate and dATP bind similarly in the N site; however, differences are observed in the position of the dihydrofuran ring as opposed to the deoxyribose, the former being closer to the Y115 side chain (Figure 4). Finally, the fourth protein complex examined was with the GS-9148 as part of the primer at its terminus, generated enzymatically (18). Thus, Lansdon et al. (18) reveal atomic details of these binary and ternary molecular complexes for GS-9148, which is an effective nucleotide reverse transcriptase inhibitor effective against a wide range of drug resistance mutations.

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