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Short communication

Mechanistic explanation to the variable degradation behaviour of stavudine and zidovudine under hydrolytic, oxidative and photolytic conditions

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Abstract

The kinetics of decomposition of zidovudine and stavudine was studied under ICH recommended stress conditions of hydrolysis, oxidation and photolysis. The two drugs, which are closely related in structure, showed the same order of sensitivity under hydrolytic conditions, viz. acid > water > alkali. But stavudine was found to hydrolyse overall much faster than zidovudine. Both drugs were almost stable under basic conditions. Stavudine showed decomposition on exposure to peroxide while zidovudine was stable. On the contrary, zidovudine showed more sensitivity to light than stavudine, which was almost photostable. Thymine was formed as a major decomposition product of both the drugs under all the three stress conditions. The observed behaviour is explained mechanistically.

Keywords: Zidovudine; Stavudine; Decomposition; Kinetics; ICH; Stress conditions

1. Introduction

Zidovudine (AZT) and stavudine (D4T), two nucleoside reverse transcriptase inhibitors, are very closely related in structure. While zidovudine is chemically 3′-azido-3′-deoxythymidine, stavudine is 2′, 3′-didehydro-3′-deoxythymidine (Scheme 1).

Recently, a stress degradation study was carried out in our laboratory [1] on these two drugs under ICH prescribed conditions [2] with an aim to establish inherent stability and stability-indicating HPLC assay methods. While carrying out the studies, vast differences were indicated between the degradation rates of the two drugs, though they are closely related structurally. The same degradation product was formed in case of both the drugs under hydrolytic, oxidative and photolytic conditions.

Therefore, the endeavour of the present study was to (i) compare the nature and the overall rate of

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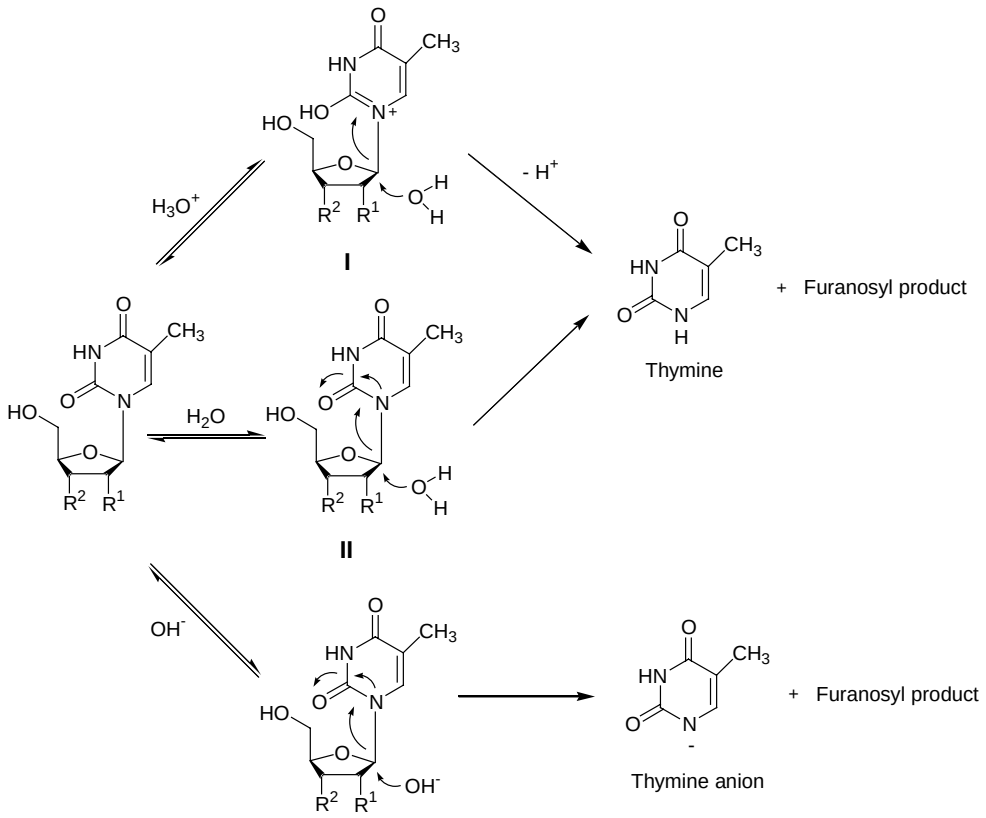


Scheme 1. Postulated mechanisms for hydrolytic decomposition of stavudine and zidovudine.

degradation, (ii) propose mechanistic explanation to variable decomposition behaviour among the two drugs and (iii) provide explanation to formation of the same single degradation product under various decomposition conditions. For the purpose, systematic comparative kinetics studies were carried out on the two drugs under select conditions. The results are presented in this communication.

There is no earlier report in literature on comparative degradation of the two drugs. The only available information is that zidovudine exhibits acid and water catalysed degradation and does not display base catalysed degradation [3]. Regarding stavudine, it is only reported to degrade to thymine in the pH range 1–9 [4].

2. Materials and methods

Zidovudine and stavudine were received gratis from Cipla Ltd. (Mumbai, India) and were used without further purification. Sodium hydroxide was purchased from Ranbaxy Laboratories (S.A.S. Nagar, India) and hydrochloric acid was procured from LOBA Chemie Pvt. Ltd. (Mumbai, India). Hydrogen peroxide was procured from s.d. Fine-chem Ltd. (Boisar, India). HPLC grade methanol was purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical reagent grade. Ultra-pure water was obtained from ELGA (Bucks, UK) water purification unit.

2.1. Decomposition studies

All decomposition studies were carried out at a drug concentration of 1 mg ml^{-1}. Hydrolytic kinetics studies were carried out in 0.1 M HCl, water and 0.1 M NaOH at 80 °C. Samples were withdrawn at intervals and subjected to analysis. Oxidative studies were carried out in 30% H_2O_2 at room temperature for 72 h. Photolytic decomposition studies were conducted in acid (0.1 M HCl for zidovudine and 0.01 M HCl for stavudine), neutral (water) and alkaline (0.1 M NaOH) solutions in a photostability chamber (KBF 240, Binder, Germany) equipped with an illumination bank in accordance with option 2 of ICH guideline Q1B [5] and set at 40 °C and 75% RH. The overall illumination at the point of placement was 6000 lx fluorescent and 0.7 W/m^2 UV light. Samples were withdrawn after 15 days.

2.2. HPLC analysis

The degraded samples were analysed by HPLC. The analyses were carried out on a system consisting of a 600E pump, a 996 photo-diode array (PDA) detector, a 717 autoinjector and a degasser module (all equipment from Waters, Milford, USA). The data were acquired and processed by use of Millennium software version 3.2. Separations were achieved on a Merck C18 column (250 mm × 4.6 mm i.d. with particle size of 5 μm) using water–methanol in the ratios of 90:10 and 77:23 in an isocratic mode for stavudine and zidovudine, respectively. The mobile phase was filtered through 0.45 μm nylon membrane and degassed before use by sonication (3210, Branson Ultrasonics Corporation, CT, USA). The flow rate was kept constant at 1 ml min^{-1}. The injection volume was 10 μl and detection wavelength was 265 nm. The PDA scans were simultaneously recorded between 210 and 400 nm.

2.3. Identification of the degradation product

As stavudine is known to degrade to thymine in the pH range of 1–9 [4], accordingly, pure thymine was procured from Lancaster (Morecambe, England) and it was spiked into the reaction solutions of stavudine, followed by HPLC analyses. The presence of the same product was also tested in reaction solutions of zidovudine.

3. Results and discussion

3.1. Degradation behaviour and kinetics under hydrolytic conditions

Both the drugs decomposed under all three hydrolytic conditions, and with the same order of sensitivity, that is, acid > neutral > alkali. Under these conditions, the semi-logarithmic plots of concentration of drug versus time were strictly linear ($r^2 > 0.99$), indicating that the reactions strictly followed pseudo first-order kinetics. The corresponding rate constants are listed in Table 1. Evidently, the rate of hydrolysis of stavudine was much higher than zidovudine under all the three conditions, by almost 2800 times in acid, 20,000 times in water and 254 times in alkali.

3.2. Behaviour and relative rates of degradation of the drugs under oxidative and photolytic conditions

Stavudine was oxidised in 30% H_2O_2 to an extent of 50% in 72 h. The corresponding decomposition of zidovudine was just ∼5%. The behaviour was opposite under photolytic conditions. On exposure in photostability chamber for 15 days, zidovudine underwent significant degradation (>80%), while there was no change in stavudine solutions. Rate of photolysis of zidovudine was highest in acid followed by base and

Table 1
Pseudo first-order rate constant, k (h^{-1}) of stavudine and zidovudine under hydrolytic conditions

Drug	Rate constant (r^2)		
	0.1 M HCl	Water	0.1 M NaOH
Zidovudine	1.382×10^{-3} (0.9988)	6.909×10^{-5} (0.9968)	4.606×10^{-5} (0.9983)
Stavudine	3.878 (0.9963)	1.389 (0.999)	0.0117 (0.9968)

then water. No degradation was observed in the control samples in dark chamber. In the case of stavudine, samples in 0.01 M HCl and water showed the same extent of degradation whether kept in light or in dark. On the other hand, the samples in 0.1 M NaOH showed no degradation in light as well as in dark. These studies, thus, indicated that light had no effect on the degradation of stavudine.

3.3. Identity of the major degradation products

Under all stress conditions where decomposition was observed in both the drugs, the fall in drug peak was accompanied by a rise in a single major decomposition product peak. Spiking study with pure thymine confirmed that it was the product formed during hydrolysis of both the drugs, and also during oxidative degradation of stavudine and photolysis of zidovudine. The PDA scanning of various reactions between 210 and 400 nm did not yield any new peak at any of the investigated wavelengths, indicating that furanosyl-type second half of the drugs was not detectable by a UV detector.

3.4. Mechanistic explanation to the formation of thymine under hydrolytic conditions

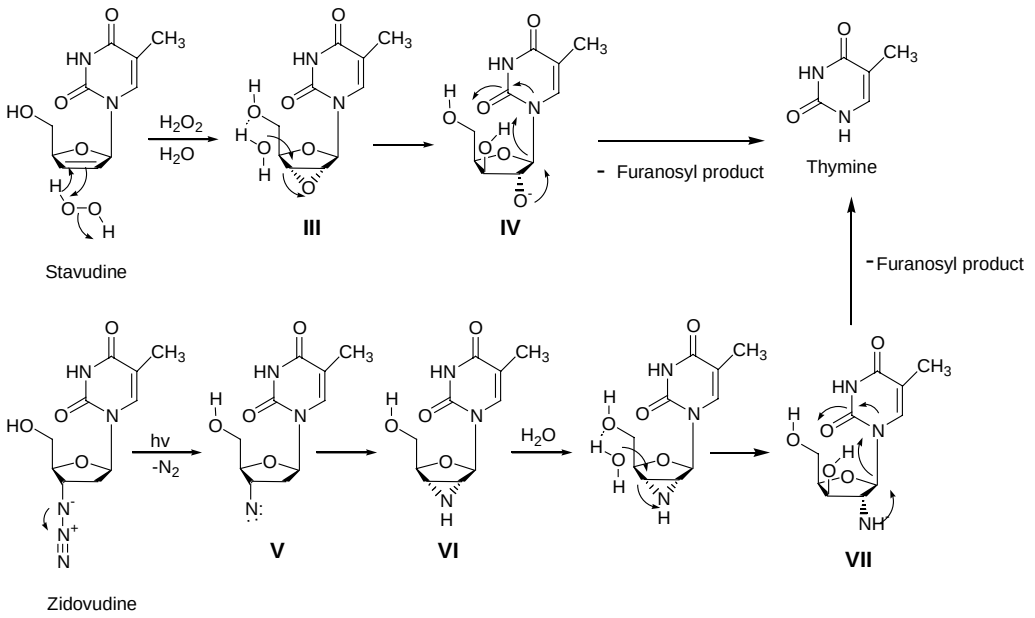
The important structural feature in stavudine and zidovudine that can be considered to be responsible for hydrolytic cleavage of the drugs to thymine under acidic, neutral and alkaline conditions is the aminal functionality at C_1' (Scheme 1). The aminal nitrogen can bear a positive charge in acidic conditions as a result of protonation of the thymine moiety, forming an enol (Structure I), which acts as a better leaving group, thus, assisting in hydrolytic cleavage. The absence of the positive charge on the aminal nitrogen under neutral condition should make the thymine moiety a relatively less effective leaving group, reducing the rate of hydrolysis compared to that observed under acidic medium. In the presence of hydroxyl anion in alkaline medium, the liberated thymine anion is rather likely to undergo electrostatic destabilization, due to which the overall rate should be less even as compared to that observed under acid or neutral conditions. These mechanisms, hence, explain the observed order of the sensitivity of the two drugs to hydrolysis, viz. acid > neutral > alkali.

Regarding the difference in relative rates amongst the two drugs (Table 1), as the hydrolysis involves a nucleophilic attack at the aminal carbon following a S_N2 process, it is expected that any structural change that might help stabilization of the pentavalent carbocationic intermediate should increase the rate of the hydrolysis. In stavudine, the double bond is incidentally present at the allylic position with respect to the aminal carbon, which helps in stabilization of the transition state. Because, zidovudine is devoid of the same, this explains the faster decomposition of stavudine under different hydrolytic conditions.

3.5. Postulated mechanism for the formation of thymine in the presence of peroxide

It is postulated that in the presence of hydrogen peroxide, the double bond in stavudine undergoes oxidation to form an epoxide **III** (Scheme 2). As the epoxide formation involves nucleophilic displacement of the hydroxyl group in hydrogen peroxide by the π-bond [6], the presence of the bulky thymine group and the hydroxymethylene moiety should direct the oxo transfer to take place in a stereoselective fashion, so that the epoxide moiety is introduced from the α face. Owing to the inherent ring strain (27 kcal/mol), epoxides are susceptible to nucleophilic ring cleavage [7] and the regio- and stereo-chemistry of the cleavage of the epoxide ring in **III** is likely to be controlled by the hydroxymethylene group. Therefore, hydrogen bond formation between water molecule and the hydroxyl group of the hydroxymethylene moiety directs the nucleophilic attack by water generating the diol anion intermediate **IV** in which the oxide anion is oriented in a antiperiplanar manner with respect to the thymine (leaving group). Thus, the oxide anion, generated from the cleavage of the intermediate epoxide, induces a neighbouring group effect for nucleophilic displacement of the thymine moiety leading to an increase in the rate of hydrolytic decomposition under oxidative condition. Thus, the same decomposition product, thymine, is produced even under oxidative conditions.

As no epoxide formation is possible for zidovudine, its decomposition under oxidative condition is perhaps the result of normal hydrolysis in the presence of water.



Scheme 2. Postulated mechanisms for the decomposition of stavudine and zidovudine in the presence of peroxide and light, respectively.

3.6. Mechanism for the observed degradation behaviour under light

Zidovudine possesses an azide group, which can be attributed for the lability of the drug under the photochemical conditions. The azide groups are known to undergo elimination of molecular nitrogen to generate nitrene as a reactive intermediate [8], which can further undergo intramolecular C–H insertion reaction leading to the formation of the aziridine [9]. Analogous to the epoxides, aziridines are also very susceptible to nucleophilic cleavage [10]. Accordingly, the nitrene of zidovudine (**V**, Scheme 2) can form aziridine (**VI**), which is expected to undergo nucleophilic attack by water, with the regio- and stereo-selectivity of the nucleophilic ring opening being controlled by the adjacent hydroxymethylene moiety. It is postulated that hydrogen bonding of water molecule with the hydroxyl group of the hydroxymethylene moiety directs the nucleophilic attack from the β face to form an intermediate **VII**. A concomitant nucleophilic displacement of the thymine moiety by the amide anion in **VII** takes place resulting in decomposition of the drug to thymine. The higher rate of photolysis in acid than alkaline or neutral conditions is explained by the possibility of protonation of the aziridine nitrogen in **VII**, making the aziridine ring more susceptible to nucleophilic attack.

In the absence of any photoreactive group, no such sequences of events are possible in stavudine. This supports the absence of appreciable change of the rate of decomposition of this drug in the presence of light.

4. Conclusions

The study brings forward new and interesting aspects on the decomposition behaviour of zidovudine and stavudine than known earlier in literature. It also provides good comparison of degradation pattern of the two drugs that are closely related in structure. It is found that both drugs hydrolyse in the order acid > water > alkali, but stavudine decomposes much faster than zidovudine. Overall, the two drugs decompose slowly in base. While stavudine undergoes oxidative decomposition, zidovudine is more sensitive to light. Under all situations, both drugs decompose to thymine as the major decomposition product.

Mechanistic explanation is provided to the observed degradation behaviour, which correlates very well with

the analytical observations and the order of decomposition of the two drugs under different conditions.

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