

## SIMPLE SPECTROPHOTOMETRIC ASSAY AND BIOLOGICAL EVALUATION OF ZALCITABINE

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Article Received on 15/11/2017

Article Revised on 05/12/2017

Article Accepted on 25/12/2017

### ABSTRACT

A Simple, precise, accurate and economical spectrophotometric method was developed and validated for simultaneous estimation of zalcitabine was quantified using their absorptivity values at selected wavelengths, viz., 268nm and 263nm respectively. The accuracy and reproducibility of the proposed method was statistically validated by recovery studies. The simultaneous equation method permits simple, rapid and direct determination of zalcitabine commercially available combined dosage form without previous separations and can therefore be used for routine analysis.

**KEYWORDS:** Zalcitabine, Simultaneous equation Method.

### INTRODUCTION

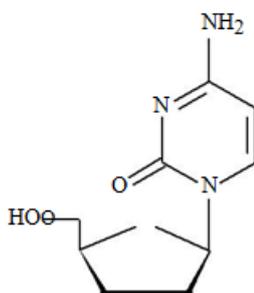
#### 1.1 Chemical and physical data

##### 1.1.1 Nomenclature

*IUPAC Systematic Name:* 2',3'-Dideoxycytidine

*Synonyms:* ddC; DDC; dideoxycytidine

##### 1.1.2 Structural and molecular formulae and relative molecular mass



$C_9H_{13}N_3O_3$

Relative molecular mass: 211.22

##### 1.1.3 Chemical and physical properties of the pure substance

(a) *Description:* White to off-white crystalline powder (American Hospital Formulary Service, 1997)

(b) *Melting-point:* 215–217°C (Budavari, 1996)

(c) *Solubility:* Soluble in water (76.4 mg/mL at 25°C) (American Hospital Formulary Service, 1997); soluble in dimethylsulfoxide (90–100 mg/mL); slightly soluble in ethanol (5–7 mg/mL) and methanol (8–10 mg/mL);

insoluble in ace-tonitrile, chloroform, butanol, ethyl acetate, and toluene (National Cancer Institute, 1992)

(d) *Optical rotation:*  $[\alpha]_{25}^{20}$ , +81° (c = 0.635 in water) (Budavari, 1996)

##### 1.1.4 Technical products and impurities

Zalcitabine is available as a 0.375- and 0.75-mg tablet. The tablets may also contain croscarmellose sodium, iron oxides (synthetic brown, black, red and yellow), lactose, macrogol, magnesium stearate, methylhydroxypropylcellulose, microcrystalline cellulose, polyethylene glycol, polysorbate 80 and titanium dioxide (Gennaro, 1995; Canadian Pharmaceutical Association, 1997; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1998; Editions du Vidal, 1998; Rote Liste Sekretariat, 1998; Thomas, 1998; US Pharmacopial Convention, 1998).

Trade names for zalcitabine include ddC Martian, Hivid and HIVID Roche (Swiss Pharmaceutical Society, 1999).

##### 1.1.5 Analysis

The *United States Pharmacopeia* specifies infrared absorption spectrophotometry with comparison to standards, liquid chromatography and thin-layer chromatography as the methods for identifying zalcitabine; liquid chromatography is used to assay its purity. In pharmaceutical preparations, zalcitabine is identified and assayed by liquid chromatography (US Pharmacopial Convention, 1997).

### 1.2 Production

Several methods have been reported for the synthesis of zalcitabine (Horwitz *et al.*, 1967; Marumoto & Honjo, 1974; Lin *et al.*, 1987).

Information available in 1999 indicated that it was manufactured and/or formu-lated in 33 countries (CIS Information Services, 1998; Swiss Pharmaceutical Society, 1999).

### 1.3 Use

Zalcitabine was among the first drugs (in the early 1990s) approved for use against human immunodeficiency virus (HIV) infection (Devineni & Gallo, 1995) but has passed out of common use in the industrialized world. Although many studies were conducted on its use in various combinations, several large clinical trials (Bartlett *et al.*, 1996; Delta Coordinating Committee, 1996; Hammer *et al.*, 1996; Schooley *et al.*, 1996; Henry *et al.*, 1998) have clearly demonstrated that zalcitabine-containing regimens are less effective than other combinations of antiviral nucleoside analogues with which it has been compared. Although it has been used to treat HIV infections in adults and children, the agent is regarded as obsolete if other nucleoside reverse trans-criptase inhibitors are available.

Zalcitabine also has two serious toxic effects: a relatively high frequency of dose-and duration-related peripheral neuropathy and an idiosyncratic syndrome of ulcerations in the mucous membranes of patients (Indorf & Pegram, 1992; Roche Laboratories, 1998).

Zalcitabine has cross-resistance with didanosine (Roche Laboratories, 1998), which is generally more effective.

### 1.4 Occurrence

Zalcitabine is not known to occur as a natural product. No data on occupational exposure were available to the Working Group.

### 1.5 Regulations and guidelines

Zalcitabine is listed in the *United States Pharmacopeia* (Swiss Pharmaceutical Society, 1999).

## 2. Studies of Cancer in Humans

In a multicentre trial in the USA, Abrams *et al.* (1994) randomly assigned 467 symptomatic HIV-infected patients with CD4 counts of  $\leq 300$  cells/ $\mu$ L plasma, who had previously received zidovudine, to treatment with either zalcitabine at 2.25 mg per day ( $n = 237$ ) or didanosine at 500 mg per day ( $n = 230$ ). The patients were recruited during 1990–91 and were followed up for a median of 1.3 years and a maxi-mum of only 1.8 years. Six cases of non-Hodgkin lymphoma were seen in the zalci-tabine-treated group and three in the didanosine-treated group. [The Working Group noted that rate ratios were not calculated, although the risk ratio for non-Hodgkin lymphoma in patients treated with zalcitabine

compared with that in patients receiving didanosine was 1.9 (95% CI, 0.49–7.7), with no adjustment for differences in survival between the two groups].

In an international randomized trial, the Delta Coordinating Committee (1996) allocated 3207 individuals with antibodies to HIV, symptoms of infection or a CD4 count of  $< 350$  cells/ $\mu$ L plasma to treatment with either zidovudine at 600 mg per day ( $n = 1055$ ), zidovudine plus didanosine at 400 mg per day ( $n = 1080$ ) or zidovudine plus zalcitabine at 2.25 mg per day ( $n = 1072$ ). The patients were followed up for a median of 2.5 years (range, 1.8–2.9), during which time 14 deaths due to cancer [not further specified] occurred; five of the deaths occurred in the group treated with zido-vudine alone, five in the group treated with zidovudine plus didanosine and four in the group treated with zidovudine plus zalcitabine.

In the study of Pluda *et al.* (1990, 1993), described in the monograph on zido-vudine, two of 18 patients receiving zalcitabine alternated with zidovudine developed a high-grade, B-cell non-Hodgkin lymphoma. [The Working Group noted that the risk is difficult to interpret in the absence of a suitable reference group consisting of AIDS patients with a similar degree of immunosuppression].

[The Working Group noted that these trials were designed to compare the efficacy of drugs in the treatment of patients with various degrees of severity of immuno-suppression. For the purposes of evaluating cancer risk, therefore, the numbers of participants were too small and the length of follow-up too short, cancer incidence may have been underascertained and cancer rates could not be analysed adequately].

## 3. Studies of Cancer in Experimental Animals

### Oral administration

#### Mouse

Groups of 10 male and 10 female B6C3F<sub>1</sub> mice, six weeks of age, were treated with zalcitabine (purity,  $> 99\%$ ) in a 0.5% methylcellulose and water suspension by gavage twice a day 6 h apart at a dose of 0 (control), 500 or 1000 mg/kg bw per day for 13 weeks, at which time all surviving mice were killed. An additional group of 10 female mice received 1000 mg/kg bw per day for 13 weeks and were then maintained without further treatment for a one-month recovery period before termination. The unexpected finding of thymic lymphomas in one female that received the low dose and one female that received the high dose prompted the authors to conduct an additional study (Sanders *et al.*, 1995).

Groups of 70 female B6C3F<sub>1</sub> mice, six weeks of age, were treated with zalcitabine (purity,  $> 99\%$ ) in a 0.5% methylcellulose and water suspension by gavage twice a day 6 h apart at a dose of 0 (control), 500 or 1000 mg/kg bw per day for 13 weeks, after which 20 animals per group were killed and necropsied. The remaining 50

mice per group were held without treatment for an additional three months before termination (recovery group). Thymic lymphomas were found in 2/19 mice that received the low dose and were necropsied at the end of the 13-week exposure period and in 3/50 and 15/50 mice at the low and high doses, respectively, that were necropsied during or at the end of the three-month recovery period. No thymic lymphomas were seen in control mice (Sanders *et al.*, 1995).

Groups of 50 male and 50 female B6C3F<sub>1</sub> mice, six weeks of age, were treated with zalcitabine (purity > 99%) in a 0.5% methylcellulose and water suspension by gavage twice a day 6 h apart at a dose of 0 (control), 500 or 1000 mg/kg bw per day for six months. An additional group at the high dose was treated for three months and killed six months after the start of the experiment (recovery group). There were no treatment-associated deaths among male mice, but marked treatment-associated and lymphoma-associated mortality was seen in female mice receiving the high dose and in the recovery group. By the end of the experiment, the mortality rates in female mice were 0% in controls, 0% at the low dose, 6% at the high dose and 12% in the recovery group. The incidences of thymic lymphoma were 0%, 14%, 20% and 12% in males and 0%, 2%, 44% and 39% in females in these groups [effective numbers not reported for either sex], respectively. The thymic lymphomas involved other lymphoid organs, such as spleen and lymph nodes. Thymic atrophy was the commonest non-neoplastic lesion in treated mice, the incidences being 0%, 2%, 18% and 0% in males in the control, low-dose, high-dose and recovery groups and 0%, 12%, 20% and 0% in females in these groups, respectively. The recovery group had a lower incidence of thymic atrophy than mice at the high dose, indicating that cessation of treatment resulted in reversal of thymic atrophy (Rao *et al.*, 1996).

Groups of 50 male and 50 female NIH Swiss mice, six weeks of age, were treated with zalcitabine (purity > 99%) in a 0.5% methylcellulose and water suspension by gavage twice a day 6 h apart at a dose of 0 (control), 500 or 1000 mg/kg bw per day for six months. An additional group at the high dose (recovery group) was treated for three months. All animals were killed six months after the start of the experiment. A treatment-related increase in mortality rate was seen in both males and females, with rates of 2%, 10%, 24% and 4% in males in the control, low-dose, high-dose and recovery groups and 0%, 14%, 50% and 46% in females in these groups, respectively. The deaths were due to thymic lymphomas in the females, whereas the male mice died from toxic effects of zalcitabine, such as anaemia. The incidences of thymic lymphoma were 0%, 15%, 55% and 47% in males in the control, low-dose, high-dose and recovery groups and 0%, 44%, 87% and 90% [effective numbers not reported for either sex] for females in these groups, respectively. Thymic atrophy was the commonest non-neoplastic lesion in treated mice, with incidences of 4%,

19%, 26% and 6% in males in the control, low-dose, high-dose and recovery groups and 0%, 2%, 10% and 2% in females in these groups, respectively. Both males and females in the recovery group had a lower incidence of thymic atrophy than those given the high dose continuously, indicating that cessation of treatment resulted in reversal of thymic atrophy (Rao *et al.*, 1996).

## Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

### 4.1 Absorption, distribution, metabolism and excretion

#### 4.1.1 Humans

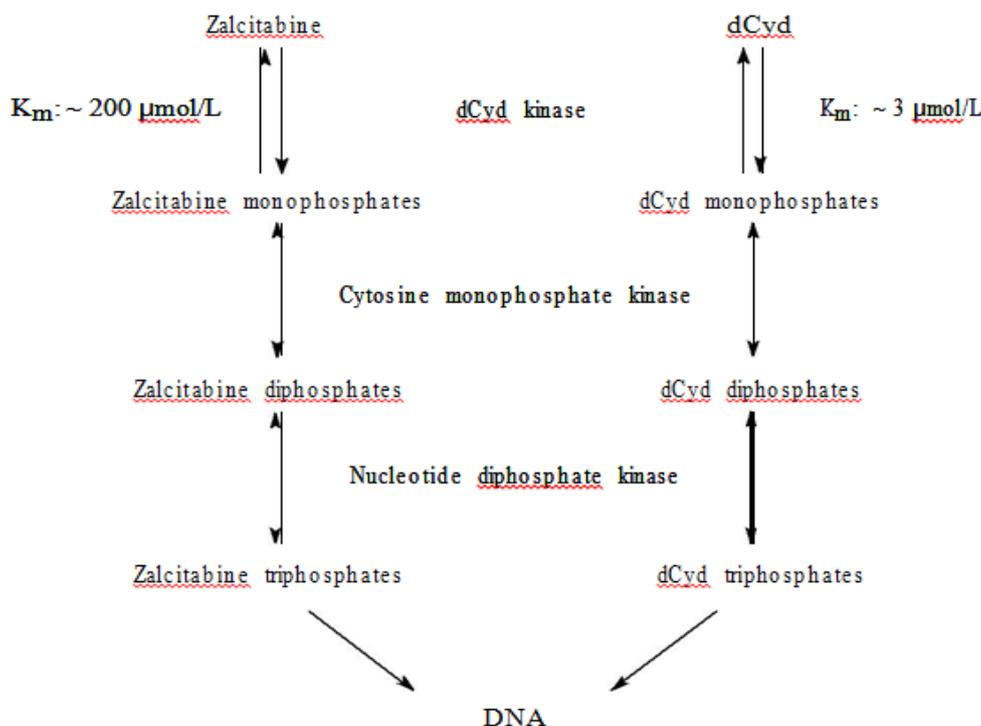
The absorption, distribution, metabolism and excretion of zalcitabine in adults have been reviewed extensively (Broder, 1990; Yarchoan *et al.*, 1990; Burger *et al.*, 1995; Devineni & Gallo, 1995; Vanhove *et al.*, 1997). Because of the toxicity of zalcitabine, the dose range that can be used is narrow, and the drug is typically given three times daily for a total of 1.0–1.5 mg. This dose range is much lower than the 400- and 600-mg daily doses of didanosine and zidovudine, respectively, but the antiviral potency of zalcitabine in cell cultures is much greater than that of these other drugs. Zalcitabine is well absorbed when administered orally, with a bioavailability of the order of 80% (Klecker *et al.*, 1988; Broder, 1990; Burger *et al.*, 1995). About 75% of an oral dose is excreted unchanged in the urine, and measurable levels have been found in plasma and cerebrospinal fluid. The peak concentration of zalcitabine in cerebrospinal fluid 2 h after dosing has been reported to be 14% of that in plasma (Klecker *et al.*, 1988; Burger *et al.*, 1995; Devineni & Gallo, 1995). Zalcitabine is transported across the cell membrane by nucleoside carrier-mediated and non-carrier-mediated mechanisms and < 5% is bound to protein (Burger *et al.*, 1995; Devineni & Gallo, 1995).

Zalcitabine is metabolized along only one pathway (Figure 1). About 10% of the drug appears in the faeces and ~75% is excreted unchanged in the urine, suggesting that renal integrity is important for clearance (Klecker *et al.*, 1988; Broder, 1990; Burger *et al.*, 1995; Beach, 1998). Hepatic metabolites have not been observed (Burger *et al.*, 1995). The antiviral action of zalcitabine, like that of zidovudine and didanosine, is dependent on phosphorylation and incorporation into DNA (Broder, 1990). The first step is the formation of zalcitabine monophosphate by the enzyme 2'-deoxycytidine kinase, which is followed by formation of the diphosphate and triphosphate metabolites through the action of the cytosine monophosphate kinase and nucleotide diphosphate kinase enzymes, respectively (Broder, 1990; Burger *et al.*, 1995). Like zidovudine and didanosine, zalcitabine targets the viral reverse transcriptase and is simultaneously incorporated into DNA, where replication is unable to proceed further owing to lack of a 3'-hydroxyl group (Broder, 1990). Although phosphorylation is critical for the antiviral activity, it

accounts for only a small fraction (probably ~1%) of the total drug disposition.

The pharmacokinetics of zalcitabine has been extensively reviewed (Yarchoan *et al.*, 1990; Burger *et al.*, 1995; Devineni & Gallo, 1995; Vanhove *et al.*, 1997). Like that of zidovudine and didanosine, the pharmacokinetics of zalcitabine appears to be linear over a broad dose range and the maximum concentration in plasma is reached by 1–2 h in adults (5–8 ng/mL after a 0.5–0.75-mg tablet orally three times a day); the plasma half-time is reported to be 1–2.7 h (Klecker *et al.*, 1988; Broder, 1990; Gustavson *et al.*, 1990; Burger *et al.*, 1995; Devineni & Gallo, 1995; Vanhove *et al.*, 1997).

Because a lower dose is given, the peak plasma concentration is only about 10% of those found with zidovudine and 20% of those found with didanosine (Yarchoan *et al.*, 1990). The mean rate of plasma clearance was 14–25 L/h, but it decreased with increasing age and weight (Yarchoan *et al.*, 1990; Vanhove *et al.*, 1997). Renal clearance is also closely linked to creatinine clearance and body weight (Burger *et al.*, 1995; Bazunga *et al.*, 1998). The pharmacokinetics appeared to be similar after an initial dose and during long-term dosing, and there were no significant interactions between zalcitabine and concomitantly administered zidovudine (Vanhove *et al.*, 1997).



**Figure 1: Pathways of anabolic phosphorylation of zalcitabine (2',3'-di-deoxycytidine, ddCyd) and its normal counterpart, 2'-deoxycytidine (dCyd).**

From Broder (1990).

The Michaelis constant for the first step of anabolic phosphorylation (mediated by 2'-deoxy-cytidine kinase) is shown for both the drug and the normal substrate.

#### 4.1.2 Experimental systems

Pregnant rhesus monkeys (*Macaca mulatta*) that were near term (146 days) received radiolabelled zalcitabine as a bolus dose of 0.6 mg/kg bw via the radial vein. During a 3-h sampling of both the mother and the fetus, the fetal:maternal ratio of the integrated area under the curve of plasma concentration–time was  $0.32 \pm 0.02$  and the fetal tissues were found to contain zalcitabine (0.05–0.8  $\mu\text{mol/L}$  equivalents) and zalcitabine monophosphate (0.008–0.09 nmol/g) (Sandberg *et al.*, 1995).

Four pregnant pigtailed macaques (*Macaca nemestrina*) that were near term (126 days) received an infusion of zalcitabine at a constant rate of 1.3  $\mu\text{g}/\text{min}$  per kg bw through the femoral vein over 30 h. The authors concluded that passive transplacental transfer of zalcitabine occurred, with a fetal:maternal concentration ratio of  $0.58 \pm 0.06$  (Tuntland *et al.*, 1996).

The absorption, distribution, metabolism and excretion of zalcitabine have been reported in microswine (Swagler *et al.*, 1991), rats (Ibrahim & Boudinot, 1989, 1991), mice (Kelley *et al.*, 1987) and monkeys (Kelley *et al.*, 1987; Qian *et al.*, 1992). A review of data for several species (Devineni & Gallo, 1995) suggested that the pharmacokinetics in experimental animals and humans were essentially similar. Virtually no zalcitabine was found in cerebrospinal fluid (< 1%) in rats, dogs or monkeys. Approximately 50–80% of the drug was

excreted unchanged in the urine, but urinary metabolites were detected only in monkeys. The half-time for drug elimination was similar in all species and averaged 1.4 h.

In four microswine given an intravenous bolus dose of 5 mg/kg bw zalcitabine (Swagler *et al.*, 1991), the rates of total and renal clearance were similar to those in humans, about 80% of the drug being excreted unchanged in the pigs and about 75% in humans (see section 4.1.1). The half-time for clearance of zalcitabine removal, 1.8 h in pigs, was also similar to that in humans (see section 4.1.1). Microswine are a good model for the pharmacokinetics of zalcitabine in humans but a less desirable model for the metabolism of zidovudine and didanosine, for which the clearance rates are significantly lower.

In rats, the pharmacokinetics of zalcitabine was stable over a dose range of 10–500 mg/kg bw. The half-time for removal of the drug from plasma and urine was 1–1.3 h. It bound to plasma proteins and 50% of the original dose was excreted unchanged in the urine. Renal clearance exceeded the glomerular filtration rate, suggesting active renal tubular secretion (Ibrahim & Budinot, 1989, 1991). Zalcitabine accumulated preferentially in the liver and spleen of rats (Makabi-Panzu *et al.*, 1994). Interspecies scaling indicated that the concentrations in humans can be predicted from the pharmacokinetics in rats.

BDF<sub>1</sub> mice were given a single dose of 100 mg/kg bw zalcitabine orally or intra-venously and continuous exposure to 47 mg/kg bw per day by Alzet pump. The plasma concentrations reached a maximum of about 15 µg/mL 45 min after oral dosing and the half-time for plasma clearance was 67 min. About 80% of the drug was recovered unchanged in the urine after intravenous dosing and about 60% of the drug was found in faeces after oral dosing. The phosphorylated metabolites constituted about 1–2% of the total dose. High concentrations of the drug were found in mouse kidney, pancreas and liver and there was low penetration to the central nervous system (Kelley *et al.*, 1987).

In three rhesus monkeys given 100 mg/kg bw zalcitabine, recovery in the urine was about 75% by five days, as in humans, but only about 9% of the drug was excreted as dideoxyuridine, which is in contrast to the human metabolic pattern. Deamination of zalcitabine to dideoxyuridine does not appear to be a significant reaction in either mice or humans but is measurable in monkeys. The half-time for clearance from plasma was 1.8 h, and the concentration in cerebrospinal fluid was < 1% of the peak plasma level (Kelley *et al.*, 1987). When a much lower dose (5 mg/kg bw) of zalcitabine was given to three monkeys, 49–61% of the drug was excreted unchanged in the urine. The half-time for plasma clearance was 1.9 h and the bioavailability was 61% (Qian *et al.*, 1992). The studies suggest that non-human primates are an appropriate model for studying the pharmacokinetics of zalcitabine in humans.

## 4.2 Toxic effects

### 4.2.1 Humans

Zalcitabine induces sensory peripheral neuropathy and a syndrome involving fever, rash and aphthous stomatitis, which are early toxic and dose-limiting effects (Yarchoan *et al.*, 1990; Burger *et al.*, 1995; Skowron, 1996; Beach, 1998). In some of the first clinical trials, peripheral neuropathy manifested as pain, numbness and weakness occurred in 70% of patients receiving doses of  $\geq 4.5$  mg per day but was reversible after discontinuation of the drug. At the lower doses used currently, the onset of neuropathy is more gradual and the symptoms resolve more rapidly (Skowron, 1996). For example, Fischl *et al.* (1995) studied 285 AIDS patients with CD4 counts of  $\leq 300$  cells/ $\mu$ L, who received 2.25 mg zalcitabine daily for 17 months (median time). Of these patients, 6% had neuropathy, 14% had anaemia or neutropenia, 6% had evidence of hepatic toxicity, 6% had stomatitis or rash and 3% had pancreatitis. Blum *et al.* (1996) reported that 34% of 79 HIV-infected individuals receiving zalcitabine at 2.25 mg/day developed neuropathy within a mean latency of 16 weeks (range, 1–51 weeks). Further reduction of the dose lessened the severity of symptoms but did not resolve the neuropathy.

In very highly compromised AIDS patients (median CD4 count, 59 cells/ $\mu$ L), toxic neuropathy was found in 27% of 51 patients receiving zalcitabine. The risk factors for peripheral neuropathy were low serum cobalamin and high alcohol consumption (Fichtenbaum *et al.*, 1995).

In contrast, two HIV-exposed health-care workers receiving prophylactic therapy that included zalcitabine and zidovudine had acute onset of rash, fever, nausea and increased activity of liver enzymes after three weeks of treatment. A liver biopsy specimen contained macrovesicular steatosis and lobular inflammation. These are known, but rare side-effects of zidovudine that may have been exacerbated by the presence of another nucleoside analogue drug, zalcitabine (Henry *et al.*, 1996).

Zalcitabine, like other anti-HIV nucleoside analogues, has been associated with a rare (1 in  $10^5$  to 1 in  $10^6$  patients) idiosyncratic syndrome of a progressive increase in the activity of liver enzymes in serum, fulminating hepatic steatosis and lactic acidosis. Failure to discontinue the drug can lead to death (US Food and Drug Administration Antiviral Advisory Committee, 1993).

### 4.2.2 Experimental systems

#### (a) Haematotoxicity

Although the haematotoxicity observed with zalcitabine is not as severe as that seen with zidovudine and is not the dose-limiting effect for zalcitabine, it is a feature of the toxic profile of zalcitabine (see section 4.2.1). It has been successfully modelled in mice, rats, dogs, rabbits and monkeys (Tsai *et al.*, 1989; Mencoboni *et al.*, 1990;

Luster *et al.*, 1991; Thompson *et al.*, 1991; Riley *et al.*, 1992; Taylor *et al.*, 1994).

Various classes of bone-marrow cells from mice given seven daily doses of 10 mg/kg bw zalcitabine were examined for 15 days after the initial exposure. By day 5, severe neutropenia was observed. The effect was greatest in committed progenitor cells of both erythroid and granulocyte-macrophage lineages and was reversible upon discontinuation of the drug (Mencoboni *et al.*, 1990). Zalcitabine-induced regenerative macrocytic anaemia, but no immunosuppressive effects, were found when the drug was administered to mice for up to 94 days at a dose of 2000 mg/kg bw per day (Luster *et al.*, 1991). Similar results were observed by Thompson *et al.* (1991) with the same dose regimen, who found macrocytic anaemia and hypoplastic bone marrow in mice and rats, the effect being generally more severe in mice than in rats. The haematological effects were reversible upon discontinuation of treatment.

Rabbits treated daily for 13–18 weeks by intubation with 10–250 mg/kg bw zalcitabine per day showed persistent lymphopenia with decreased red and white blood cell counts, haematocrit and haemoglobin concentration. Most animals had non-regenerative macrocytic anaemia of bone-marrow origin and a progressive loss of lymphocytes until death (Riley *et al.*, 1992).

Pigtailed macaques were given zalcitabine at 15 or 30 mg/kg bw per day intra-venously, either as a 24-h continuous infusion or a daily bolus dose for 10–12 days. All animals showed leukopenia, anaemia, lethargy and reduced appetite, and those given the bolus doses also had exfoliative dermatitis and peripheral neuropathy (Tsai *et al.*, 1989). In rhesus monkeys given lower doses (0.06–6.0 mg/kg bw per day) for up to 243 days, transient decreases in CD4 T and CD20 B cell counts were observed after 20 days of dosing, but few other haematological effects were found (Taylor *et al.*, 1994).

#### (b) Neurotoxicity

The neurotoxicity of zalcitabine in rabbits was modelled in a series of studies (Anderson *et al.*, 1991; Feldman *et al.*, 1992; Anderson *et al.*, 1994; Feldman & Anderson, 1994), which suggested that the underlying mechanism was mitochondrial damage (see section 4.5). New Zealand white rabbits were given zalcitabine at 0–250 mg/kg bw per day for 13–18 weeks. Rabbits at doses > 50 mg/kg bw per day developed hind-limb paresis and gait abnormalities and a 30–50% decrease in nerve conduction. Histological examination (Anderson *et al.*, 1991) revealed myelin splitting, demyelination and axonal loss in the sciatic nerve, but no alterations in the.

#### ACKNOWLEDGEMENT

➤ It's my pleasure to express my thanks to P.RAVEENDRAREDDY department of Chemistry for giving an opportunity to do research.

- I express my sincere thanks to P.RAVISANKARA REDDY (Sr.Executive in Biological E.Ltd company, shameerpet, Hyd), who is giving valuable guidance during my research.
- I express my sincere thanks to M.VENUGOPAL who is giving valuable guidance during my research.
- I express my sincere thanks to ABDUL HAQ who is giving valuable guidance during my research.

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