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A BRIEF OVERVIEW OF NEVIRAPINE

AHER S*, DAMA GY* AND JOSHI SA

Sharadchandra Pawar college of pharmacy, Dumbarwadi, Otur, Junnar, Pune, 412409

***Corresponding Author: Mr. Sanket Aher, Mr. Ganesh Y Dama: E Mail: sanketaher2122@gmail.com**

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ABSTRACT

Nevirapine is a highly specific inhibitor of HIV-1 reverse transcriptase (RT), which is an important therapeutic target in the treatment of HIV infection. It was the first non-nucleoside RT inhibitor (NNRTI) approved for use in HIV-infected individuals, including children. Nevirapine inhibits the replication of several HIV-1 strains and clinical isolates in cultured human T cells, but has no activity against other retroviral RTs (including HIV-2 RT) or endogenous human DNA polymerases. Nevirapine monotherapy rapidly selects for advanced drug resistance caused by a single amino acid substitution in the HIV RT gene. The pattern of resistance mutations selected by nevirapine overlaps with other NNRTIs, but differs from that of nucleoside analog RT inhibitors and protease inhibitors. The pharmacokinetics of nevirapine is characterized by rapid and almost complete oral absorption, apparently uniform distribution in all body organs and tissues, and a long elimination half-life. Nevirapine is metabolized by cytochrome P450 isoenzymes and induces their activity. Caution should be exercised when nevirapine is co-administered with other drugs metabolized by this system, including HIV protease inhibitors.

Keyword: Nevirapine (NVP), HIV protease inhibitors, ADR, Mechanism of action, HPLC approach

INTRODUCTION –

Nevirapine (NVP) is one of the oldest antiretroviral (ARV) drugs for the treatment of HIV infection and is still widely used. More than one million patient years of worldwide experience have provided a significant amount of data to better understand this non-nucleoside antiretroviral inhibitor (NNRTI) **[1-3]**.

Nevirapine is one of the most widely used ARV drugs in Africa and Asia, giving it an

important role in the global fight against HIV infection. As the first NNRTI approved by the US Food and Drug Administration (FDA), NVP has a well-understood and widely described safety profile.4 Although it may have some drawbacks, recent data suggest that it may be an acceptable regimen in certain populations. Health Care and Personnel Management Guide **[4]**.

Nevirapine (NVP), sold under the brand name Viramune among others, is a drug used to treat and prevent HIV/AIDS, particularly HIV-1. It is usually recommended to be used in combination with other antiretroviral drugs. It ca n be used to prevent mother-to-child transmission during childbirth, but is not recommended after exposure to others. It is taken orally. Common side effects include rash, headache, nausea, fatigue and liver problems.

Liver problems and rash may be serious and should be monitored during the first months of treatment. It appears to be safe to use during pregnancy. It is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and blocks the activity of the reverse transcri ptase enzyme. Nevirapine was approved for medical use in the United States in 1996. It is on the World Health Organization's List of Essential Medicines **[5, 6]**.

ADVERSE EFFECT-

The most common adverse effect of nevirapine is the development of mild or moderate rash (13%). Severe or life-threatening skin reactions have been observed in 1.5% of patients, including Stevens–Johnson syndrome, toxic epidermal necrolysis and hypersensitivity. Nevirapine may cause severe or life-threatening liver toxicity, usually emerging in the first six weeks of treatment **[7]**.

In 2000, the U.S. Food and Drug Administration issued a black box warning on nevirapine, warning that it could cause life-threatening liver toxicity and skin reactions. Unacceptably high risk of serious liver symptoms in certain patient groups (women with CD4 count >250 and men >400) has led the U.S **[8]**.

DHHS to recommend the restriction of nevirapine use to those at lower risk, unless the benefit to the patient clearly outweighs the risk. Although in the 2NN study which found these CD4 limits, the effect was seen only in patients recruited from Thailand. The nevirapine drug substance is a white to off-white crystalline powder. Nevirapine is highly lipophilic. It is only slightly soluble in water (0.1 mg/ml), forming a clear colorless solution, and is relatively insoluble in non-polar media **[9, 10]**.

The nevirapine used in the formation of the tablets is anhydrous with a molecular weight of 266.3 g/mol. It is a low molecular weight compound that is lipophilic (partition coefficient = 83) and has a weak base (pKa =

2.8). At pH values below the pKa, nevirapine is very soluble in an aqueous buffer **[11, 12]**. At higher pH values, the water solubility of nevirapine decreases asymptotically to about 0.1 mg/ml. Nevirapine is a weak base because of the two pyridine nitrogens. The ionization constants measured are pKa1 = 2.8; pKa2 = −0.4. The first and second ionization constants were determined by spectrophotometry (UV) and by NMR, respectively. Nevirapine exhibits solubility in chloroform; sparingly soluble in methanol **[13]**.

MECHANISM OF ACTION-

Nevirapine belongs to the non-nucleoside reverse transcriptase inhibitor (NNRTI) class of antiretroviral drugs. Both nucleoside and non-nucleoside RTIs inhibit the same target, reverse transcriptase, an important viral enzyme that transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind to the polymerase active site, NNRTIs bind to a hydrophobic pocket in the p66 subdomain about 10 angstroms from the active site (known as the NNRTI pocket). Therefore, this NNRTI-binding pocket inhibits reverse transcription in a manner different from NRTIs. Nevirapine is not effective against HIV-2 because the reverse transcriptase pocket of HIV-2 has a different structure that confers intrinsic resistance to the NNRTI class. Resistance to nevirapine develops rapidly if viral replication is not completely inhibited **[14, 15]**.

The most commonly observed mutations after nevirapine treatment are Y181C and K103N, which are also seen with other NNRTIs. Because all NNRTIs bind in the same pocket, viral strains resistant to nevirapine are usually also resistant to the other NNRTIs, efavirenz and delavirdine. However, second-generation NNRTIs such as rilpivirine and etravirine are effective in the treatment of HIV strains resistant to nevirapine and other first-generation drugs of the same class **[16-18]**.

CHEMISTRY –

Nevirapine has two chemical names: de 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b:2',3'[1,41-diazepin-6-one and 5 , 11-dihydro-6H-11-cyclopropyl-4-methyldipyrido-[3,2-b:2',3'-e1[1,41-diazepin-6-one **[19-21]**.

The molecular formula is ClsH14N40. Nevirapine medication is a white or off-white crystalline powder.

Nevirapine is highly lipophilic. It is slightly soluble (0.1 mg/ml) in water to form a clear colorless solution and is relatively insoluble in non-polar media. The nevirapine used in the composition of the tablet is anhydrous and has a molecular weight of 266.3. Nevirapine has no possible geometric isomers, and since it has no asymmetric center, optical isomers cannot exist. Nevirapine is a weak base due to the two pyridine nitrogens **[22]**.

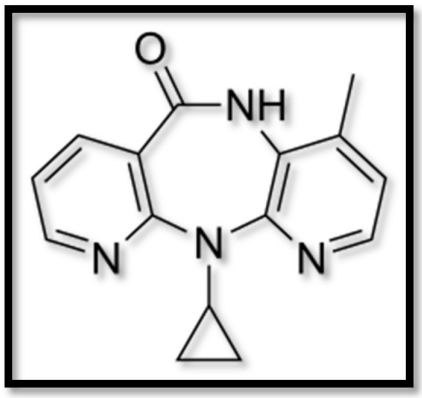

Figure 1: Chemical structure of Nevirapine

The measured ionization constants are pKa1 = 2.8; pKa2 = -0.4. The first and second ionization constants were determined spectrophotometrically (W) and NMR, respectively. Nevirapine is a very stable compound. At pH 3 and pH 11, half-lives were determined to be approximately 1200 and 700 days, respectively **[23]**.

In the stability studies of the bulk drug, no degradation of the drug was observed for five years. No special storage requirements are necessary. The shelf life of Nevirapine 200 mg tablets in plastic bottles is 30 months and the shelf life of 200 mg tablets in blister packs is 24 months if stored at 15-30^{0}C **[24]**.

The commercial 200 mg tablet is identical to the formulation used in clinical trials. Other formulations of nevirapine prepared for clinical trials include other tablets, oral suspension, intravenous injection, and oral solution prepared at the clinical site **[25]**.

PHARMACODYNAMICS

PROPERTIES-

Dipyridodiazepinonevirapine is a non-nucleoside inhibitor (NNRTI) of HIV-1 reverse transcriptase (RT). Nevirapine binds directly to the HIV-1 RT, slowing the rate of viral DNA synthesis before insertion into the host cell genome, thus preventing viral replication in acutely infected cells **[26]**.

Nevirapine inhibited the replication of several HIV-1 strains and clinical isolates in human T cells cultured in vitro with a 50% inhibitory concentration (IC50) of approximately 40 nmol/L (10.6 μg/L) as determined by cytopathic inhibition of a virus. effect It did not inhibit other retroviral RTs, including HIV-2 RT or human endogenous DNA polymerases. Nevirapine had very low cytotoxicity in uninfected human cells **[27]**.

Complete suppression of viral replication was achieved when nevirapine was added to cultured cells within 24 hours of HIV-1 infection, but activity was limited when the drug was added later. This is consistent with inhibition of the early stages of the retroviral life cycle and activity against acute HIV infection. Short-term administration of nevirapine to chimpanzees, initiated

immediately before HIV-1 vaccination, was sufficient to protect animals from productive or chronic infection, although evidence of proviral integration was observed **[28]**.

The antiretroviral activity of nevirapine was synergistic with zidovudine, lamivudine, or stavudine against wild-type HIV-1 in vitro. Nevirapine was effective alone or synergistically with lamivudine or stavudine against zidovudine-resistant virus. Nevirapine reduced the accumulation of HIV-1 reverse transcriptase in cell-free virions, which appears to be required for efficient virion infectivity. This activity affects the control of HIV transmission of cell-free virions in physiological fluids such as semen, cervical secretions, blood plasma and breast milk.

NNRTIs, including nevirapine, are associated with the rapid development of drug-resistant viral mutants when used as monotherapy in HIV-infected patients or after limited transmission of HIV-1 in the presence of an inhibitor in vitro **[29, 30]**.

The most common HIV-1 RT mutation selected by nevirapine both in vitro and in vivo is a tyrosine to cysteine change at residue 181 (Y181C). This variant is more than 100-fold less sensitive to nevirapine than the wild-type virus and confers cross-resistance to other NNRTIs. Acquisition of the Y181C mutation renders zidovudine-resistant HIV variants susceptible to NRTIs. Nevirapine-resistant variants selected for zidovudine resistance contained a valine to alanine change at residue 106 (V106A) instead of Y181C. This virus variant is resistant to both nevirapine and zidovudine **[31, 32]**.

PHARMACOKINETIC PROPERTIES-

Nevirapine pharmacokinetics in adults are characterized by rapid and nearly complete oral absorption, an apparently even distribution throughout all organs and tissues in the body, and a long elimination half-life (t1⁄2) of approximately 40 hours. The recommended adult dosage of nevirapine 200mg twice daily produced an average steady-state plasma concentration of 5.5 mg/L in healthy volunteers. An oral suspension of nevirapine has shown bioavailability similar to that of the tablet in doses up to 200mg. Nevirapine suspension was rapidly absorbed after administration of single oral doses of 7.5 to 120 mg/m2 in 9 HIV-infected children. Maximum plasma concentrations (C max) were achieved within 4 hours and reached 0.3 to 2.9 mg/L (1 to 10 μ mol/L), up to 273 times higher than the nevirapine IC50 for wild-type virus **[33]**.

Nevirapine increases its metabolism by inducing cytochrome P450 (CYP) isoenzymes (mainly CYP3A). This results in an approximately twofold increase in the systemic clearance of nevirapine in both adults and children after repeated administration for 2-4 weeks. Young children (and over 6 years of age) appear to eliminate

nevirapine more quickly than older children, suggesting that the dose should be adjusted according to age. Population kinetic analyzes indicate that nevirapine 7 mg/kg or 150 mg/m2 twice daily in children 8 years of age and 4 mg/kg or 120 mg/m2 in children 8 years and older would produce nevirapine concentrations equivalent to 200 mg in adults **[34]**.

Radioactivity studies in healthy male volunteers showed that approximately 81.3% of a total oral dose of nevirapine was excreted in the urine and 10.1% in the face, mainly as hydroxylase glucuronide metabolites **[35]**.

Nevirapine was found to cross the placenta efficiently after a single oral 200mg dose to the mother at the onset of labour. This resulted in cord blood nevirapine concentrations well above the target concentration of 100 μg/L (10 times the in vitro IC50 for HIV-1) thought to be necessary for prevention of perinatal HIV transmission. The median t1⁄2 of nevirapine in the mothers was 61.3 to 65.7 hours. In infants, median t1⁄2 was 45.4 to 72.1 hours for elimination of the maternal nevirapine dose, and 36.8 to 46.5 hours for elimination of a single 2 mg/kg neonatal dose **[36, 37]**.

CLINICAL EFFICACY-

PREVENTION OF PERINATAL HIV TRANSMISSION-

The favorable pharmacokinetic profile of nevirapine prompted its evaluation as a single-dose regimen for the prevention of late intrauterine and perinatal transmission of HIV. HIVNET 012, a phase IIB/III randomized, open-label study, evaluated the efficacy of nevirapine or ultrashort-course zidovudine in preventing HIV transmission from infected pregnant women (n = 626) to their newborns. Nevirapine treatment consisted of a single 200 mg oral tablet taken by the mother at the onset of labor and a single dose of nevirapine suspension (2 mg/kg) administered to the neonate within 72 hours after birth (median 24–30 hours). Zidovudine therapy was started with an oral dose of 600 mg at the onset of labor, followed by 300 mg every 3 hours during labor **[38]**.

A neonate received oral zidovudine syrup (4 mg/kg) twice daily for 7 days after birth. At 6-8 and 14-16 weeks postpartum, HIV infection was significantly higher in the zidovudine group than in the nevirapine group (25.1 vs. 13.1%). HIV-free survival 14-16 years. week was equally higher in the nevirapine group than in the zidovudine group (85.6 vs. 72.4%). Thus, the risk of infant perinatal HIV infection or death in the first 4 months was reduced by 47% with nevirapine therapy in this predominantly (98.8%) breastfed population **[39, 40]**.

TREATMENT OF PAEDIATRIC HIV INFECTION-

Although antiviral efficacy of nevirapine has been demonstrated in randomized controlled trials in adult patients, studies of its therapeutic use in children are more limited.

One randomized, open-label study with nevirapine included pediatric patients (ACTG 245). Antiretroviral-treated patients with advanced disease (age 6 months to 20 years; n = 432) were randomized to triple therapy with nevirapine, zidovudine, and didanosine or dual therapy with either nevirapine and didanosine or zidovudine and didanosine (no dose manipulation). An interim analysis of 136 patients in patients showed that the triple therapy group achieved significantly greater reductions in mean plasma HIV RNA levels over 48 weeks than either of the dual therapy groups. Triple therapy also resulted in a sustained reduction in CSF viral load in patients with HIV-related encephalopathy. Initiation of triple therapy with nevirapine, zidovudine, and didanosine before 4 months of age in asymptomatic or mildly symptomatic perinatally HIV-infected infants significantly reduced viral load in a phase I/II open-label study **[41]**.

Plasma HIV RNA levels decreased by 1.5 log10 copies/mL in 5 of 6 children within 2 to 4 weeks of starting therapy and remained below baseline during 6 months of therapy. Another phase I/II trial showed the efficacy of triple therapy with nevirapine, zidovudine, and lamivudine in reducing viral load ≥ 2 log10 copies/mL, which was sustained for 12 weeks in 12 of 15 children **[42, 43]**.

TOLERABILITY-

In clinical trials, nevirapine was reasonably well tolerated in children at doses of 240-400 mg/m2/day. Drug-related adverse reactions reported in pediatric studies of nevirapine were similar to those reported in adults. Rash, the most commonly reported adverse reaction, occurred in 17% of adult patients in controlled phase II/III studies and occasionally progressed to a severe or life-threatening rash (Stevens-Johnson syndrome/toxic epidermal necrolysis). In small clinical trials, rash occurred in 24% of children. Most cases occurred within the first 6 weeks of treatment; A reduced starting dose (120 mg/m2/day in children) during the first 2-4 weeks has been shown to reduce the incidence of rash during nevirapine metabolic autoinduction in both adults and children **[44, 45]**.

Granulocytopenia was the second most common adverse event in children (incidence 16%); this was the only side effect that differed from the side effects commonly reported in adults. Other commonly reported (≥ 5%) adverse reactions in pediatric clinical trials were vomiting, fatigue, nausea, nervousness, headache, dizziness, somnolence, abdominal pain, diarrhea, fever, and hyperkinesia **[46]**.

Serious or life-threatening hepatotoxicity has also occurred in patients treated with nevirapine, indicating that liver function should be closely monitored during nevirapine therapy. No serious drug-related adverse events were reported in women and infants who received a single dose of

nevirapine for the prevention of perinatal HIV infection. The incidence of rash in mothers was low (<2%) and no serious cases of rash were reported **[47, 48]**.

DOSAGE AND ADMINISTRATION-

To prevent perinatal HIV infection, HIV-infected pregnant women naïve to antiretroviral therapy may receive a single oral dose of 200 mg of nevirapine during labor. Subsequently, the HIV-infected neonate should receive a single dose of 2 mg/kg nevirapine oral suspension within 72 hours of delivery. Nevirapine is available as an oral suspension for children. The recommended dose for children aged 2 months to 8 years is 4 mg/kg once a day for 2 weeks, followed by 7 mg/kg twice a day **[49]**.

METABOLISM AND ELIMINATION-

Nevirapine increases its metabolism by inducing cytochrome P450 (CYP) isoenzymes (mainly CYP3A), which results in an approximately twofold increase in nevirapine systemic clearance during 2-4 weeks of repeated therapy. CYP3A autoinduction also results in a reduction in elimination half-life (t1⁄2) from approximately 40 hours after a single dose to <30 hours at steady state in adult patients. The ACTG 180 study showed that, as in adults, repeated dosing of nevirapine (120-240 mg/m2/day) in HIV-infected children (n = 21) resulted in a 1.5- to 2-fold increase in nevirapine clearance (CL). compared to a single dose **[50]**.

The mean CL for a single dose of nevirapine was 0.9 L/m2/h (36.8 ml/kg/h) and the terminal half-life was 30.6 hours. Children younger than 6 years had a higher CL than older children (7-14 years; CL/F = 42.6 vs 29.5 ml/kg/h), suggesting that the dose should be adjusted according to age. Radiolabeling studies in healthy male adults showed that approximately 81.3% of a total oral dose of nevirapine was excreted in urine and 10.1% in feces, mainly as hydroxylated glucuronide metabolites (primarily 2-, 3-, and 12-hydroxyvirapine glucuronides); only 3.3% of the total dose was excreted unchanged in the urine **[51, 52]**.

Table 1: HPLC approach of Drug Nevirapine

Instrument	Standard Validation Solution	Mobile phase	Columns specification	Injection volume	Reference
Method development and validation was carried out by RP-HPLC (Shimadzu) with PDA detector module with auto-sampler and data recorded using LC Solutions software.	100mg of drug and trxansfer it into 10ml volumetric flask and make up the volume up to 10ml using diluent and sonicated for 5 minutes. Pipette out 1ml of above solution into 10ml volumetric flask, make up the volume with diluent. Obtained standard concentration is 100µg/ml.	Methanol: Water 50:50	Agilent Eclipse XBD (150*4.6 * 5µm),	1 ml	[53, 54]
HPLC systems were mainly used in this work. System 1, which was primarily used, was purchased from Waters Corporation (Milford, MA) and consisted of a model 616 four solvent delivery system and controller, a 717 WISP sample injector, a model 996 photodiode array detector (PDA), and a 486 NEC (Melville, NY) computer.	Approximately 24 mg of Nevirapine reference standard was transferred into a 100-mL volumetric flask. Then, 4 mL of acetonitrile and approximately 80 mL of mobile phase were added. The flask was sonicated until all material was dissolved (as indicated by no visible powder at the bottom of the flask). The solution (100% level) was cooled to ambient temperature and then diluted to volume with mobile phase.	Methanol: Water (60:40)	Supercoil LC-ABZ (150 × 4.6 mm, 5-µm particle size, Supelco, Bellefonte, PA).	0.25 ml	[55, 56]
HPLC apparatus consisted of a Knauer (Germany) Model, 1000 HPLC pump, 2500 variable wave length UV detector, Rheodyne injector, and ezchrom elite software.	Stock solutions of ZDV, LMV, NVR, and IS were prepared by dissolving accurate amount of standard compound in methanol to obtain concentration of 100 mg/ml. Working solutions were made by diluting with appropriate volume of mobile phase. All stock solutions and working solutions were stored in polypropylene vials at 20 C.	0.1 M ammonium acetate in 0.5% acetic acid, v/v	C18 (250 mm x 4.6 mm, 5 mm particle size	0.85 ml	[57, 58]
HPLC system (Merck-Hitachi LaChrom®, USA) equipped with pump (L-7100), autosampler (L-7200), column oven (L-7300), ultraviolet (UV) detector (L-7400) and vacuum degasser (L-7612) was used for all analysis. The connection between all system components and the specific software was made by an interface D-7000 for data collection and processing (chromatographic station software, HPLC system manager, version 4.1, 1994- 2001, P/N 810866201).	250 mg of d4T into a 100 mL volumetric flask. A mixture of purified water and methanol (80:20 v/v, %) was added for solubilization. The same procedure was made for 3TC (625 mg) and AZT (625 mg). Thus, the concentration of the stock solutions prepared were 2.5 mg/mL for d4T, 6.25 mg/mL for 3TC and AZT.	acetonitrile and methanol in the ratio of 90:7:3 (v/v/v, %)	A Gemini C18 Phenomenex® column (150 mm x 4.6 mm x 5 µm)	01.00	[59]

CONCLUSION-

Nevirapine is the first representative of a new class of antiretroviral compounds, non-nucleoside reverse transcriptase inhibitors, approved for use in HW-1 -infected individuals. Although clinical endpoint data were not completed, analysis of surrogate marker data indicates that nevirapine therapy in combination with nucleosides is associated with sustained antiviral and CD4 cell counts. Nevirapine is synergistic, and can be used safely with nucleoside analogs. Resistance to nevirapine is rapid and common when is administered intravenously. When used in combination with one or more nucleosides at the recommended dose, the development of clinically relevant resistance is reduced or delayed. Nevirapine is a stable and bioavailable substance that penetrates most tissues, including the central nervous system. It has favorable pharmacokinetics, allowing to be administered twice daily, except for the predictable and usually mild to moderate rash, nevirapine is safe and very well tolerated with few other side effects.

CONFLICT OF INTEREST-

Nill

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