Molecular basis of HIV resistance to zidovudine and its detection by polymerase chain reaction (PCR)

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Zidovudine (AZT) therapy decreases both mortality and the incidence of opportunistic infections in the patients infected with human immunodeficiency virus (HIV). The resistance to AZT, however, is an important problem. This resistance is associated with the point mutations at codon 41 (Met to Leu), 67 (Asp to Asn), 70 (Lys to Arg), 215 (Thr to Phe or Tyr) and 219 (Lys to Glu) in the reverse transcriptase coding region of HIV pol gene. PCR amplification technique is used most commonly to determine these mutations. [Journal of Turgut Özal Medical Center 1(4):321-323,1994]

***Key Words**: Zidovudine , HIV, PCR..*

Zidovudine karşı HIV rezistansının moleküler temeli ve rezistansın polimeraz zincir reaksiyonu ile saptanması

Zidovudin ile tedavi, human immunodeficiency virus (HIV) ile infekte hastalarda gözlenen fırsatçı infeksiyonların insidansını ve mortaliteyi azaltmaktadır. Ancak zidovudine karşı direnç gelişimi önemli bir problem oluşturmaktadır. Bu rezistans; HIV pol gen bölgesinde revers transkriptaz enzimini kodlayan alandaki 41 (Met→Leu)'inci, 67 (Asp→Asn)'inci, 70 (Lys→Arg)'inci, 215 (Thr→Phe veya Thy)'inci ve 219 (Lys→Glu)'uncu kolonlarda meydana gelen nokta mutasyonlarla ilgilidir. Bu mutasyonların saptanmasında PCR amplifikasyon tekniği çok yaygın olarak kullanılmaktadır. [Turgut Özal Tıp Merkezi Dergisi 1(4):321-323,1994]

***Anahtar Kelimeler:** Zidovudine, PCR, HIV*

Zidovudine (3'-azido 3-deoxytymidine=AZT) is an effective agent against human immunodeficiency virus. Compounds such as AZT act as competitive inhibitor of reverse transcriptase (RT) by being incorporated into newly synthesized viral DNA in the place of the usual analogue, e.g, thymidine. When this happens, the formation of proviral DNA is arrested[1,2].

The randomized clinical studies have demonstrated that zidovudine therapy delays progression to AIDS in the individuals infected with HIV and prolongs survival in the patients with advanced HIV infection[1,3]. As with all chemotherapeutic agents, the clinical use of the AZT has prompted considerable interest in the emergence of antiviral resistance. HIV isolated from patients undergoing prolonged therapy with AZT shows reduced susceptibility[4,5]. HIV drug resistance occurs most commonly in patients with low CD_4 cells counts and with more serious forms of disease[1,3].

Molecular basis of zidovudine resistance

The phenotypic changes in AZT-resistant strains are associated with a set of point mutations in the RT-coding region of the HIV *pol* gene. Four mutations at codons 67, 70, 215 and 219 appear common. The presence of high level resistance is most often accompanied by the presence of at least three of these mutations. Recently, another mutation that apparently plays an important role in conferring high-level resistance to AZT has been identified at codon 41[1,2,6].

Introduction of these substitutions singly and in

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various combinations into the wild-type genetic background of the *pol* gene sufficed to convert these viruses from a drug-sensitive to drug-resistant phenotype[1,4,7]. Mutation at codon 70 (Lys to Arg) is often found as a first mutation, but this is replaced by a mutation at codon 215 (Thr to Phe or Tyr). Mutation at codon 41 (Met to Leu) can occur after the appearance of the mutation at codon 215. The remaining mutations at codons 67 (Asp to Asn) and codon 219 (Lys to Glu) appear less frequently[2,8]. Table I presents the mutation sites and corresponding amino acid changes thus far identified, as responsible for HIV resistance to zidovudine.

Table I. Human immunodeficiency virus type 1 pol gene mutations associated with zidovudine resistance.

Mutation site	Wild-type amino acid	Substituted amino acid
41	Methionine (Met)	Leucine (Leu)
67	Aspartic acid (Asp)	Asparagine (Asn)
70	Lysine (Lys)	Arginine (Arg)
215	Threonine (Thr)	Tyrosine (Tyr) or Phenylalanine (Phe)
219	Lysine (Lys)	Glutamine (Glu) or Glutamic acid (Gln)

Detection of zidovudine resistance

There are at least three methods used for demonstrating the existence of HIV drug resistance.

1-Determination of the 50 % of inhibitory concentration (IC_{50}) : Most of the researches on resistance of zidovudine use the 50 % inhibitory concentration to describe degree of resistance to AZT. The IC_{50} represents the concentration of a drug required to inhibit 50 % of virus replication. Before any exposure to AZT, most HIV isolates display a narrow range of zidovudine susceptibility with IC_{50} in the range of 0.01 and 0.08μM. Following initiation of therapy, IC_{50} begins to increase and by 18 months it may rise to 0.1-6.0μM[1,4]. Some investigators defined zidovudine resistance as follows: Partial resistance as an IC_{50} 0.05 to 1μM and high level resistance as an IC_{50} of more than 1.0 μM[9-11]. To determine IC_{50}, a HeLa cell line (HT4-6C) expressing the human CD_4 receptor on its surface has been used. By demonstrating that viruses from patients receiving zidovudine therapy can form syncytial foci of infection (plaques) in HT4-6C cells, even though AZT is present in the culture medium, it is apparent that resistance to zidovudine occurred[1,3,4].

2- Determination of the reverse transcriptase activity: This method is to compare reverse transcriptase activity (counts/min/ml) of HIV-1 isolates between different period of drug therapy and then contrasts reverse transcriptase activity in the absence of zidovudine[12].

3-Detection of specific mutations associated with zidovudine resistance: Many investigaters had cloned and sequenced the RT-coding region of the HIV *pol* gene and identified a number of mutations that could be related to HIV drug resistance[6,13,14]. Polymerase chain reaction (PCR) technique is used to determine these specific mutations. Total proviral DNA is extracted from cells infected with HIV and the specific fragment of the reverse transcriptase coding region that encompasses all the point mutations is amplified by PCR. After amplification, the specific mutations can be detected by one of the following ways.

A-Second amplification with mutant selective primers.
B-Hybridization with mutant specific probes.
C-Cloning and nucleotide sequence analysis.

Second amplification with mutant selective primers: Amplification product from first PCR is reamplified by using two master mixes per codon containing a wild-type or mutant selective primer paired with a common primer. Amplification products are visualized by running approximately 10μl of the sample in agarose gel containing 1μg of ethidium bromide per ml. Each sample is scored as wild-type, mutant and mixture according to the presence of specific DNA bands[6,8].

Hybridization with mutant specific probes: Blots are prepared in duplicate for hybridization with probes specific for wild-type or mutant. PCR amplification product is resolved by electrophoresis using agarose gel, then it transfers to nylon membranes. After denaturation and neutralizing, hybridization is done with kinased probes specific for wild-type or mutant type. Then, auto radiography is used for detection of hybridization. Hybridization observed with mutant specific probe means that there are point mutations at this codon[9].

Cloning and nucleotide sequence analysis: After amplification with PCR, cloning is done to increase the amount of DNA. T-vector can be used for this purpose. This vector has protruding " *T*" ends and can be ligated with PCR amplification product which has protruding "*A*" ends, directly. Following ligation, T-vector is transformed into competent cells (Escherichia coli MC1061) which can not grow on ampicillin plates (50 μg/ml), without recombinant

T-vector. Colonies on ampicillin plates are picked up and examined with colony hybridization for identification of correct recombinant constract. Restriction enzyme analysis also can be used for this purpose. DNA is extracted from the right clones and single-stranded DNA is prepared for sequencing. The dideoxynucleoside chain termination method is used for sequencing[15]. Mutations in RT-coding region can be demonstrated by the way of comparing the sequencing results of the wild type HIV-1 gene and those of new isolates.

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