ABSTRACT

Most of the anticancer molecules are supposed to induce their effects at the DNA level through intercalation. The work presented here aimed at evaluating the anticancer potency of three structural analogues of Pyrimido[4',5':4,5]selenolo (2,3-b)quinoline (PSQ) series having 4-amino and 4-benzylamino groups at 4th position (APSQ and BAPSQ) and 8-methyl-4-(3-diethylaminopropylamino) group at 8th position (MDPSQ) which are structurally related to ellipticine (a known anticancer drug). The entire work is presented in two parts: (i) evaluation of the biophysical interaction of PSQ analogues with DNA and (ii) enumeration of the biological impact of the interaction in terms of cytotoxicity.

Biophysical interaction was studied employing Circular dichroism, hydrodynamic methods, absorbance and fluorescence titrations. On binding to DNA, the absorption spectrum underwent bathochromic and hypochromic shifts, the fluorescence intensity was quenched. These compounds were able to bind to DNA with an affinity of about $10^4$-$10^5M^{-1}$ for calf thymus DNA at ionic strength of 0.01M and their intercalating characteristic (lengthening of the DNA) depends upon the length of the chain. Binding to the GC-rich DNA micrococcus lysodeikticus was stronger than the binding to calf thymus DNA at ionic strength 0.01M. The experiments also show that electrostatic binding played an important role in the interaction of these compounds with DNA. An increase in viscosity was observed in sonicated DNA fragments upon addition of PSQ analogues indicating DNA helix extension due to monointercalation ($m$ values ranging from 1.02 to 1.39). The DNA melting studies clearly showed that the value of $T_m$ of calf thymus DNA was increased from 3 °C to 7 °C with a single transition. On addition of APSQ,
BAPSQ and MDPTQ to a solution of the DNA, significant bathochromic and hypochromic shifts were observed in their respective CD spectra. Such changes are likely to result from structural alterations induced by the drug into the polynucleotide helical structure. Further the positive CD signal in the range of 320 to 350nm suggests an intercalation of PSQ analogues between adjacent base pairs with its long axis perpendicular to the long axis of the DNA helix.

Biological impact of the intercalation of PSQ analogues into DNA was analyzed using various techniques in vitro. Cytotoxicity of these compounds was tested on six tumor cell lines. Significant decrease in colony forming efficiency was observed when compared to untreated controls and normal bone marrow cells. IC_{50} values obtained from MTT assay ranged from 46 – 650 μM. All three PSQ analogues induced cytotoxicity in all six cell lines in a time and dose dependent manner, but their effect of leukemic cell lines K-562 and REH were highly significant. All three PSQ analogues induced significant micronuclei formation (CBPI values ranging from 0.437- 0.672) and comet with long tails. BAPSQ and MDPSQ molecules induced higher micronuclei formation while MDPSQ induced longer tail comet formation when compared to APSQ. Treatment with PSQ analogues lead to increased generation of ROS and fall in mitochondrial membrane potential (MMP) in both K-562 and REH cells. MDPSQ and BAPSQ induced higher levels of ROS generation than APSQ in K-562 cells when compared to REH cells. A marked fall in MMP was observed in both cell lines on treatment with APSQ and BAPSQ in a time dependent manner when compared to MDPSQ. All three PSQ analogues exhibited very small (not significant) increase in caspase 3-activity in both cell lines when compared to their controls. Similarly very small
increase in PARP cleavage was observed on treatment with PSQ analogues. No significant cell cycle arrest was observed. Fluorescence cytochemical studies using AO-ETBR staining method exhibited large number of orange to red stained cells in all treated groups indicating typical morphological features of necrosis and late apoptosis. Externalization of phosphatidyl serine was assessed using Annexin V-Alexa fluor/ PI double staining and found that a significant increase in Pt cells and Pt⁺- Annexin⁺ (double positive) was observed in all treatment groups in both cell lines, suggesting necrosis as mode of cell death due to complete disruption of cell membrane. This was further supported by significant increase in LDH release by all three PSQ molecules in both cell lines.

To support the above findings expression of various proteins involved in DNA double strand break repair (Ku80, DNAPKcs, MRE11, RAD50 and NBS1), apoptotic pathways (Caspase 3, Caspase 9, Bcl-2, FAS) and DNA damage check point proteins (p53, p73) were checked in K-562 cells upon treatment with MDPSQ. A time dependent increase in Ku80, DNAPKcs, MRE11, RAD50 and NBS1 was observed that confirms DNA damage and fragmentation. A weak PARP cleavage, Caspase 3 and 9 activation was observed. Levels of Bcl-2 did not change significantly, while expression of FAS ligand exhibited a significant down regulation, suggesting exclusion of intrinsic or extrinsic apoptosis. Further complete down-regulation of p53 and p73 DNA (damage sensor proteins that lead to apoptotic pathway) was observed.

In conclusion various lines of experimental evidences suggest that MDPSQ, APSQ and BAPSQ activates necrosis to induce cytotoxicity in K-562 and REH cells.