Korean J. Environ. Biol. 23(4) : 379~382(2005)

DNA Damage Induced by New Pophyrins of Different Chemical Structure

Hovhannisyan Galina, Haroutiunian Samvel[1], Margaryan Kristina, Ghazaryan Robert[2], Aroutiounian Rouben*

Department of Genetics and Cytology; [1]Department of Molecular Physics, Yerevan State University, Yerevan, Armenia;
[2]Department of Pharmacological Chemistry, Yerevan Medical State University, Yerevan, Armenia

Abstract - The new cationic meso-substituted N-quarternized 4-pyridylporphyrins and their metal derivatives were synthesized as novel chemotherapeutics. The level of DNA damage induced by porphyrins TOEt4PyP, TOBut4PyP, TOEt4PyPMn and TOBut4PyPMn and its dependence on the chemical structure of compounds were analyzed by the Comet-assay. On the base of data obtained, the investigated porphyrins may be arranged by their genotoxic activity in the following order: TOEt4PyP>TOEt4PyPMn>TOBut4PyP>TOBut4PyPMn. Thus, i) the genotoxicity of the Mn-derivatives of TOEt4PyP and TOBut4PyP is higher than the original porphyrins and ii) the genotoxicity of TOEt4PyP and TOEt4PyPMn is increased after substitution of a butyl radical for ethyl one. The applied Comet-assay permits to reveal the dependence of DNA damage induction on the chemical structure of porphyrins.

Key words : Porphyrins, Comet-assay, human leukocytes, DNA strand breaks

INTRODUCTION

Porphyrins are applied in the cancer therapy. Several porphyrins have been proposed as highly promising boron delivery agents for the boron neutron capture therapy of tumors (Barth *et al.* 1999; Vicente 2001).

Porphyrins were shown to be effectively accumulated within tumor cells for long period of time (Vicente, 2001). This feature of porphyrins is the basis for their use in another binary method, the photodynamic therapy (PDT) of tumors. PDT is based on photoactivation at a given wavelength of sensitizer selectively retained by cancer cells, which produces reactive oxygen species that can destroy tumor cells (Dougherty *et al.* 1998).

*Corresponding author: Aroutiounian Rouben
Tel. +37-410-55-2352, Fax. +37-410-55-4641,
E-mail. genetik@ysu.am

A series of cationic porphyrins has been identified as G-quadriduplex interactive agents that stabilize telomeric G-quadriduplex and thereby inhibit human telomerase (Izbichka *et al.* 1999). A significant level of telomerase activity has been detected in >85% tumors (Kim *et al.* 1994). Thus, the telomerase presents a target with a potentially good selectivity for tumor over healthy tissue, and telomerase inhibition has been proposed as a new approach to the cancer therapy (Morin 1995).

The new porphyrins and their metal derivatives applied in our research were synthesized as potential chemotherapeutics (Ghazaryan *et al.* 2004a, b). In our research their capacity to induce DNA damage was investigated by the Comet-assay (single cell gel electrophoresis), based on the principle of quantifying the amount of DNA fragments migrating out of the cell nuclei during electrophoresis. This technique is commonly used in pharmaceutical industries as a standard tool to assess the safety of new preparations.

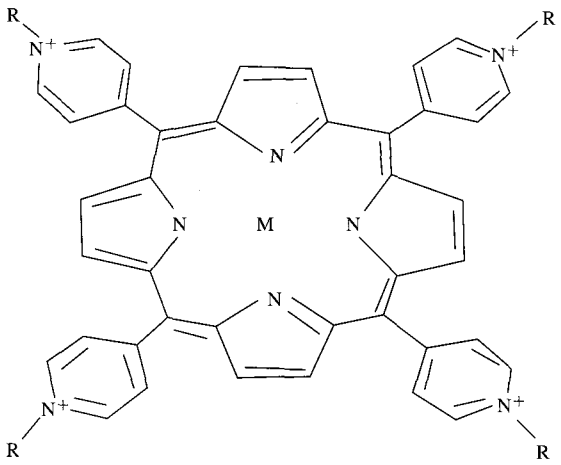

Fig.1. Schematic structure of porphyrins investigated (R = CH_2-CH_2-OH in TOEt4PyP, R = CH_2-CH_2-CH_2-CH_3 in TBut4PyP, M = Mn in the Mn-derivatives of TOEt4PyP and TBut4PyP).

The aim of the present study was to evaluate potential genotoxicity of new porphyrins of different chemical structure by the Comet-assay in human leukocytes.

MATERIALS AND METHODS

1. Cells

Human peripheral blood samples were obtained from the same healthy female 25 years old donor.

2. Test chemicals and treatment of cells

The schematic chemical structure of new cationic meso-substituted N-quarternized 4-pyridylporphyrins: TOEt4PyP (tetraoxyethyl-4-pyridylporphyrin), TBut4PyP (tetrabuthyl-4-pyridylporphyrin) and their Mn-derivatives used in our study is shown in Fig. 1.

Test drug candidates were supplied from Department of Pharmaceutical Chemistry (Medical State University Yerevan, Armenia). The structure of synthesized compounds was determined by the nuclear-magnetic resonance (NMR) and electronic adsorption spectroscopy. The optimal range of concentrations investigated and exposure time were determined in the preliminary experiments. All substances were dissolved in water.

3. Comet-assay

The Comet-assay was performed under alkaline conditions according to the procedure of Singh *et al.* (1988). Whole human blood was treated for 2 h with aliquots of porphyrins solutions to the final concentration: 10^{-7}, 10^{-6} and 10^{-5} M and additionally 10^{-8}, 10^{-9} and 10^{-10} only for MnTBut4PyP. Then 10 µL of blood was dissolved in 0.5% LMA and spreaded on microscope slides precoated with 1% normal melting agarose. Untreated cells were used as control variants. Then the cells were lysed for 1 hour at 4°C in a solution consisting of 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, pH 10. The slides were placed in a horizontal electrophoresis box allowing DNA to unwind for 40 min in electrophoresis buffer consisting of 300 mM NaOH, 1 mM EDTA, >pH 13. Electrophoresis was conducted at 4°C for 25 min in electric field at 26 V, 300 mA. The slides were then neutralized with 0.4 M Tris, pH 7.5 and stained with 20 µg mL^{-1} ethidium bromide.

4. Comet scoring

The comets were observed at 360 × magnification under a fluorescence microscope equipped with 560 nm excitation filter and 590 nm barrier filter. For each dose at least 150 cells, 50 cells from each of three replicate slides were examined.

The image analysis program CASP was used to analyze various Comet measurement parameters. The measures chosen for presentation were % of DNA tail (relative tail fluorescence intensity) and tail moment (DNA product in the tail multiplied by tail length).

5. Statistical analysis

The significance of the effect of each treatment dose versus the control was evaluated by the nonparametric Mann-Whitney test. This test was performed on tail moment only and treatment groups were considered significantly different from the control at $p < 0.05$.

RESULTS AND DISCUSSIONS

Data on % DNA in tail are presented in Fig. 2. Data on Tail moment are presented in Tables 1 and 2. TOEtPyP at concentrations 10^{-5} and 10^{-6} M induced a significant increase in the tail moment ($p < 0.05$). Concentration 10^{-7} M was not genotoxic for human leukocytes. TButPyP induced statis-

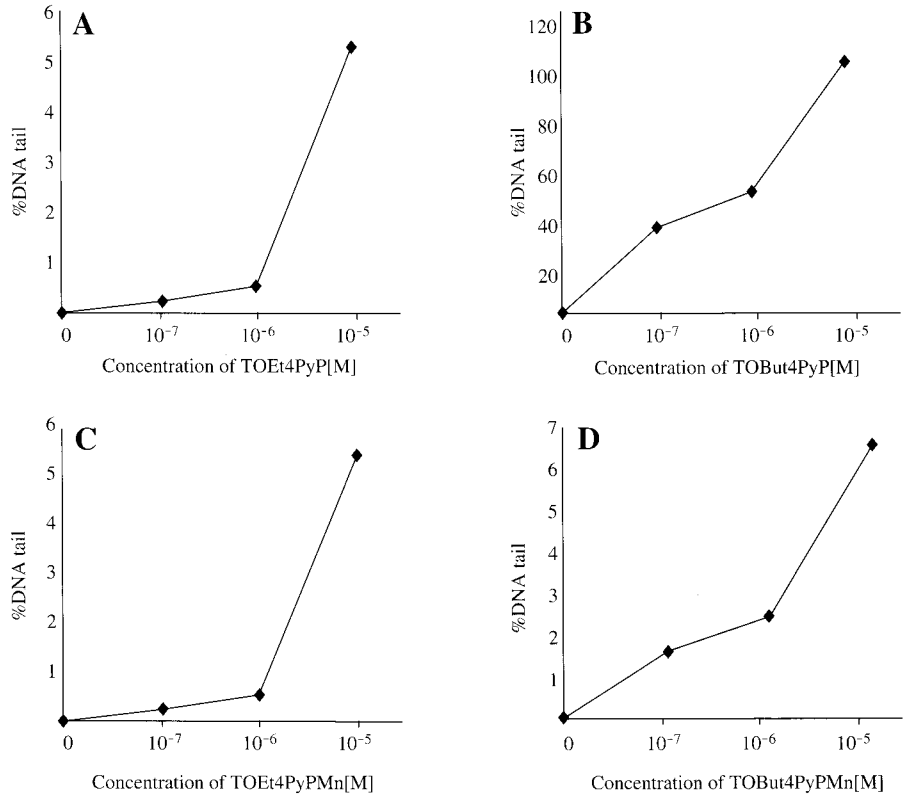

Fig. 2. Percentage of DNA in the tail of human leukocytes treated with TOEt4PyP (A), TBut4PyP (B), MnTOEt4PyP (C) and MnTBut4PyP (D).

Table 1. Genotoxic effect of TOEt4PyP and TBut4PyP on human leukocytes

Treatments	TOEt4PyP	TBut4PyP
	Mean ± SE	Mean ± SE
Control	0.04 ± 0.01	0.04 ± 0.01
10^{-7} M	0.10 ± 0.02^{ns}	71.87 ± 4.93*
10^{-6} M	0.30 ± 0.06*	157.06 ± 6.02*
10^{-5} M	2.67 ± 0.68*	100% DNA degradation

Results are presented as tail moment.
Ns, Not significantly different from the control.
*, Significantly different from the control ($p < 0.05$).

Table 2. Genotoxic effect of MnTOET4PyP and MnTBut4PyP on human leukocytes

Treatments	MnTOEt4PyP	Treatments	MnTBut4PyP
	Mean ± SE		Mean ± SE
Control	0.09 ± 0.03	Control	0.11 ± 0.02
10^{-7} M	0.06 ± 0.01^{ns}	10^{-10} M	0.09 ± 0.12^{ns}
10^{-6} M	0.47 ± 0.09*	10^{-9} M	0.14 ± 0.14^{ns}
10^{-5} M	13.35 ± 0.00*	10^{-8} M	0.72 ± 0.73*

Results are presented as tail moment.
Ns, Not significantly different from the control
*, Significantly different from the control ($p < 0.05$)

tically significant increase in the tail moment at 10^{-6} and 10^{-7} M ($p < 0.05$). At the highest dose (10^{-5} M) it destroyed the majority of DNA in cells. TOEt4PyPMn at high doses (10^{-5} and 10^{-6} M) caused a significant increase in the tail moment ($p < 0.05$). At 10^{-7} M porphyrin was ineffective in the induction of DNA damage.

In the cells treated with TBut4PyPMn at the concentrations 10^{-5}, 10^{-6} and 10^{-7} M the total degradation of DNA in majority of cells in all variants was revealed. That is why the concentrations 10^{-8}, 10^{-9} and 10^{-10} M were additionally tested. TBut4PyPMn induced a significant increase of DNA damage only at concentration of 10^{-8} M ($p < 0.05$).

The comparison of genotoxicity of different porphyrins showed that TBut4PyP at 10^{-6} and 10^{-7} M induced the significantly higher level of DNA damage than TOEt4PyP. TBut4PyP at 10^{-5} M completely destroyed DNA, whereas TOEt4PyP at the same concentration induced comet with a tail moment of 2.66 ± 0.67.

The Mn-derivative of TOEt4PyP is more genotoxic compared with the original porphyrin TOEt4PyP only at

the highest concentration studied (10^{-5} M). Our results indicate that all the porphyrins are genotoxic in the most of applied concentrations in human leukocytes as assessed by the Comet assay.

The investigated porphyrins may be arranged by their genotoxic activity in the following order: TOEt4PyP < TOEt4PyPMn < TBut4PyP < TBut4PyPMn. Thus, i) the genotoxicity of the Mn-derivatives of TOEt4PyP and TBut4PyP is higher than original porphyrins and ii) the genotoxicity of TOEt4PyP and TOEt4PyPMn is increased after substitution of a butyl radical for oxyethyl one.

The results obtained showed the complete fragmentation of chromatin and formation of cells with non-detectable nuclei (NDCN) in variants treated with TButPyP at 10^{-5} M and TBut4PyPMn at 10^{-7} M, 10^{-6} and 10^{-5} M. In the Comet-assay this morphology is typical for dead or dying cells and, according to the recommendation of Hartman *et al.* (2001), can be used together with other tests to obtain information about cytotoxicity.

It can be supposed that the chemical structure, size and conformation of TBut4PyPMn are the best among porphyrins investigated to permit its penetration into the cell and/or interaction with DNA.

The Comet-assay application permits to make preliminary conclusion concerning the dependence of DNA damage induction on the chemical structure of the new porphyrins. The results obtained can be useful for further synthesis of porphyrins with desired properties.

ACKNOWLEDGEMENTS

This study was supported by ISTC grant A-301.2.

REFERENCES

Barth RF, AH Soloway, JH Goodman, RA Gahbauer, N Gupta, TE Blue, W Yang and W Tjarks. 1999. Boron neutron capture therapy of brain tumors: an emerging therapeutic modality. Neurosurgery 44:433-451.

Dougherty TG, CJ Gomer, BW Henderson, G Jori, D Kessel, M Korbelik, J Moan and Q Peng. 1998. Photodynamic therapy, J. Natl. Cancer Inst. 90:889-905.

Ghazaryan RK, AG Tovmasyan, RH Hakobyan, SG Vardanyan, A Shahkhatuni and TS Kurtikyan. 2004. Synthesis of the meso-substituted pyridyl-phenyl metalloporphyrins-specific sensors and adsorbents. p. 125. In Abstracts of 7th International seminar "Scientific Advances in Chemistry: Heterocycles, Catalysis and Polymers as Driving Forces" Ekaterinburg. Russia.

Ghazaryan RK, LA Sahakyan, AG Tovmasyan, SG Vardanyan, A Gazaryan, L Aloyan, GV Gyulkhandanyan and VE Hovhannesyan. 2004. Synthesis of new water-soluble cationic porphyrins and their catalytic and biological activity. p. 40. In Abstracts of 7th International seminar "Scientific Advances in Chemistry: Heterocycles, Catalysis and Polymers as Driving Forces" Ekaterinburg. Russia.

Hartman A, E Kiskinis, A Fjallman and W Suter. 2001. Influence of cytotoxicity and compounds precipitation on test results in the alkaline comet assay. Mut. Res. 497:199-212.

Izbichka E, RT Wheelhouse, E Raymond, KK Davidson, RA Lawrence, D Sun, BE Windle, LH Huley and DD Von Hoff. 1999. Effects of cationic porphyrins as G-quadruplex interactive agents in human tumor cells. Cancer Res. 59:639-644.

Kim NW, AM Piatyszek, KR Prowse, CB Harley, MD West, PLC Ho, GM Coviello, WE Wright, SL Weinrich and JW Shay. 1994. Specific association of human telomerase activity with immortal cells and cancer, Science 266: 2011-2015.

Morin G. 1995. Is telomerase a universal cancer target? J. Natl. Cancer Inst. 87:859-861.

Singh PN, TM McCoy, RR Tice and EL Schneider. 1988. A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res. 175:184-191.

Vicente MGH. 2001. Porphyrin-based sensitrizers in the detection and treatment of cancer: recent progress. Current Med. Chem., Anti-Cancer Agents 1:175-194.

Manuscript Received: October 26, 2005
Revision Accepted: November 14, 2005
Responsible Editorial Member: Jin Kyu Kim (KAERI)