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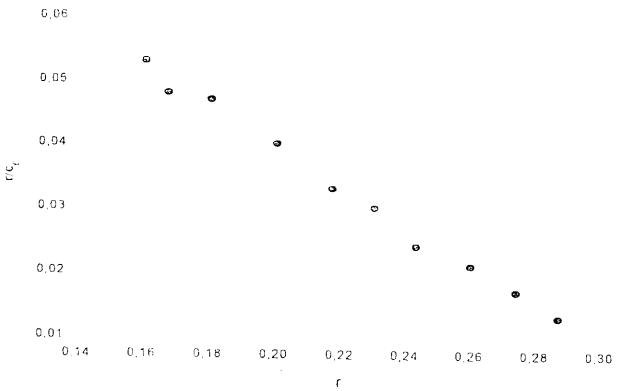

Figure 2. The analysis of experimental data with adsorption isotherm of Krozers-Gursky. (6). The values of r/c_f are plotted on ordinate axis in M^{-1}.

It should be noted that the proposed method for determination of K and n within the domains of low fillings is less effort-consuming and costly and does not require large quantities of ligand agent and DNA. It should be also noted that the proposed method is much faster if you need to scan many types of agents and determine their binding constant or to determine the number of base pairs n that are bound with one ligand molecule.

References:

1.Crothers D. Biopolymers. 1968, v.6, p.574-580.
2.Zasedatelev A.S., Gurski G. V., Volkenshtejn M. V.,Mol. Biol.(Russia). 1974, v.5, p.245-252.
3. McGee J.D., Von Hoppel P.H.J.Mol.Biol. 1974, v. 86, p.469-489.
4.Nechipurenko Y.D.,Gurski G.V., Biophisical Chemistry. 1986, v. 24, p.195-209.
5.Chaires J.B. Struct. Biol. 1998, v.8, p.314-320.
6.Krugh T.R. Curr. Opin. Struct. Biol. 1994, v. 4, p.351-364.
7.Vardevanyan P. O., Antonyan A. P., et al. Exp. Mol. Medicine, 2001, v.33, N4, p.205 – 208.
8.Arakelyan V.B., Babayan S.Yu., et al. // J. of Biomol. Struct. and Dyn., 2006, v.23, N4, p. 479-483.
9.Vardevanyan P.O., Antonyan A.P., et al. // Exp. and Mol. Medicine, 2003, v. 35, N6, p. 527-533.
10.Minasyan S.G., Tavadyan L.A., Davtyan H.G., Parsadanyan M.A., Antonyan A.P., Vardevanyan P.O.//Bioelectrochemistry, 2006, v.68, p.48-55.
11.Haroutiunian S.,Diss. doct.phys.-mat. sci., 1993, Yerevan.

12. Babayan Yu.S., Manzini G., Quadrifoglio F. Mol. Biol. (Russian). 1988, v.22, p.898-910.
13.Babayan Yu.S., Manzini G., Quadrifoglio F. Mol. Nucleic Acids Res.1987, v.15, p.5803-5812.
14.Nechipurenko, Y.D., Zasedatelev, A.S., Gurski, G.V., *Mol. Biol. (Russia) 18*, 798-812 (1984).
15.Arakelyan V.B., Babayan Yu.S., Potikyan G. Journal Biomolecular Structure and Dynamics, 2000, v.18, N2, p.231-235.
16.Strothkamp K.G., Strothkamp R. J. Chem. Educ. 1994, v.71, p.77.
17.Waring M.J. J. Mol. Biol. 1965, v. 13, p.269-282.
18.LePeeq J.B., Paoletti C. J. Mol. Biol. 1967, v. 27, p.87-106.
19.Alonso A., Almendral M.J., Curto Y., Criado J.J., Rodriguez E., Manzano J.L. Analyticaql Biochemistry. 2006, v.355, p.157-164.
20.Cantor Ch., Schimmel P., Biophysical chemistry, W.H.Freeman and company, 1980, Part 3, P.354.

ASSESSMENT OF GENOTOXICITY OF POTENTIAL PHARMACEUTICAL PREPAPATIONS: *in vitro - in vivo* APPROACH

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Introduction

Application of alternative approach based on *in vitro* models instead experimental animal tests is known to provide valuable information about potential toxicity of any substance investigated. In the fields of toxicology and drug discovery and development numerous programs of identification of novel potential antitumor agents using batteries of tumor cell lines were developed. In this context normal cells *in vitro* can be applied to predict possible side effects. Selected promising compounds should be then evaluated *in vivo* to precise the mode of their action in the organism.

The application of known parallelogram approach permits to predict the risks of chemicals for humans by extrapolation of data from animal studies [1]. In this approach, the data obtained *in vitro* can be compared with or partially substituted for animal-derived data. The application of human cells *in vitro* allows comparing between the

DNA damage was microscopically quantified. Images were captured to a PC running Komet 4 software (Kineticimaging, UK). The % DNA in tail was detected to estimate level of DNA damage. The data presented in means and medians, but statistics are carried out on median values.

Statistical analysis

The comparison of data obtained was realized using a Mann-Whitney test.

Results

The experimental results about levels of DNA damage induced with porphyrins investigated in human and rat lymphocytes *in vitro* and in rat lymphocytes *in vivo* are shown in Tables 1, 2 and 3.

Table 1. Levels of DNA damage in human leukocytes treated for 2h *in vitro* with Ag and Zn-derivatives of TOEtPyP

Porphyrins	% DNA Tail	
	Mean ± SE	Median
AgTOEtPyP		
0M	1.83 ± 0.12	1.55
10^{-7}M	1.96 ± 0.14	1.67 ^{ns}
10^{-6}M	2.19 ± 0.16	1.79 ^{ns}
10^{-5}M	18.78 ± 0.69	16.97*
ZnTOEtPyP		
0M	2.03 ± 0.12	1.72
10^{-7}M	2.68 ± 0.19	1.99 ^{ns}
10^{-6}M	5.64 ± 0.22	5.41*
10^{-5}M	23.97 ± 0.77	23.63*

Table 2. Levels of DNA damage in rat leukocytes treated for 2h *in vitro* with Ag and Zn-derivatives of TOEtPyP

Porphyrins	% DNA Tail	
	Mean ± SE	Median
Control		
1	6.38 ± 0.87	4.34
2	6.11± 0.86	5.10
3	6.46 ± 0.91	5.44
AgTOEtPyP-10^{-7}M		
1	14.47 ± 1.27	12.54*
2	11.31 ± 1.38	8.85*
3	8.61 ± 0.93	7.02*
AgTOEtPyP-10^{-6}M		
1	21.97 ± 1.85	19.69*
2	18.14 ± 1.40	16.87*
3	20.63 ± 1.94	16.82*
AgTOEtPyP-10^{-5}M	Total DNA degradation	
ZnTOEtPyP-10^{-7}M		
1	13.44 ± 1.34	12.27*
2	13.20 ± 1.53	10.66*
3	10.18 ± 1.44	8.85*
ZnTOEtPyP-10^{-6}M		
1	25.21 ± 2.23	23.86*
2	23.71 ± 2.15	20.64*
3	23.73 ± 2.17	20.38*
ZnTOEtPyP-10^{-5}M	Total DNA degradation	

Table 3. Levels of DNA damage leukocytes of rats exposed for 10 days *in vivo* to 15mg/kg of ZnTOEtPyP and 10mg/kg of AgTOEtPyP

Porphyrins	% DNA Tail	
	Mean ± SE	Median
Control		
1	4.21 ± 0.74	3.16
2	4.39 ± 0.77	3.22
3	4.47 ± 0.82	3.49
AgTOEt4PyP		
1	8.64 ± 1.54	5.11 *
2	7.74 ± 1.27	4.97 *
3	7.21 ± 1.02	4.67 *
ZnTOEt4PyP		
1	7.85 ± 1.43	4.54*
2	8.39 ± 1.27	6.22*
3	7.22 ± 1.16	5.43*

* Significantly different from control ($p<0.05$)

ns Not significantly different from control ($p>0.05$)

1, 2, 3 serial numbers of animals

Human lymphocytes in vitro. AgTOEtPyP significantly increased the level of % DNA tail in human lymphocytes *in vitro* only at highest concentration (10^{-5}M). ZnTOEtPyP displayed its genotoxic properties at doses 10^{-6} M and 10^{-5}M.

Rat lymphocytes in vitro. In rat lymphocytes both porphyrins induced significant increase of DNA damage at concentrations of 10^{-5} and 10^{-6}M and provoked total degradation of DNA at the highest concentration (10^{-5}M).

Rat lymphocytes in vivo. After treatment of rats *in vivo* with porphyrins the % DNA in tail was significantly increased for ZnTOEtPyP and AgTOEtPyP for all animals.

Discussion

In vitro approach is widely applied for hazard estimation and risk assessment of genotoxic compounds. At the same time it is clear that results of this approach is not final and exhaustive. The *in vitro* data on genotoxic effects caused by an agent are not necessarily in agreement with the response of the whole organism. That is why the *in vitro* investigation should be combined and supplemented with animal tests. In this case results of both tests can be compared and extrapolated by the parallelogram approach [1]. It allows collating results of any experiments, in particular cultured cell- and animal-derived data. The differences between *in vitro* and *in vivo* situation were taken into account in these comparisons. The results of this analysis can be used for the extrapolation of results to humans.

The data obtained by the Comet assay *in vitro* and *in vivo* are supposed to be used for extrapolation by parallelogram approach.

In human and rat cells *in vitro* AgTOEtPyP and ZnTOEtPyP displayed certain level (up to 25% DNA tail) of genotoxicity. At the same time we revealed considerable difference between sensitivity of human and rat blood cells toward porphyrins at identical experimental conditions. The increase of DNA damage was recorded at lower concentrations of AgTOEtPyP and ZnTOEtPyP in rat cells compared with human ones. The highest concentration of porphyrins (10^{-5} M) induced total degradation of DNA in rat cells. This difference reflects variations in DNA sensitivity of different species.

In rats *in vivo* AgTOEtPyP and ZnTOEtPyP at IC50 concentrations also displayed genotoxic effects. The results obtained suggested

that both porphyrins are about 3 times less genotoxic for rat cell *in vivo* than *in vitro* conditions.

The discrepancy between *in vivo* and *in vitro* models could be explained by metabolic and mechanistic issues and should be taken into consideration for future extrapolations.

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REFERENCES

1. Blaauboer BJ, Wortelboer HM, Mennes WC: ATLA, 1990, 18, 251–258.
2. Pool-Zobel BL, Lotzmann N, et al.: Environmental and Molecular Mutagenesis, 1994, 24, 23–45.
3. Pool-Zobel BL, Leucht U: Mutation Research, 1997, 375, 105–116.
4. Sobels FH: Archives of Toxicology, 1980, 46, 21-30.
5. Gasparyan G, Hovhannisyan G, et al.: International Journal of Toxicology, 2007, 26, 1-6.
6. Tice RR, Agurell E, Anderson D et al.: Mol. Mutagen. 2000, 35,206-221
7. Ghazaryan RK, Sahakyan LA, Tovmasyan AG: Patent of the Republic of Armenia No 1715 A2. 2006.
8. Singh NP, McCoy MT, Tice RR, Schneider EL: Exp. Cell Res., 1988, 175, 184-191.
9. Hartmann A, Agurell E, et al.: Mutagenesis, 2003, 18, 1, 45-51.

BIOLOGY, GENETIC VARIABILITY AND BIOTECHNOLOGICAL APPLICATION OF SEVERAL MEDICINAL MUSHROOMS

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Introduction:

Extensive research on biology and genetics of Basidiomycetes mushrooms has markedly increased owing to their potential use in biotechnology. Mushrooms are rich in proteins, carbohydrates, fibers, unsaturated fatty acids, vitamins, minerals and considered a very good dietary food [1]. They are also regarded as sources of a wide range of bioactive metabolites (polysaccharides, glucans, terpenoic, indolic and phenolic compounds, etc.) and different enzymes used in medicine, food and cosmetic industries.

Since ancient times, anti-inflammatory, analgesic, blood-coagulating and wound-healing properties of several mushrooms (*Ganoderma lucidum, Lentinula edodes, Coriolus versicolor, Cordyceps sinensis, Grifola frondosa, Auriculeria auricula- judae,* and *Schizophyllum commune*) were recognized in China, Korea,