Poster Session 2 – Pharmacology

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Antioxidant activity of 5-arylidene-2,4-thiazolidinedione-3-alkanoic acid derivatives

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Initiation of free-radical lipid peroxidation is a trigger in various oxygen-deficient processes, which play a main role in many diseases states. Drugs with antioxidant mechanisms are widely proposed as bases for development of new approaches for pharmacological regulation of peroxidative-antioxidative homeostasis.

To reach this aim, a number of 5-arylidene-4-thiazolidone-3-alkanoic acids were synthesized as potential antioxidants (Zimenkovsky et al 1998; Lesyk et al 2001). They were studied in a special series of screening investigations using the model of unsaturated fatty acid methyl esters autooxidation initiated by Fe^{2+} ions (Fernandez et al 1982). Samples of the incubation medium were selected at 20-, 40- and 60-min intervals after initiation of lipid peroxidation by Fe^{2+}. Lipoperoxides were detected by the degree of active products accumulating, by their reaction with thiobarbituric acid (TBA) (Ohkawa et al 1979). The results of the screening are listed in Table 1.

Table 1 In-vitro comparative evaluation of antioxidant activity of studied compounds

Compound	Concn of lipoperoxides after Fe^{2+} addition (mmol $L^{-1} \times 10^{-3}$)			
	0 min	20 min	40 min	60 min
C. 1	193 ± 8	243 ± 17	258 ± 13	320 ± 23
R.s.	152 ± 12	135 ± 6	168 ± 9	265 ± 2
Les 1	59.8 ± 8	150 ± 9	122 ± 11	205 ± 7
Les 2	247 ± 13	240 ± 10	258 ± 10	200 ± 12
Les 3	138 ± 4	166 ± 5	193 ± 12	297 ± 11
C.2	213 ± 2	157 ± 5	222 ± 1	224 ± 2
R.s.	227 ± 9	252 ± 2	175 ± 3	123 ± 7
Les 4	132 ± 9	280 ± 13	352 ± 9	153 ± 9
Les 5	77 ± 1	267 ± 3	288 ± 15	173 ± 10
Les 6	103 ± 4	371 ± 4	257 ± 19	166 ± 11
Les 10	161 ± 5	367 ± 10	248 ± 30	190 ± 16
Les 11	19 ± 11	415 ± 14	525 ± 17	210 ± 9

0 min, evaluation of iBA – products in peroxidative system before Fe^{2+} addition. Controls (C.1 – water, C.2 – dimethyl sulfoxide). R.s. (reference substance) – tocopherol acetate

Analysis of obtained results revealed that the most active compound was Les-1 since it possessed the highest antioxidative activity (in comparison with standard compounds) and low toxicity for warm-blooded animals. Les-1 belongs to the group of 5-phenylpropenylidene-2,4-thiazolidinedione-3-alcanoic acids. Results of the experiment allowed the identification of possible pharmacophores, among which, the 5-phenylpropenylidene fragment is the most active antioxidant. On the basis of the lead compound, a group of related compounds was synthesized for detailed elaboration of structure–activity relationship and modelling of optimal chemical structure for antioxidative activity.

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Lesyk, R., et al (2001) *Farm. Zhurnal* **2:** 57–61 (in Ukrainian)
Ohkawa, H., et al (1979) *Anal. Biochem.* **95:** 351–358
Zimenkovsky, B., et al (1998) *J. Pharm. Pharmacol.* **50** (Suppl.): 240

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In-vivo evaluation of piperine and its analogues as potential treatment for vitiligo

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Vitiligo is the most commonly acquired hypomelanosis of skin, characterised by the development of depigmented patches due to loss of functional melanocytes, first in the epidermis and later in the follicular reservoir where most melanocytic stem cells (MSC) are probably situated. Consequently, identification of stimuli for the activation of MSC has a key relevance for the treatment of this disease.

In a program analysing traditional plant remedies for vitiligo (Lin et al 1999a), we showed that the black pepper alkaloid piperine (PIP) has a potent stimulatory effect on mouse melanocyte proliferation and dendricity in-vitro by a mechanism involving PKC signaling (Lin et al 1999b). This led to a programme of research involving synthesis of analogues of piperine and their preliminary screening in-vitro followed by selection of particular candidates for in-vivo testing.

In-vitro testing of over 30 synthetic analogues provided 2 lead compounds for in-vivo studies, namely a tetrahydro version (THP) and a cyclohexylamido derivative (CHP) of piperine. These were tested along with piperine (PIP) in a hairless, sparsely pigmented mouse strain used as a model of the low melanocyte population of vitiligo patches. Mice back skin and ears were treated topically with 175 mM PIP, THP or CHP solutions twice daily. Topical treatment with PIP or THP (but not CHP) induced greater visible pigmentation than solvent alone after 4 weeks of treatment, with little concomitant irritation or inflammation, defined by Kipp et al (1998) as less than 20% increase in skin fold thickness. Histological analysis revealed a statistically significant increase in DOPA + melanocytes as a consequence of treatment ($P < 0.05$). Furthermore, there was no evidence for the development of melanoma in these mice.

These results provide strong support for the use of this group of compounds as novel treatments for vitiligo.

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Cellular uptake and activity of a novel dendrimer delivery system covalently attached to antisense oligonucleotides targeting epidermal growth factor receptor

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We have recently shown that antisense oligonucleotides (AODNs) designed by a novel DNA-chip methodology have been shown to be effective inhibitors of epidermal growth factor receptor (EGFR), which is known to be over-expressed in several tumours including those of the breast and brain. In this study, we aimed to improve the delivery of AODNs to cancer cells by using a novel dendrimer delivery system based on a pentaerythritol building bloc structure.

Each dendrimer was synthesised to house nine molecules of the 21-mer phosphorothioate-modified antisense oligonucleotide sequence. Cellular uptake of AODNs was significantly enhanced (~ 4 fold) in A431 and U87-MG tumour cells when delivered as the dendrimer delivery system compared to the free AODN. Uptake was temperature and time dependent in both cell types. Metabolic and fluorescence microscopy studies suggested an adsorptive endocytic mechanism of cellular entry for this supramolecular construct. The dendrimer system housing nine AODNs targeting the EGFR mRNA effectively cleaved a 560bp EGFR transcript in the presence of RNaseH in vitro suggesting that oligonucleotide

hybridization with the target mRNA was not sterically hindered and that liberation of AODN from the dendrimer was not essential for activity. This was confirmed in cell culture where a dose-dependent inhibition of EGFR protein was demonstrated using Western blotting.

These data suggest that oligonucleotide delivery to tumour cells can be enhanced by covalent attachment to a pentaerythritol-based dendrimer, probably by increasing adsorptive endocytosis. Release or detachment of AODNs from the supramolecular structure was not essential for hybridization to target mRNA as assessed by RNaseH-mediated cleavage assays in-vitro and effective antisense inhibition of EGFR protein levels in cell culture.