

Pharmacological and cellular characterization of GRI977143, a novel nonlipid LPA₂ receptor agonist identified by virtual screening

Ph.D. thesis

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2. Abbreviations

Akt	protein kinase B
Bax	Bcl-2-associated X protein
CM	conditioned medium
DKO	double knockout
EC	effective concentration
ERK1/2	extracellular signal regulated kinases 1/2
G _i	inhibitory G protein
GPCR	G protein-coupled receptor
GRI	Genome Research Institute
GRI977143	[2-((3-(1,3-dioxo-1 <i>H</i> benzo[de]isoquinolin-2(3 <i>H</i>)-yl)propyl)thio)benzoic acid]
Gy	Gray (radiation unit)
H2L	Hit2Lead
H2L5547924	[4,5-dichloro-2-((9-oxo-9 <i>H</i> fluoren-2-yl)carbamoyl)benzoic acid]
H2L5828102	[2-((9,10-dioxo-9,10-dihydroanthracen-2-yl)carbamoyl)benzoic acid]
HUVEC	human umbilical vein endothelial cells
IEC-6	rat intestinal epithelial cell line 6
LD	lethal dose
LIM	Lin-11, Isl-1 and Mec-3 proteins
LPA	lysophosphatidic acid
LPAR	LPA receptor
MEF	mouse embryonic fibroblast cell
MEK	mitogen-activated protein kinase/extracellular signal regulated kinase
MM1	rat hepatoma cells
NF κ B	nuclear factor κ B
NHERF2	Na ⁺ -H ⁺ exchange regulatory factor 2
NSC12404	[2-((9-oxo-9 <i>H</i> fluoren-2-yl)carbamoyl)benzoic acid]
OTP	octadecenyl thiophosphate
PARP-1	poly (ADP-ribose) polymerase 1
PDZ	PSD95/Dlg/ZO-1 domain
PI3K	phosphoinositide-3-kinase
PPAR γ	peroxisome proliferator-activated receptor gamma

PSD95	postsynaptic density protein 95
QPCR	real-time quantitative polymerase chain reaction
TNF- α	tumor necrosis factor α
TRIP6	thyroid receptor interacting protein 6
UC-DDC	University of Cincinnati Drug Discovery Center
U937	human monocyte lymphoma cell line

3. Introduction

Lysophosphatidic acid (LPA) species regulate many fundamental cellular responses, ranging from cell survival through cell proliferation to cell motility and migration. Biological activities of LPA species are mediated through interactions with specific G protein-coupled plasma membrane receptors (GPCR; LPA receptor 1-6, LPAR 1-6) and the nuclear peroxisome proliferator-activated receptor gamma (PPAR γ ; Tigyi, 2010).

Dysregulated LPA signaling has been suggested to play a role in the pathogenesis of various human disorders. In the cardiovascular system activation of LPA₁ and LPA₃ receptors has been shown to influence cardiomyocyte contraction (Cremers et al., 2003) and lead to cardiac hypertrophy (Chen et al., 2008). LPA promotes tumor cell invasion, metastasis and angiogenesis via stimulation of LPA₂ and LPA₃ receptors (Kato et al., 2012; Pustilnik et al., 1999), while LPA₁ might act as a tumor suppressor (Yamada et al., 2009). Different studies revealed the connection between activation of LPA₁ receptor and the development of organ fibrosis (Tager et al., 2008), neuropathic pain (Inoue et al., 2004), osteoarthritis (Mototani et al., 2008), the absence of LPA₁ and schizophrenia (Harrison et al., 2003), as well as the inhibitory role of LPA₂ receptor in cholera toxin-induced secretory diarrhea (Li et al., 2005).

Discovery of individual LPA receptors followed by the effort to develop receptor-subtype selective agonists and antagonists accelerated understanding of LPA signaling and raised the possibility of pharmacotherapeutic LPAR modulation (Im, 2010). Despite the lack of ligand-bound GPCR crystal structures, mutagenesis studies combined with computational analysis led to the generation of numerous LPAR agonist and antagonist candidates (Im, 2010). Development of LPA-based drug candidates has been limited to the discovery of lipid-like ligands, which is understandable due to the hydrophobic environment of the LPA GPCR ligand binding pockets (Valentine et al., 2008). Only a few LPA receptor ligands break away from lipid-like structural features, among which Ki16425, an LPA_{1/2/3} antagonist (Ohta et al., 2003), and the AM095-152 series of LPA₁-selective compounds are of importance (Swaney et al., 2011).

A decade ago, we had already shown that LPA has profound activity in preventing apoptosis and can also rescue apoptotically condemned cells from the progression of the apoptotic cascade (Deng et al., 2002; Deng et al., 2007). We developed a long-acting LPA mimic, octadecenyl thiophosphate (OTP; Durgam et al., 2006), which has superior efficacy compared to LPA *in vitro* and *in vivo* in rescuing cells and animals from radiation-induced apoptosis (Deng et al., 2007). LPA and OTP not only prevented apoptosis induced by radiation injury when applied prior to the irradiation of the cells but acted as radiomitigators by rescuing apoptotically condemned cells when applied two hours postirradiation (Deng et al., 2002). Prosurvival effects of OTP and LPA were mediated by the LPA₂ receptor subtype. However, in spite of OTP's effectiveness in animal models of acute radiation syndromes, it is lipid-like with suboptimal partition coefficient from a drug development standpoint and it lacks receptor subtype specificity. The pan-agonist properties of OTP might lead to suboptimal antiapoptotic efficacy in cells coexpressing LPA₁ and LPA₂ receptors because the former receptor has been shown to promote apoptosis via anoikis (Funke et al., 2012).

Our laboratory focused on developing metabolically stabilized analogs of LPA that could be used as long-acting stimulators of the prosurvival signaling mediated by LPA receptors. These studies led to the previously unrealized role of the LPA₂ GPCR as a center of a macromolecular signaling complex mediated through unique sequence motifs present in its C-terminal domain (E et al., 2009; Lin et al., 2007). We discovered that LPA₂ via a C₃₁₁xxC half zinc-finger-like motif binds the proapoptotic protein Siva-1 from the Lin-11, Isl-1 and Mec-3 (LIM) family of proteins and this complex is withdrawn from GPCR recycling, undergoes polyubiquitination and is degraded in the proteasome (Lin et al., 2007). In a subsequent study, we have determined that the LPA₂ GPCR makes a ternary complex with two other PSD-95, DlgA, and ZO-1 (PDZ) binding domain containing proteins, the thyroid receptor interacting protein 6 (TRIP6) and the Na⁺-H⁺ exchange regulatory factor 2 (NHERF2). The ternary complex consisting of LPA₂ – TRIP6 – 2x(NHERF2) is formed upon LPA stimulation of the GPCR leading to enhanced, long-lasting activation of the mitogen activated protein kinase/extracellular signal regulated kinase (MEK)-extracellular signal regulated kinases 1/2 (ERK1/2) and phosphoinositide-3-kinase (PI3K)-protein kinase B (Akt)-nuclear factor κB (NFκB) prosurvival pathways required for the LPA₂-mediated antiapoptotic effect (E et al., 2009). Ternary complex formation upon LPA₂ receptor activation plays a unique role in chemoresistance (Tigyi et al., 2010).

4. Aims of the study

- 4.1 Identify novel nonlipid and drug-like hits specific for the LPA₂ receptor subtype:
 - 4.1.1 Similarity searching of NSC12404 [2-((9-oxo-9H-fluoren-2-yl)carbamoyl)benzoic acid], a serendipitously identified, week nonlipid LPA₂ receptor agonist using the University of Cincinnati Drug Discovery Center (UC-DDC) chemical library database.
 - 4.1.2 Experimentally characterize new nonlipid LPA₂ agonist analogs at the LPA₁₋₅, GPR87, and P2Y10 receptors using stable cell lines individually expressing these LPA receptors, vector-transfected control cells and receptor mediated Ca²⁺ mobilization assay.
- 4.2 Determine the effect of the lead compound on cell growth using vector- and LPA₂-transduced mouse embryonic fibroblast (MEF) cells derived from LPA₁ and LPA₂ double knockout (DKO) mice.
- 4.3 Determine the effect of the lead compound on tumor cell invasion using invasive rat hepatoma (MM1) cells and human umbilical vein endothelial cell (HUVEC) monolayers.
- 4.4 Characterize the selected nonlipid LPA₂ agonist for antiapoptotic action using different intrinsic and extrinsic apoptosis models.
 - 4.4.1 Determine anti-apoptotic efficacy of the lead compound in a model of Adriamycin-, and serum withdrawal-induced apoptosis, using vector- and LPA₂-transduced MEF cells derived from LPA₁ and LPA₂ DKO mice, based on Bcl-2-associated X protein (Bax) translocation, caspase 3, 7, 8, 9 activation, poly (ADP-ribose) polymerase 1 (PARP-1) cleavage and DNA fragmentation assays.
 - 4.4.2 Determine anti-apoptotic efficacy of the lead compound in a model of tumor necrosis factor α (TNF- α)-induced apoptosis, using the rat intestinal epithelial cell line 6 (IEC-6), based on DNA fragmentation assay.
- 4.5 Characterize the selected nonlipid LPA₂ agonist for radiomitigating action *in vitro* and *in vivo*:
 - 4.5.1 Determine radiomitigative efficacy of the lead compound in a model of γ -irradiation-induced apoptosis using vector- and LPA₂-transduced MEF cells derived from LPA₁ and LPA₂ DKO mice, based on Bax translocation, caspase 3, 7, 8, 9 activation, PARP-1 cleavage and DNA fragmentation assays.
 - 4.5.2 Determine anti-apoptotic efficacy of the lead compound in a model of γ -irradiation-induced bystander apoptosis using unirradiated IEC-6 cells and conditioned medium (CM) of γ -irradiated human monocyte lymphoma cell line (U937), based on caspase 3 and 7 activation.
 - 4.5.3 Determine the radiomitigative effect of the lead compound on the hematopoietic acute radiation syndrome using C57BL6 mice exposed to 6.6 Gray (Gy) total body γ -irradiation (~ lethal dose, LD_{100/20}).
 - 4.5.4 Determine the effect of the lead compound on malignant transformation of γ -irradiated cells using LPA₂-transduced MEF cells derived from LPA₁ and LPA₂ DKO mice and soft agar assay.

4.6 Characterize prosurvival signaling mechanisms activated by the selected nonlipid LPA₂ agonist:

4.6.1 Determine ERK1/2 activation in the presence of the lead compound using vector- and LPA₂-transduced mouse MEF cells derived from LPA₁ and LPA₂ DKO mice and immunoblot analysis.

4.6.2 Determine ligand induced supramolecular complex formation between LPA₂, TRIP6 and NHERF2 in the presence of the lead compound using HEK293T cells, pull-down assay, and immunoblot analysis.

5. Discussion and Conclusions

Our previous work aimed at the virtual discovery of LPA₁-specific compounds has serendipitously identified NSC12404, which is a weak but specific agonist of LPA₂ (Perygin, 2010). In the present study, we used this hit for virtual screening of the UC-DDC chemical library database.

This approach identified three new selective nonlipid LPA₂ agonists:

GRI977143 [2-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)thio)- benzoic acid], H2L5547924 [4,5-dichloro-2-((9-oxo-9H-fluoren-2-yl)carbamoyl) benzoic acid], and H2L5828102 [2-((9,10-dioxo-9,10-dihydroanthracen-2-yl)- carbamoyl) benzoic acid]. In receptor mediated Ca²⁺ mobilization assays NSC12404, H2L5547924, H2L5828102, and GRI977143 only activated LPA₂ and failed to activate any of the other established and putative LPA GPCRs when applied up to 10 μM. A 10 μM concentration of these compounds have also been tested for the inhibition of the Ca²⁺ response elicited by the ~EC₇₅ concentration of LPA 18:1 at those receptors that the compound failed to activate when applied at 10 μM. We found that at this high concentration NSC12404 and GRI977143 inhibited LPA₃ but none of the other receptors we tested were either activated or inhibited by these two compounds. H2L5547924 activated LPA₂ but partially inhibited LPA₁, LPA₃, LPA₄, GPR87, and P2Y10. H2L5828102 although was a specific agonist of LPA₂ but fully inhibited LPA₃ and partially inhibited LPA₁, GPR87 and P2Y10. Based on its lower EC₅₀ concentration to activate the LPA₂ receptor compared to NSC12404 and because it only inhibited the LPA₃ receptor compared to the H2L compounds we selected GRI977143 for further characterization in cell-based assays.

We showed that specific stimulation of the LPA₂ receptor subtype promotes cell growth. This is the first pharmacological evidence that this receptor subtype mediates mitogenesis. Surprisingly, the LPA receptor panagonist OTP and GRI977143 had equally robust activity on cell proliferation. We note that OTP and GRI977143 after 3 days also promoted the growth of vector-transduced MEF cells, which might be due to off-target or indirect effects. We cannot exclude the possibility that OTP and GRI977143 somehow potentiated the effect of the 1.5% serum present in the medium. There might be differences in the pharmacokinetic properties of these ligands, which could explain the differences we noted. Future experiments will have to address the differences on cell growth observed between these ligands.

LPA has been shown to promote cancer cell invasion and metastasis (Kato et al., 2012; Pustilnik et al., 1999). We tested the effect of GRI977143 in an *in vitro* invasion model that has been considered a realistic model of metastasis (Mukai et al., 2005; Uchiyama et al., 2007). Stimulation of MM1 hepatocarcinoma cells with GRI977143 elicited a dose-dependent increase in the number of cells that penetrated the HUVEC monolayer. However, this effect, although significant at a 10 μM concentration of GRI977143, was modest compared to that of LPA. The MM1 cells express LPA₂ >> LPA₁ > LPA₆ > LPA₅ > LPA₄ transcripts, whereas HUVECs express LPA₅ >> LPA₄ > GPR87 ~ LPA₁ > LPA₂ transcripts determined by quantitative RT-PCR (Lee & Tigyi – unpublished). The increase in GRI977143-induced invasion of MM1 cells is likely to represent the effect of selective stimulation of LPA₂ in the invading MM1 cells rather than in HUVEC due to the very low expression of this receptor subtype in the cells of the monolayer (Gupte et al., 2011).

Studies have already established the role of the LPA₂ receptor in protecting cells from programmed cell death (Deng et al., 2002; E et al., 2009; Lin et al., 2007). The LPA₂-specific agonist properties of GRI977143 allowed us to test this hypothesis in the LPA₂ knock-in MEF cells and in IEC-6 cells, the latter of which endogenously expresses multiple LPA GPCRs (Deng et al., 2002; Deng et al., 2007). Our experiments showed that by activating the LPA₂ receptor GRI977143 effectively reduces cytosolic Bax translocation, activation of initiator and effector caspases, DNA fragmentation and PARP-1 cleavage associated with Adriamycin-, serum withdrawal-, or γ -irradiation induced intrinsic apoptosis. GRI977143 had no effect in the vector-transduced MEF cells with the exception of a minimal reduction in DNA fragmentation in the Adriamycin model of apoptosis, which might be due to some yet unknown off-target effect of the compound. There was no such detectable effect of GRI977143 in the serum withdrawal- or γ -irradiation-induced apoptosis models. In contrast to GRI977143, we have also noticed that LPA and OTP had a slight attenuating effect in vector-transfected MEF cells. Quantitative RT-PCR analysis showed that the MEF cells derived from LPA₁ and LPA₂ double KO mouse embryos express appreciable amounts of LPA_{4/5/6} and P2Y₁₀, which can explain the antiapoptotic effect of LPA and OTP. It is also important to recognize that GRI977143 protected IEC-6 cells from apoptosis, which endogenously express multiple LPA GPCR subtypes. This result is the first evidence that we know of in the literature that specific activation of LPA₂ is sufficient to evoke an antiapoptotic effect and this effect is not limited to the LPA₂ knock-in MEF cells. Thus, we propose that specific activation of LPA₂ is sufficient to protect cells from apoptosis. The specific agonist properties of GRI977143 might represent an advantage over LPA and other receptor-nonspecific LPA mimics that also stimulate LPA₁ receptor subtype activation, which has been shown to promote cell death via anoikis in tumor cells (Furui et al., 1999), in cardiac myocytes (Chen et al., 2006), and in pulmonary epithelial cells (Funke et al., 2012).

We also examined the effect of LPA and GRI977143 in a model of radiation-induced bystander apoptosis *in vitro*. This model has relevance to the radiomitigative action of LPA analogs because in the animal experiments the LPA analogs were not present during the first 24 h postirradiation when the initial wave of radiation-elicited apoptosis takes place. Nevertheless, administration of GRI977143 or OTP (Deng et al., 2007) at +24 h postirradiation is effective in protecting the lives of the animals. We hypothesize that GRI977143-mediated activation of LPA₂ receptors in the tissues exerts some of its protective action by attenuating bystander effects of radiation injury that occur 24 – 48 h post injury and are possibly mediated by agents similar to those present in the CM of irradiated U937 cells in our *in vitro* model (Kim et al., 2008). Our results obtained with GRI977143 in the different γ -irradiation injury models consistently suggest that this compound exerts a radiomitigative action and is capable of rescuing apoptotically condemned cells *in vitro* and *in vivo*. In this context we were surprised to find that malignant transformation of the irradiated and rescued MEF cells did not show enhancement after GRI977143 treatment. This observation will need to be followed up *in vivo* but already hints that GRI977143-treated cells have been able to repair DNA damage that otherwise could have led to a high-rate of transformation revealed by growth in soft agar.

LPA₂-mediated activation of the ERK1/2 prosurvival kinases is a required event in antiapoptotic signaling (E et al., 2009; Lin et al., 2007). Consistent with our previous results obtained

with LPA and OTP (Deng et al., 2007; E et al., 2009; Lin et al., 2007), GRI977143 treatment resulted in a robust ERK1/2 activation. We have previously shown that in addition to the Gi protein-mediated signals demonstrated by the partial pertussis toxin-sensitivity of the effect (Deng et al., 2002), the LPA₂-mediated antiapoptotic effect requires additional ligand-induced assembly of a C-terminal macromolecular complex consisting of LPA₂, TRIP6, and a homodimer of NHERF2 (E et al., 2009; Lin et al., 2007). We found that GRI977143 elicited the assembly of this signalosome, which can explain the concomitant robust ERK1/2 activation.

Taken altogether, the present findings indicate that nonlipid LPA₂-specific agonists, such as those described here, represent an excellent starting point for the development of lead compounds with radiomitigative effect and potential therapeutic utility for the prevention of programmed cell death involved in many types of degenerative and inflammatory diseases.

6. Summary of scientific results

- 6.1 Identification of novel nonlipid compounds, GRI977143, H2L5547924, and H2L5828102, that are specific agonists of LPA₂ and do not activate other LPA GPCRs including LPA_{1/3/4/5}, GPR87, or P2Y10.
- 6.2 Lead compound GRI977143 is less potent but equally efficacious as LPA and OTP in protecting cells against different forms of intrinsic and extrinsic apoptosis in vitro.
- 6.3 GRI977143 promotes carcinoma cell invasion of human umbilical vein endothelial cell monolayers and fibroblast proliferation, however, it does not induce malignant transformation of the irradiated and rescued MEF cells.
- 6.4 GRI977144 shows the features of a radiomitigator:
 - 6.4.1 It rescues apoptotically condemned cells in vitro from high-dose γ -irradiation injury when administered 1 h after radiation exposure.
 - 6.4.2 It is effective in rescuing the lives of mice from deadly levels of radiation when administered 24 h after radiation exposure.
- 6.5 GRI977143 inhibits bystander apoptosis elicited by soluble proapoptotic mediators produced by irradiated cells.
- 6.6 By specifically activating LPA₂ receptors GRI977143 robustly activates the ERK1/2 survival pathway and leads to the assembly of a macromolecular signalosome consisting of LPA₂, TRIP6, and NHERF2, required for the prosurvival signaling elicited via this receptor subtype.

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8. Publications of the author/A szerző közleményei

8.1 Publications/Közlemények

2012

Kiss GN, Fells JI, Gupte R, Lee SC, Liu J, Nusser N, Lim KG, Ray RM, Lin FT, Parrill AL, Sumegi B, Miller DD, Tigyi GJ (2012) Virtual Screening for LPA2-Specific Agonists Identifies a Nonlipid Compound with Antiapoptotic Actions. *Mol Pharmacol* **82**(6):1162-1173.

Kiss GN, Lee SC, Fells JI, Liu J, Valentine WJ, Fujiwara Y, Thompson KE, Yates CR, Sumegi B, Tigyi G (2013) Mitigation of Radiation Injury by Selective Stimulation of the LPA2 Receptor. *Biochim Biophys Acta-Mol Cell Biol Lipids* **1831**(1):117-125.

2010

Valentine WJ and Kiss GN, Liu J, E S, Gotoh M, Murakami-Murofushi K, Pham TC, Baker DL, Parrill AL, Lu X, Sun C, Bittman R, Pyne NJ, Tigyi G (2010) (S)-FTY720 vinylphosphonate, an analogue of the immunosuppressive agent FTY720, is a pan-antagonist of sphingosine 1-phosphate GPCR signaling and inhibits autotaxin activity. *CellSignal* **22**(10):1543-1553.

2009

Kovacs K, Hanto K, Bogнар Z, Tapodi A, Bogнар E, Kiss GN, Szabo A, Rappai G, Kiss T, Sumegi B, Gallyas F Jr (2009) Prevalent role of Akt and ERK activation in cardioprotective effect of Ca(2+) channel- and beta-adrenergic receptor blockers. *Mol Cell Biochem* **321**(1-2):155-164.

2008

Bartha E, Kiss GN, Kalman E, Kulcsár G, Kálai T, Hideg K, Habon T, Sumegi B, Toth K, Halmosi R (2008) Effect of L-2286, a poly(ADP-ribose) polymerase inhibitor and enalapril on myocardial remodeling and heart failure. *J Cardiovasc Pharmacol* **52**(3):253-261.

8.2 Presentations, posters/Előadások, poszterek

2011

Tigyi G, Kiss GN, Fells J, Liu D, Yue J, Thompson KE, Yates R, E S, Lai YJ, Lin FT, Parrill AL, Deng W, Gupte R, Miller DD (2011) Development of Radiomitigators Targeting the Lysophosphatidic Acid Receptors. *14th International Congress of Radiation Research, Warsaw, Poland, poster*

Tigyi G, Kiss GN, Valentine W, E S, Fells J, Liu J, Yue J, Thompson KE, Yates R, Parrill A, Lai YJ, Lin FT, Deng W (2011) Computational Design of a Non-lipid LPA2 Receptor Agonist with Antiapoptotic and Radioprotective Action. *Experimental Biology Meeting, Washington, D.C., poster*

2010

Tigyi G, Kiss GN, Valentine W, E S, Fells J, Liu J, Yue J, Thompson KE, Yates R, Parrill A, Lai YJ, Lin FT, Deng W (2010) Radiomitigative signaling by lysophospholipid receptors. (2010) *56th Annual Meeting of the Radiation Research Society, Maui, Hawaii, poster*

Valentine WJ, Kiss G, Liu J, E S, Lu X, Sun C, Bittman R, Tigyi G (2010) (S)-ene Phosphonate Analog of the Immunosuppressive Agent FTY720 is a Pan-antagonist of the S1P GPCR. *Keystone Symposium on Sphingolipid Signaling, Kyoto*, **poster**

Valentine WJ, Kiss GN, Liu J, Lu X, Sun C, Bittman R, Tigyi G (2010) FTY720 S-ene-phosphonate is a novel pan-antagonist of the S1P receptors that inhibits lymphocyte egress. *Experimental Biology Annual Meeting, San Diego Ca*, **poster**

2008

Bognar E, Kiss GN, Sarszegi Zs, Bartha E, Solti I, Sumegi B, Berente Z (2008) Poly (ADP-ribose) Polymerase (PARP) Inhibitor HO3089 Enhanced Post Ischemic Myocardial Glucose Uptake Mostly By Activation Of AMP-activated Protein Kinase (AMPK). *World Congress of Cardiology*, **poster**

2007

Bartha E, Halmosi R, Kiss GN, Solti I, Bognar E, Kalman E, Kalai T, Hideg K, Sumegi B, Toth K (2007) Effect of PARP and ACE inhibitors on rat chronic heart failure model. *XXXVII.Membrane Transport Conference*, **poster**

Bartha E, Halmosi R, Kulcsar G, Kiss GN, Kalman E, Sumegi B, Kalai T, Hideg K, Toth K (2007) Effect of PARP-inhibitors and ACE-inhibitors on the progression of the isoproterenol-induced heart failure. *European Heart Journal* vol. 28, Abstract Suppl.: 48-49, **poster**

Bartha E, Palfi A, Mark L, Kiss GN, Halmosi R, Szabados E, Sumegi B (2007) Effect of an alcohol free red wine extract on the myocardial remodeling induced by isoproterenol. *Hungarian Society of Cardiology Scientific Conference*, **presentation**

Berente Z, Kiss GN, Bognar E, Sumegi B (2007) Relationship between the cardioprotective effect of certain poly(ADP-ribose) polymerase inhibitors and the glucose metabolism of the postischemic heart. *Hungarian Biochemical Society Annual Meeting*, **presentation**

Berente Z, Kiss GN, Radnai B, Sumegi B (2007) In situ and in vivo applications of nuclear magnetic resonance for biomarker search. *Symposium on Medicinal Chemistry and Technology*, **presentation**

Kovacs K, Szabo A, Bognar E, Kiss GN, Kiss T, Sarszegi Z, Sumegi B, Gallyas F (2007) Identification of novel drug targets preventing ischemic heart diseases. *Bridges in Life Sciences Annual Scientific Review, Regional Cooperation for Health, Science and Technology*, vol.1, page 42., **presentation**

Szabo A, Kovacs K, Bognar E, Kiss GN, Sumegi B, Gallyas F (2007) Role of Akt and ERK activation in the cardioprotective effect of poly(ADP-ribose) polymerase inhibitors, Ca²⁺ channel- and β -blockers. *Hungarian Biochemical Society Annual Meeting*, **presentation**

2006

Bartha E, Palfi A, Mark L, Kiss G, Halmosi R, Szabados E, Kalman E, Pajor L, Toth K, Sumegi B (2006) Effect of Alcohol-free red wine extract on isoproterenol-induced cardiac remodeling in rats. *Exp Clin Cardiol* 11(3):246, **poster**

Bognar E, Kiss GN, Solti I, Hanto K, Kovacs K, Sumegi B, Gallyas F (2006) Comparison of 4-hydroxy-quinazoline, Verapamil and Metoprolol in heart protection during ischemia- reperfusion. *Exp Clin Cardiol* **11**(3):247, **poster**

Bognar E, Solti I, Nemeth V, Bartha E, Tucsek Zs, Veto S, Hocsak E, Kiss GN, Sumegi B, Berente Z (2006) Study of glucose uptake and related cell signaling pathways in isolated rat heart model. *Hungarian Biochemical Society Annual Meeting*, **poster**

Kiss G, Deres P, Hanto K, Bognar E, Bartha E, Sumegi B, Berente Z (2006) Do poly(ADP-ribose) polymerase (PARP) inhibitors affect myocardial metabolism? *European Heart Journal* vol. 27, Abstract Suppl.:410, **poster**

Kiss GN, Bognar E, Solti I, Hanto K, Kovacs K, Gallyas F, Sumegi B (2006) Molecular mechanisms of cardioprotection afforded by poly(ADP- ribose) polymerase inhibitors, Ca²⁺ channel- and β -blockers in ischemia-reperfusion: a comparative ex vivo study. *Semmelweis Symposium Nitric Oxide and Nitrosative Stress in the Cardiovascular system*, **poster**

Solti I, Bognar Z, Nemeth V, Veto S, Hocsak E, Kiss GN, Szanto A, Varbiro G, Sumegi B (2006) Effect of Taxol on the mitochondrion and the ROS production. *Hungarian Biochemical Society Annual Meeting*, **poster**

2005

Hanto K, Tapodi A, Kiss G, Varbiro G, Gallyas F, Hideg K, Sumegi B, Toth K (2005) Enhanced Akt activation induced by PARP inhibition in H9c2 cells during oxidative stress. *Hungarian Society of Cardiology Scientific Conference*, **presentation**

Hanto K, Tapodi A, Kiss G, Varbiro G, Gallyas F, Hideg K, Sumegi B, Toth K (2005) PARP inhibition induced Akt activation is dominantly responsible for the cytoprotection against oxidative stress in H9c2 cells. *VI. Hungarian Genetic Congress; XIII. Cell and Developmental Biology Conference*, **poster**

Kiss G (2005) Effect of poly (ADP-ribose) polymerase (PARP) inhibitors on the glucose uptake of isolated perfused rat hearts. *Student Science Work Conference, University of Pecs, Faculty of Medicine*, **presentation**

Resko A, Kiss GN (2005) Cardioprotective effects of poly(ADP-ribose)polymerase inhibitors on chronic rat heart failure. *Student Science Work Conference, University of Pecs, Faculty of Medicine*, **presentation**

2004

Kiss G, Pozsgay E, Resko A (2004) Study of glucose uptake and related cell signaling pathways on isolated rat heart model during ischemia-reperfusion. *Student Science Work Conference, University of Pecs, Faculty of Medicine*, **presentation**

Pozsgay E, Kiss G, Resko A (2004) Experimental antioxidant compounds decrease acute cardiotoxicity induced by Doxorubicin. *Student Science Work Conference, University of Pecs, Faculty of Medicine*, **presentation**

Deres P, Kiss G, Pozsgay E, Toth A, Halmosi R, Kovacs K, Palfi A, Hideg K, Sumegi B, Toth (2004) Experimental antioxidant compounds protect against acute cardiotoxicity induced by Doxorubicin. *Hungarian Society of Cardiology Scientific Conference*, **poster**

Deres P, Hanto K, Kovacs K, Halmosi R, Palfi A, Kiss G, Resko A, Pozsgay E, Sumegi B, Toth (2004) Protective effect of poly(ADP-ribose) polymerase inhibitors on Doxorubicin induced acute cardiotoxicity. *XXXIV. Membrane Transport Conference*, **poster**

2003

Kiss G, Hanto K (2003) Effect of experimental antioxidant compounds on the activation of cardioprotective cell signaling pathways during rat heart ischemia-reperfusion. *Student Science Work Conference, University of Pecs, Faculty of Medicine*, **presentation**