Biochemical and Biophysical Research Communications 460 (2015) 446–450

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

ATP-γ-S-(α,β-CH_2) protects against oxidative stress and amyloid beta toxicity in neuronal culture

Ortal Danino [a], Shlomo Grossman [b], Bilha Fischer [a, *]

[a] Department of Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel
[b] The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

ARTICLE INFO

Article history:
Received 3 February 2015
Available online 19 March 2015

Keywords:
Nucleotide
Antioxidant
Neuroprotectant
Amyloid beta

ABSTRACT

Amyloid beta (Aβ) oligomers and oxidative stress, typical of Alzheimer's disease, are highly neurotoxic. Previously we identified ATP-γ-S as a most promising antioxidant and neuroprotectant. To further improve both potency and metabolic stability of ATP-γ-S, we designed a related analogue, ATP-γ-S-(α,β-CH_2). We found that ATP-γ-S-(α,β-CH_2) effectively inhibited ROS formation in PC12 cells subjected to Fe(II)-oxidation, slightly better than ATP-γ-S (IC_{50} 0.18 and 0.20 μM, respectively). Moreover, ATP-γ-S-(α,β-CH_2) rescued primary neurons from $Aβ_{42}$ toxicity, 4-fold more potently than ATP-γ-S, (IC_{50} 0.2 and 0.8 μM, respectively). In addition, the metabolic stability of ATP-γ-S-(α,β-CH_2) in PC12 cells during 4 h of incubation, was up to 20% greater than that of ATP-γ-S and ATP. Previously, we found that ATP-γ-S-(α,β-CH_2) resisted hydrolysis by ecto-nucleotidases such as, NPPs and TNAP, and was found to be ~7-fold more potent agonist than ATP at P2Y11 receptor. Therefore, we propose ATP-γ-S-(α,β-CH_2) as a promising agent for rescue of neurons from insults typical of Alzheimer's disease.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

The main features of Alzheimer's disease (AD) include amyloid plaques, neurofibrillary tangles, oxidative stress [1], and neuro-inflammatory processes [2]. Aβ (amyloid beta) forms oligomers which give rise to fibrils. Aβ binds Cu(II), Fe(III) and Zn(II) ions with high affinity [3]. High concentrations of Zn(II), Cu(II), and Fe(II) ions have been found in senile plaques. When excess iron ions are present, their redox activity might lead to significant oxidative damage via reactive oxygen species (ROS) production within the brain [4].

The effect of naturally occurring purine nucleotides on Fe(II)-induced OH radical production from either O_2 or H_2O_2 (Fenton reaction), was studied earlier [5–7]. We have shown that nucleotides modulate Fenton reaction in a concentration-dependent biphasic fashion [8]. In addition to natural nucleotides, we investigated a series of phosphate-modified nucleotides, dinucleotides, and inorganic phosphates, as potential biocompatible anti-oxidants in in-vitro and cellular systems [9]. The purine nucleotide scaffold contains metal binding sites at both the phosphate chain and purine ring (N7-nitrogen atom), and makes a natural divalent metal ion chelator [8]. Indeed we showed that nucleotides are efficient Fe(II)-chelators [10].

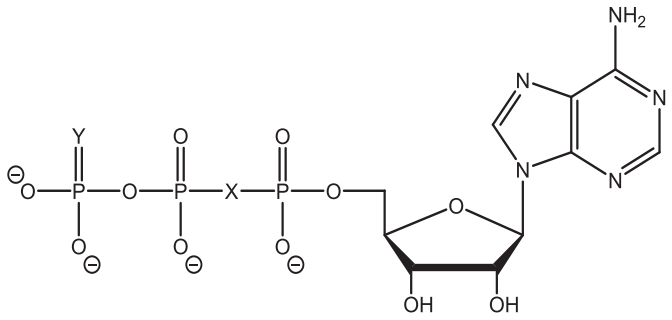
Previously we also found that nucleotides containing phosphorothioate moieties inhibited ROS formation in Fe(II)-treated PC12 cells up to 300-fold better than natural nucleotides and were up to 4.5-fold more metabolically stable [9]. We identified ATP-γ-S (Fig. 1) as a highly potent antioxidant that inhibited Fe(II)-induced ROS production under Fenton reaction conditions, in both non-cellular and cellular system, IC_{50} 14 μM [8] and 180 nM [9], respectively.

We reported that nucleotides are also capable of dissolution of $Aβ_{40/42}$-Cu(I)/Cu(II)/Zn(II) aggregates. Specifically, ATP-γ-S-(α,β-CH_2) (Fig. 1) effectively dissolved $Aβ_{40}$-Cu(I) and $Aβ_{42}$-Cu(I)/Zn(II) aggregates [10] better than ATP-γ-S or ATP, as monitored by ^{1}H NMR, TEM, and turbidity assays. Furthermore, ATP-γ-S-(α,β-CH_2) re-solubilized $Aβ_{40}$–Cu(II) aggregates more efficiently than EDTA [10].

The potential pharmaceutical use of nucleotides as therapeutic agents is limited because of their rapid dephosphorylation in extracellular media by hydrolyzing enzymes such as nucleotide pyrophosphatases (NPPs), ecto-nucleoside triphosphate diphosphohydrolases (NTPDases), and alkaline phosphatase (AP) [11]. Recently, we reported that ATP analogues modified at Pα/Pβ/Pγ

* Corresponding author. Fax: +972 3 6354907.
E-mail addresses: ortal5000@gmail.com (O. Danino), shlomo.grossman@biu.ac.il (S. Grossman), bilha.fischer@biu.ac.il (B. Fischer).

http://dx.doi.org/10.1016/j.bbrc.2015.03.053
0006-291X/© 2015 Elsevier Inc. All rights reserved.



	X	Y
ATP	O	O
ATP-γ-S	O	S
ATP-γ-S-(α,β-CH_2)	CH_2	S

Fig. 1. Structures of ATP, ATP-γ-S, and ATP-γ-S-(α,β-CH_2).

positions by bridging methylene and thiophosphate moieties, such as ATP-γ-S-(α,β-CH_2), at 100 μM were stable to hydrolysis by NTPDase1,2,3,8 as compared to ATP (4.4–5.5% hydrolysis vs. 45–74%, respectively). Likewise, ATP-γ-S-(α,β-CH_2) at 100 μM was not catabolized by NPP1 or NPP3 and was highly stable to tissue non-specific alkaline phosphatase (TNAP) hydrolysis. Furthermore, ATP-γ-S-(α,β-CH_2) was found to be an potent NPP1 inhibitor, Ki 20 nM [12].

Adenine nucleotides are not only substrates of ectonucleotidases but also potent agonists at P2Y1, P2Y2, and P2Y11 G-protein-coupled receptors [13]. These receptors are distributed in a large variety of tissues and are widely expressed in the nervous system [14]. These receptors trigger neuroprotective effects due to their activation by adenine nucleotides during brain injury, i.e. following ischemia, necrosis or trauma, in which massive release of ATP occurs [15].

Recently we reported that ATP-γ-S-(α,β-CH_2) was a 6.7-fold more potent P2Y11-R agonist than the endogenous agonist, ATP (EC_{50} 1 and 6.7 μM, respectively), and was selective to P2Y11-R exhibiting no activity at P2Y1 or P2Y2 receptors [12].

The highly promising properties of ATP-γ-S-(α,β-CH_2) as a Fenton reaction inhibitor [10], an effective ion chelator [16], a powerful agent for in-vitro dissolution of $A\beta_{40/42}$-M(II) oligomers and aggregates [10], together with its enzymatic stability, and its high activity at P2Y11-R [12], encouraged us to explore the potential of ATP-γ-S-(α,β-CH_2) for the rescue of primary neurons subjected to insults typical of Alzheimer's disease.

2. Materials and methods

2.1. General

ATP-γ-S-(α,β-CH_2) was prepared according to Nadel et al. [12]. Trolox was obtained from Acros Organics (Morris Plains, NJ). DMEM medium, glutamine, and penicillin-streptomycin-nystatin were obtained from Biological Industries, Inc. (Kibbutz Beit Haemek, Israel). DCFH, Cytosine β-D-arabinofuranoside (Ara-C), DMSO, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT), $FeSO_4$, ATP and ATP-γ-S were obtained from Sigma Chemical Co. (St. Louis, MO). $A\beta_{42}$ was obtained from GL-Biochem (Shanghai, China). PC12 cells were obtained from the American Type Culture Collection (Manassas, VA). Absorbance was read by a spectrometer, Spectrafluor plus (Tecan, Switzerland). The metabolic stability of nucleotides was evaluated by HPLC (Merck-Hitachi) using an analytical reverse-phase column (Gemini 5u C-18 110A, 150 × 4.60 mm; 5 micron; Phenomenex, Torrance, CA).

2.2. Determination of ROS production in cultured PC12 cells in the presence of $FeSO_4$ and nucleotide analogues

Determination of ROS production in cultured PC12 cells was performed according to our previous protocol [9]. Oxidation was initiated by the addition of $FeSO_4$ (0.16 μM) to the wells in the presence of nucleotide analogues at 0.2–200 μM. The plates were incubated for 1 h at 37 °C, and read at 485/530 nm.

2.3. Determination of metabolic stability of the tested nucleotides in PC12 cell culture

Determination of metabolic stability was performed according to our previous protocol [9].

2.4. Primary neuron cultures

Preparation of primary neuron culture was performed according to our previous protocol [17].

2.5. Evaluation of the effect of nucleotide analogues on primary neuron-cultures subjected to $FeSO_4$ insult

Primary ortical neurons were seeded in 96-well plates and treated with $FeSO_4$ at a final concentration of 3 μM and nucleotide analogues at various concentrations (0.008–200 μM), after 24 h the cells were tested for cell viability by MTT assay. Percentage of control of cell viability was plotted against nucleotide concentration. IC_{50} values were obtained by "Sigma Plot" software. The value of nucleotide concentration at 50% inhibition was determined as IC_{50}.

2.6. Evaluation of the effect of nucleotide analogues on primary neuron-cell cultures subjected to $A\beta_{42}$ insult

Amyloid-beta (1–42) oligomers were prepared according to Amir et al. [10]. Briefly, $A\beta_{42}$ (3 mg) was dissolved in 10 mM NaOH, sonicated for 3 min, and then freeze-dried. To evaluate the presence of oligomers, we measured the size of $A\beta_{42}$ aggregates by dynamic light scattering (DLS) and TEM. A primary mixed culture of neurons and astrocytes was prepared in a similar way to that used to prepare the primary neuron-cell cultures, but without the treatment with Ara-C, in order to create conditions resembling those in animal models. Next, 50 μM (final) of $A\beta_{42}$ was added to the cells in the presence or absence of nucleotide analogues at 0.04–25 μM, for 48 h. After 48 h, cells were tested for viability by dyeing them with trypan blue, and counting the vital cells.

2.7. Statistical analysis

All experiments were performed at least three times. Where appropriate, the data were expressed as the mean ± standard error of the mean. Probability (P) was calculated using a Student t-test. P values lower than 0.05 were considered significant.

3. Results

3.1. ATP-γ-S-(α,β-CH_2) reduced ROS production in PC12 cells under oxidizing conditions

We evaluated the inhibition of ROS production in PC12 cells in the presence of $FeSO_4$ by ATP-γ-S-(α,β-CH_2), using the DCFH-DA [9]

assay, and compared it to ATP and ATP-γ-S. The antioxidant activity of ATP-γ-S-(α,β-CH_2) was almost similar to that of ATP-γ-S, with IC_{50} values of 0.18 and 0.20 μM, respectively. However, ATP, up to 200 μM, did not achieve 50% reduction in the ROS production. Fig. S1 shows the inhibition percentage of ROS production by ATP-γ-S-(α,β-CH_2), ATP-γ-S, and ATP (at the concentration range of 0.2–200 μM).

3.2. ATP-γ-S-(α,β-CH_2) rescued primary neurons from $FeSO_4$-induced cell death

The above finding that ATP-γ-S-(α,β-CH_2) is a potent antioxidant at PC12 cells, encouraged us to evaluate its neuroprotective activity, vs. ATP-γ-S and ATP, in primary cortical neurons subjected to $FeSO_4$. Specifically, neuronal cells were treated with ATP-γ-S-(α,β-CH_2), ATP-γ-S, and ATP at increasing concentrations (0.008–25 μM) simultaneously with $FeSO_4$ (3 μM) for 24 h. ATP-γ-S and ATP-γ-S-(α,β-CH_2), maintained the vitality of 50% of the neuronal culture at 0.01 and 0.04 μM, respectively. At 25 μM, both compounds showed 80% cell viability. ATP proved to be a poor neuroprotectant with IC_{50} value of 30 μM (Fig. 2).

3.3. ATP-γ-S-(α,β-CH_2) rescued a primary mixed culture of neurons and astrocytes from $A\beta_{42}$ toxicity

Iron chelators have been shown to protect neuron cells against the pro-apoptotic signaling of amyloid beta [18]. In order to study the protective activity of ATP-γ-S-(α,β-CH_2) against $A\beta_{42}$-induced cell death, we used a primary mixed culture of neurons and astrocytes to create conditions resembling those in animal models [19]. $A\beta_{42}$ oligomers [10] induced a concentration-dependent decrease in cell viability, as assessed by trypan blue staining and counting the vital cells following 48 h of exposure. At 50 μM $A\beta_{42}$, 60% cell loss was observed (data not shown).

The neuroprotective effect of ATP-γ-S-(α,β-CH_2) was evaluated and compared to ATP-γ-S and ATP in a primary mixed culture exposed to 50 μM $A\beta_{42}$ for 48 h. ATP-γ-S-(α,β-CH_2) proved to be a 4-and-125 fold more potent neuroprotectant vs. ATP-γ-S and ATP, IC_{50} 0.2, 0.8 and 25 μM, respectively (Fig. 3). At 5 μM, ATP-γ-S brought about almost 80% of the cell survival, while addition of 5 μM ATP-γ-S-(α,β-CH_2) resulted in 65% survival.

3.4. ATP-γ-S (α,β-CH_2) was relatively metabolically stable in PC12 cell culture and human blood serum

The potential pharmaceutical use of nucleotide analogues depends on their resistance to degradation processes by ectoenzymes of a given cell type. Here, we used PC12 cell line as a physiological model to evaluate the stability of studied nucleotides.

ATP-γ-S-(α,β-CH_2), ATP-γ-S, and ATP were incubated at 37 °C with or without PC12 cells for 0.2–24 h. The stability of the nucleotides was evaluated and monitored by HPLC for dephosphorylation products. After 2 h of incubation in the presence of PC12 cells, the amounts of ATP-γ-S(α,β-CH_2), ATP-γ-S, and ATP decreased by 22, 40, and 77%, respectively. After 24 h, ATP-γ-S and ATP were more than 90% degraded as compared to only 50% degradation of ATP-γ-S-(α,β-CH_2) (Fig. S2a).

When ATP-γ-S-(α,β-CH_2), ATP-γ-S, and ATP were incubated cell media without PC12 cells, the amount of ATP remained almost unchanged after 24 h, while the amounts of ATP-γ-S-(α,β-CH_2) and ATP-γ-S, decreased by 10 and 20%, respectively (Fig. S2b). In human blood serum after 24 h incubation, ATP-γ-S-(α,β-CH_2) ($t_{1/2}$ 14 h), was found to be 30% more stable than ATP ($t_{1/2}$ 5 h) (data not shown), consistent with previous findings [20].

4. Discussion

Our working hypothesis here was that protection of neurons from Fe(II)-oxidation or $A\beta_{42}$ insult may be achieved by biocompatible, metabolically stable and effective Fe(II)-chelators. Hence, our approach to the development of drug candidates for treating typical AD insults was based on ATP scaffold. The latter was chemically modified to improve its metabolic stability, activity as Fe(II)-chelator, and activity as an agonist of P2Y-receptor involved in neuroprotection.

Indeed, ATP-γ-S-(α,β-CH_2) selected here for this purpose, was found to be an antioxidant equipotent to ATP-γ-S and reduced 50% of ROS formation in PC12 cells under oxidative stress at 0.18 μM.

ATP promotes cell proliferation, growth, and development [21] and may play a beneficial role in various neurological disorders [21,22]. Therefore, here we evaluated the possible neuroprotective

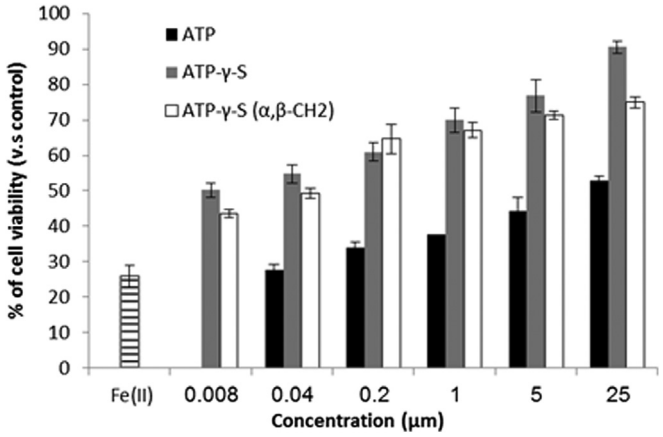


Fig. 2. Neuroprotective activity of ATP-γ-S-(α,β-CH_2), ATP-γ-S, and ATP. Cortical neurons were treated with various concentrations (0.008–200 μM) of nucleotide analogues and 3 μM $FeSO_4$ for 24 h. After 24 h, cell viability was measured by MTT assay. The viability of untreated cells (control) was taken as 100%, all other experimental values were determined as percentage of control relative to this reference. The results shown are the mean ± S.D. of three independent experiments performed in triplicate (*P < 0.05).

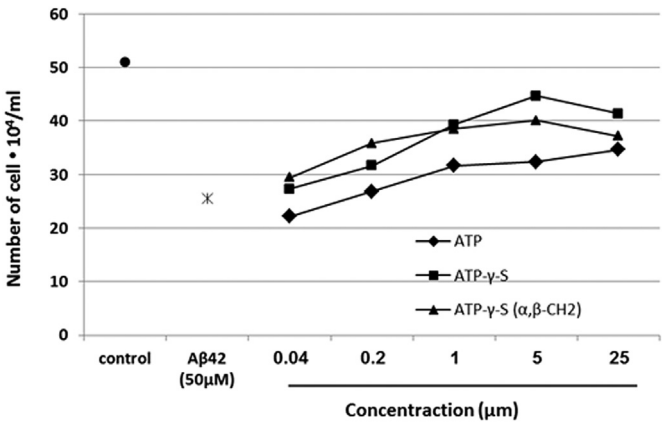


Fig. 3. Rescue of neurons and astrocytes culture from $A\beta_{42}$ toxicity by ATP-γ-S-(α,β-CH_2), ATP-γ-S, and ATP. Primary neuronal cells were cultured in 96-well plates. After 24 h, the cells were treated with 50 μM $A\beta_{42}$ in PBS and various concentrations (0.04–25 μM) of ATP-γ-S-(α,β-CH_2), ATP-γ-S, and ATP for 48 h. Cell viability was measured by dyeing the cells with trypan blue and counting the vital cells. The results shown are the mean ± S.D. of three independent experiments performed in triplicate (*P < 0.05 vs. $A\beta_{42}$ treatment).

activity of ATP-γ-S-(α,β-CH_2) in primary neuron culture exposed to $FeSO_4$ insult. Indeed, ATP-γ-S-(α,β-CH_2) rescued primary neurons under oxidizing conditions with IC_{50} of 0.04 μM. Yet, ATP-γ-S was found to be more effective. At 0.01 μM it maintained the vitality of 50% of the neuron culture, while ATP-γ-S-(α,β-CH_2), at the same concentration, maintained only 40% cell viability. At 25 μM, both compounds showed 80% cell viability. The ability of ATP-γ-S-(α,β-CH_2) and ATP-γ-S to protect neurons better than ATP, could be linked to their role as agonists of P2Y receptors. Both ATP and ATP-γ-S activate P2Y11 receptor [23] with ATP-γ-S being the most potent agonist of the two [24].

P2Y1 receptor signaling enhances the neuroprotective capacity of astrocytes against oxidative stress [25]. The P2Y1 and P2Y11 receptors were found to be co-expressed by several tissues, including specific cell types in the brain [26], smooth muscle cells, and cells of the immune system [14]. In HEK293 cells, the P2Y11 receptor was found to be functionally associated with endogenous P2Y1 receptors [27]. Both receptors are phylogenetically related [28], yet, respond to different adenine nucleotide derivatives [29]. Hence, we assume that the P2Y1 and P2Y11 receptors are possibly related in their function as promoters of neuroprotection. Indeed, previously we found that ATP-γ-S-(α,β-CH_2) is a 7-fold more potent agonist than ATP at P2Y11 receptor [12]. This finding is also in agreement with our reported model of hP2Y11-R and the preference of the latter for ATP-γ-S over ATP, as a result of the tighter fit of the larger P_γ-S moiety [30]. The above data imply that one of the mechanisms of action of ATP-γ-S-(α,β-CH_2) as a neuroprotectant could be the activation of P2Y11/P2Y1 receptors.

Here we have shown that ATP-γ-S-(α,β-CH_2) also rescues neurons from $Aβ_{42}$ toxicity 4- and 125-fold better than ATP-γ-S and ATP, respectively (IC_{50} 0.2, 0.8 and 25 μM, respectively). This activity is probably due to the dual activity of ATP-γ-S-(α,β-CH_2) as both a metal ion chelator and radical scavenger. Recently, we have reported the ability of ATP-γ-S-(α,β-CH_2) to dissolve $Aβ_{40}$-Cu(I) and $Aβ_{42}$-Cu(II)/Zn(II) aggregates, much more effectively than ATP-γ-S, ATP, or EDTA [10]. We have also determined its high affinity to Zn(II)-ions [16]. Hence, we assume that the ability of ATP-γ-S-(α,β-CH_2) to dissolve Aβ-M(II) aggregates, and consequently rescue neurons from Aβ toxicity, results from metal-ions chelation.

On the other hand, various antioxidants have been shown to protect cells from Aβ toxicity [31–33]. The antioxidant activity of these agents resulted in decreased protein oxidation and lipid peroxidation, decreased radical formation, and elevated mitochondrial function vs. that in the presence of Aβ alone. Antioxidants reduce Aβ toxicity by various mechanisms such as suppression of expression of inflammatory cytokines [31], and increase of intracellular GSH levels [32] and heme oxygenase-1 and heat shock protein 72 levels. The latter play a pivotal role in the cytoprotection of neuronal cells against Aβ toxicity [33].

Importantly, we found that ATP-γ-S-(α,β-CH_2) was 20% more stable in PC12 cells than ATP-γ-S or ATP, during the first 4 h of incubation. After 24 h, ATP-γ-S and ATP were more than 90% degraded as compared to 50% degradation of ATP-γ-S-(α,β-CH_2). Furthermore, the latter ($t_{1/2}$ 14 h) proved to be 30% more stable than ATP in human blood serum.

In summary, the replacement of Pα-Pβ bridging oxygen atom in ATP-γ-S by a methylene group resulted in a biocompatible, relatively metabolically stable and potent agent that rescues primary neurons from oxidative damage and $Aβ_{42}$-toxicity in the culture. We propose that the mechanisms of action of ATP-γ-S-(α,β-CH_2) involve Fe(II)-chelation and to a lesser extent, radical scavenging [8]. Furthermore, ATP-γ-S-(α,β-CH_2) rescues neurons by breaking toxic Aβ-aggregates [10], possibly by removing metal-ions from $Aβ_{42}$ oligomers/aggregates. Hence, we suggest ATP-γ-S-(α,β-CH_2) as a lead structure for rescuing neurons from typical AD insults.

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.053.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.053.

References

[1] D. Pratico, Oxidative stress hypothesis in Alzheimer's disease: a reappraisal, Trends Pharmacol. Sci. 29 (2008) 609–615.

[2] H. Akiyama, S. Barger, S. Barnum, B. Bradt, J. Bauer, G.M. Cole, N.R. Cooper, P. Eikelenboom, M. Emmerling, B.L. Fiebich, C.E. Finch, S. Frautschy, W.S.T. Griffin, H. Hampel, M. Hull, G. Landreth, L.F. Lue, R. Mrak, I.R. Mackenzie, P.L. McGeer, M.K. O'Banion, J. Pachter, G. Pasinetti, C. Plata-Salaman, J. Rogers, R. Rydel, Y. Shen, W. Streit, R. Strohmeyer, I. Tooyoma, F.L. Van Muiswinkel, R. Veerhuis, D. Walker, S. Webster, B. Wegrzyniak, G. Wenk, T. Wyss-Coray, N.W. Grp, Inflammation and Alzheimer's disease, Neurobiol. Aging 21 (2000) 383–421.

[3] W. Garzon-Rodriguez, A.K. Yatsimirsky, C.G. Glabe, Binding of Zn(II), Cu(II), and Fe(II) ions to alzheimer's Aß peptide studied by fluorescence, Bioorg. Med. Chem. Lett. 9 (1999) 2243–2248.

[4] J.F. Schenck, E.A. Zimmerman, High-field magnetic resonance imaging of brain iron: birth of a biomarker? NMR Biomed. 17 (2004) 433–445.

[5] A.V. Kachur, Y. Manevich, J.E. Biaglow, Effect of purine nucleoside phosphates on OH radical generation by reaction of Fe^{2+} with oxygen, Free Radic. Res. 26 (1997) 399–408.

[6] J.E. Biaglow, K.D. Held, Y. Manevich, S. Tuttle, A. Kachur, F. Uckun, Role of guanosine triphosphate in ferric ion-linked Fenton chemistry, Radiat. Res. 145 (1996) 554–562.

[7] E.L.R. Swennen, P.C. Dagnelie, A. Bast, ATP inhibits hydroxyl radical formation and the inflammatory response of stimulated whole blood even under circumstances of severe oxidative stress, Free Radic. Res. 40 (2006) 53–58.

[8] Y. Richter, B. Fischer, Nucleotides and inorganic phosphates as potential antioxidants, J. Biol. Inorg. Chem. 11 (2006) 1063–1074.

[9] O. Danino, S. Grossman, B. Fischer, Nucleoside 5'-phosphorothioate derivatives as oxidative stress protectants in PC12 cells, Nucleos. Nucleot. Nucl. 32 (2013) 333–353.

[10] A. Amir, E. Shmuel, R. Zagalsky, A.H. Sayer, Y. Nadel, B. Fischer, Nucleoside-5'-phosphorothioate analogues are biocompatible antioxidants dissolving efficiently amyloid beta-metal ion aggregates, Dalton Trans. 41 (2012) 8539–8549.

[11] B. Grobben, P. Claes, D. Roymans, E.L. Esmans, H. Van Onckelen, H. Slegers, Ecto-nucleotide pyrophosphatase modulates the purinoceptor-mediated signal transduction and is inhibited by purinoceptor antagonists, Br. J. Pharmacol 130 (2000) 139–145.

[12] Y. Nadel, J. Lecka, Y. Gilad, G. Ben-David, D. Förster, G. Reiser, S. Kenigsberg, J. Camden, G.A. Weisman, H. Senderowitz, Highly potent and selective ecto-nucleotide pyrophosphatase/phosphodiesterase I inhibitors based on an adenosine 5'-(α or γ) thio,(α, β-or β, γ)-methylene triphosphate scaffold, J. Med. Chem. 57 (2014) 4677–4691.

[13] D. Erlinge, Chapter 13-P2Y receptors in health and disease, in: A.J. Kenneth, L. Joel (Eds.), Adv. Pharmacol, vol. 61, Academic Press, 2011, pp. 417–439.

[14] M.P. Abbracchio, G. Burnstock, J.-M. Boeynaems, E.A. Barnard, J.L. Boyer, C. Kennedy, G.E. Knight, M. Fumagalli, C. Gachet, K.A. Jacobson, G.A. Weisman, International union of pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy, Pharmacol. Rev. 58 (2006) 281–341.

[15] R.D. Fields, G. Burnstock, Purinergic signalling in neuron-glia interactions, Nat. Rev. Neurosci. 7 (2006) 423–436.

[16] A.H. Sayer, Y. Itzhakov, N. Stern, Y. Nadel, B. Fischer, Characterization of complexes of nucleoside-5'-phosphorothioate analogues with zinc ions, Inorg. Chem. 52 (2013) 10886–10896.

[17] O. Danino, N. Giladi, S. Grossman, B. Fischer, Nucleoside 5′-phosphorothioate derivatives are highly effective neuroprotectants, Biochem. Pharmacol. 88 (2014) 384–392.

[18] F. Kuperstein, E. Yavin, Pro-apoptotic signaling in neuronal cells following iron and amyloid beta peptide neurotoxicity, J. Neurochem. 86 (2003) 114–125.

[19] P. Maiti, R. Piacentini, C. Ripoli, C. Grassi, G. Bitan, Surprising toxicity and assembly behaviour of amyloid beta-protein oxidized to sulfone, Biochem. J. 433 (2011) 323–332.

[20] S.E. Eliahu, J. Camden, J. Lecka, G.A. Weisman, J. Sevigny, S. Gelinas, B. Fischer, Identification of hydrolytically stable and selective P2Y(1) receptor agonists, Eur. J. Med. Chem. 44 (2009) 1525–1536.
[21] M.P. Abbracchio, G. Burnstock, A. Verkhratsky, H. Zimmermann, Purinergic signalling in the nervous system: an overview, Trends Neurosci. 32 (2009) 19–29.
[22] G. Burnstock, Physiology and pathophysiology of purinergic neurotransmission, Physiol. Rev. 87 (2007) 659–797.
[23] D. Houston, S. Costanzi, K.A. Jacobson, T.K. Harden, Development of selective high affinity antagonists, agonists, and radioligands for the P2Y(1) receptor, Comb. Chem. High. Throughput Screen 11 (2008) 410–419.
[24] K.A. Jacobson, A.A. Ivanov, S. de Castro, T.K. Harden, H. Ko, Development of selective agonists and antagonists of P2Y receptors, Purin. Signal 5 (2009) 75–89.
[25] T. Fujita, H. Tozaki-Saitoh, K. Inoue, P2Y1 receptor signaling enhances neuroprotection by astrocytes against oxidative stress via IL-6 release in hippocampal cultures, Glia 57 (2009) 244–257.
[26] D.J. Moore, J.K. Chambers, J.P. Wahlin, K.B. Tan, G.B. Moore, O. Jenkins, P.C. Emson, P.R. Murdock, Expression pattern of human P2Y receptor subtypes: a quantitative reverse transcription-polymerase chain reaction study, Biochim. Biophys. Acta 1521 (2001) 107–119.
[27] D. Ecke, T. Hanck, M. Tulapurkar, R. Schafer, M. Kassack, R. Stricker, G. Reiser, Hetero-oligomerization of the P2Y11 receptor with the P2Y1 receptor controls the internalization and ligand selectivity of the P2Y11 receptor, Biochem. J. 409 (2008) 107–116.
[28] S. Costanzi, L. Mamedova, Z.-G. Gao, K.A. Jacobson, Architecture of P2Y nucleotide receptors: structural comparison based on sequence analysis, mutagenesis, and homology modeling, J. Med. Chem. 47 (2004) 5393–5404.
[29] D. Ecke, M.E. Tulapurkar, V. Nahum, B. Fischer, G. Reiser, Opposite diastereoselective activation of P2Y1 and P2Y11 nucleotide receptors by adenosine 5'-O-(alpha-boranotriphosphate) analogues, Br. J. Pharmacol 149 (2006) 416–423.
[30] J. Zylberg, D. Ecke, B. Fischer, G. Reiser, Structure and ligand-binding site characteristics of the human P2Y11 nucleotide receptor deduced from computational modelling and mutational analysis, J. Biochem. 405 (2007) 277–286.
[31] G.P. Lim, T. Chu, F. Yang, W. Beech, S.A. Frautschy, G.M. Cole, The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse,, J. Neurosci. 21 (2001) 8370–8377.
[32] R. Sultana, S. Newman, H. Mohmmad-Abdul, J.N. Keller, D.A. Butterfield, Protective effect of the xanthate, D609, on Alzheimer's amyloid beta-peptide (1-42)-induced oxidative stress in primary neuronal cells, Free Radic. Res. 38 (2004) 449–458.
[33] R. Sultana, A. Ravagna, H. Mohmmad-Abdul, V. Calabrese, D.A. Butterfield, Ferulic acid ethyl ester protects neurons against amyloid β-peptide (1–42)-induced oxidative stress and neurotoxicity: relationship to antioxidant activity, J. Neurochem. 92 (2005) 749–758.