Journal of Biological Sciences 6 (1): 140-145, 2006
ISSN 1727-3048

Aβ(25-35) Peptide Induces Cell Death in PC12 Cells via Mitochondrial Damage and Cytochrome *c* Release

[1]Cristiana Carelli Alinovi, [1]Michela Pezzotti, [2]Daniele Mezzogori,
[3]M. Elisabetta Clementi, [4]Francesco Misiti, [1,3]Bruno Giardina and [1]Federica Orsini
[1]Faculty of Medicine, Institute of Biochemistry and Clinical Biochemistry,
Catholic University Largo F. Vito 1, 00168 Rome, Italy
[2]Faculty of Medicine, Institute of Physiology,
Catholic University Largo F. Vito 1, 00168 Rome, Italy
[3]Faculty of Medicine, Institute Chimica del Riconoscimento Molecolare,
CNR, Catholic University Largo F. Vito 1, 00168 Rome, Italy
[4]Department of Scienze Motorie e della Salute, Faculty of Scienze Motorie,
University of Cassino, Viale Bonomi, 03043 Cassino (Fr), Italy

Abstract: The pathological features of Alzheimer's disease include deposition of senile plaques in different brain zones formed by aggregates of fibrillar Aβ peptide (AβP), a neurotoxic metabolic product. In this study we used the soluble form of fragment 25-35 of AβP, that includes methionine 35, side chain of AβP, to investigate the role of redox state of Met-35 on the pathogenesis of AD, because this residue in AβP is the most susceptible to oxidation *in vivo*. The data obtained evidenced that Aβ(25-35) peptide determines a loss of PC12 cells viability determining mitochondrial damage with a possible trigger of pro-apoptotic signals. In particular, the following parameters were examined: cytochrome *c* release, mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial respiration. In this study, three different peptides have been used: Aβ(25-35) with methionine 35 in the reduced state, oxidized to sulfoxide and/or substituted with norleucine. We conclude that alteration in the mitochondrial functionality might be a contributing factor to the pathogenesis of AD and the amplitude of the effects elicited by Aβ peptide is modulated by the redox state of methionine.

Key words: Alzheimer's disease, Aβ(25-35) fragment, apoptosis, cytochrome *c*

INTRODUCTION

Alzheimer's disease is a multifaceted neurodegenerative disorder characterized by the progressive deterioration of cognition and memory in association with deposit of senile plaques formed by aggregates of fibrillar AβP in several parts of brain[1]. The Amyloid-β Peptide (AβP) is a product formed in cells through the proteolytic processing of the amyloid precursor protein (APP). APP is present in soluble form in plasma and is constitutively expressed in many cells of normal individuals. Neuronal cell death is associated with the cumulating of amyloid plaques formed by aggregates of fibrillar Aβ peptides, due either to excessive production or reduced clearance of Aβ[2].

Aβ is a 39-42-residue peptide but a smaller 11-residue fragment of Aβ, the Aβ(25-35) fragment possesses much of the biological activity of the full-length peptide. The fibrillar aggregates of fragment of Aβ(25-35), both *in vivo* and *in vitro*, are highly cytotoxic to neuronal cells[3].

One of the best known hypothesis is that Aβ-dependent-neurotoxicity may be mediated by free radicals and/or reactive oxygen species (ROS)[4]. One of the earliest pathological events in AD is oxidative damage that is not limited to the AD lesions but it involves also the neuronal cytoplasm and mitochondrial enzymes[5]. It has been evidenced that Met-35 is the residue in Aβ most susceptible to oxidation *in vivo*, producing methionine sulfoxide in biological systems[6]. Elevated levels of oxidised AβP were found in AD brains[7]. For these reasons in this study we used the fragment 25-35 of AβP that includes methionine 35 at C-terminal of AβP to investigate the role of oxidation state of methionine 35 on the toxic and pro-apoptotic effects in PC 12 cells. Aβ(25-35) may exist in two different forms: the aggregated and soluble ones[8]. The first one is not able to

Corresponding Author: Federica Orsini, Faculty of Medicine, Institute of Biochemistry and Clinical Biochemistry,
Catholic University Largo F. Vito 1, 00168 Rome, Italy
Tel: +39 6 3015 4215 Fax: +39 6 3015 4309

be internalised within the PC12 cells and its addiction to neuronal cell cultures produces changes in the activity of various membrane proteins, including ion channels and receptors, potentially as a result of spontaneous intercaling into the membrane bilayer[9,10]. These findings indicate that this peptide, highly lipophilic, interacts with the membrane inducing apoptotic effects independently and/or dependently of a receptor mediated pathway. On the contrary the soluble form is able to cross the plasmatic membranes triggering cell death pathways[11,12]. It has been also recently demonstrated[13] that Aβ regulates specific genes that are relevant to the induction of apoptotic pathways.

In this study, we have evidenced that PC12 cells (a line of rat pheochromocytoma) treated with Aβ(25-35) show loss of cell viability, probably due to an altered mitochondrial functionality, as evidenced by the change of mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial respiration. Therefore, it has been evidenced that the amplitude of these effects changes according to the redox state of Methionine-35[14,15].

MATERIALS AND METHODS

Preparation of peptides: When this study was conducted, Aβ(25-35) peptides with methionine, which is either in the oxidized and reduced form or substituted with norleucine (were -S group of methionine is replaced by a $-CH_2$) were used. All peptides were synthesized and purchased from Peptide Speciality Laboratories GmbH (Heidelberg, Germany). Peptides were dissolved in dimethylsulfoxide (DMSO) at a concentration of 25 mM and stored at -80°C. In previous studies[8,16] these condition have been shown to lead to the predominance of the soluble monomeric form of these peptides. In any case, in order to verify the non-aggregated form of the peptides, quantitative measurement of Congo red (from Sigma) binding was carried out as described by Wood[17]. In all control experiments, the concentration of DMSO (i.e.,< 0.5%) was the same of the solutions.

Cell culture and Aβ treatment: The PC12 were cultured in according to Iuvone *et al.*[18]. For determination of cytotoxicity of Aβ peptides, PC12 (rat pheochromocytoma) were initially plated in 96-well plates at a density of 10,000 cells/well and maintained 16 h in complete medium. Cells were then incubated for 24, 36 and 48 h in the absence (controls) or presence of 40 μM Aβ(25-35) peptides with methionine in the oxidized and unoxidized form and with Met→Nle substituted Aβ peptides. 10 μM staurosporine was used as positive control of 100% of cellular death (data not shown).

Direct toxicity study: Cell viability was determined by MTS test (Cell Titer 96 Promega Corporation Madison, USA)[19]. PC12 cells were plated in 96-well plates at a density of 10,000 cells/well and maintained for 16 h in complete medium. Cells were then incubated in the absence (control) or presence of 40 μM Aβ(25-35) with reduced, oxidized and norleucine-substituted methionine 35. 10 μM staurosporine was used as positive control of 100% of cellular death (data not shown). After 48 h of peptide incubation, 20 μM of MTS reagent (2.0 mg mL^{-1}) was added to each well.

Mitochondria preparation: All experiments were conducted according to the European Communities Council Directive of 24 November 1986 (86/609/EEC) and all efforts were made to minimize the number of animals used and their suffering.

Non-synaptic brain mitochondria were prepared as previously reported[14].

Analysis of mitochondrial oxygen consumption: Mitochondrial respiration were measured using for complex I, glutamate (1.7 mM) and malate (1.7 mM) and for complex II, succinate (2.5 mM) with NADH dehydrogenase inhibitor (2 μM rotenone) as previously reported[14]. Oxygen consumption was measured at 37°C with a Clark-type oxygen electrode (Strathkelvin Instr., Glasgow, UK) under continuous stirring.

Mitochondrial transmembrane potential determination: $\Delta\psi_m$ was measured by fluorimetry in the presence of rhodamine 123 (excitation 490 nm/emission 530 nm) according to Scaduto and Grotyohann[20]. Fluorescence measurements were conducted on mitochondria suspension previously incubated with peptides under analysis at a final concentration of 1 mg mL^{-1} for 30 min at 37°C. The concentration of Aβ(25-35) and Aβ(25-35)Met35^{ox} was 40 μM. Substrates were added at a concentration of 2.5 mM for succinate (in the presence of 2 μM rotenone) and of 1.7 mM glutamate plus 1.7 mM of malate.

Detection of cytochrome *c* release: Freshly isolated mitochondria were incubated in the absence (control) or presence of Aβ(25-35) and Aβ(25-35)Met35^{ox} at a concentration of 40 μM. Detection of cytochrome *c* release was performed by Western blotting analysis[13] utilizing an anti cytochrome *c* mAb (clone 7H8.2C12; PharMinger, San Diego, CA, USA).

Statistical analysis: Data were analysed for statistical differences by one-way analysis of variance (ANOVA) as

well as by the two-tailed Student's t-test; a p-value of less than 0.05 was considered significant.

RESULTS

The toxicity of Aβ(25-35) peptide with reduced Aβ(25-35)Met-35, oxidized Aβ(25-35)Met-35^{ox} and Norleucine-substituted Aβ(25-35)Met-35^{Nle} methionine residue in a cell line of neuronal origin, PC12, was examined. Cell viability was determined by MTS reduction assay. The Aβ peptides were prepared in DMSO, as described in the material and methods section, in order to avoid formation of aggregates in Aβ(25-35) peptide samples[8,16]. The results depicted in Table 1, show that Aβ(25-35)Met-35 induces an higher level of cell death with respect to that evidenced by Aβ(25-35) Met-35^{ox}-treatment. Aβ(25-35) Met-35^{Nle} has the lowest toxicity, as demonstrated by the low level of toxicity degree. Importantly, neurotoxic effects were already statistically significant after 24 h incubation.

In order to identify the intracellular target of Aβ peptides, we investigated the effects on mitochondria functionality after exposure of PC12 cells to Aβ(25-35) peptides. Firstly, we analysed the mitochondrial oxygen consumption, using as substrates both succinate

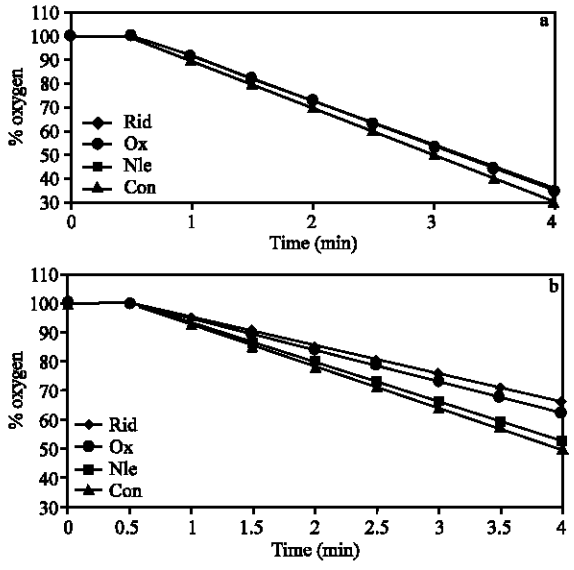

Fig. 1: Oxygen consumption (expressed as percentage of control) for isolated rat brain mitochondria (1 mg mL^{-1}) in the presence of 40 μM peptides. Arrows show addition of substrate. (A) Representative oxygen electrode traces in the presence of succinate as substrate plus 2 μM rotenone. (B) Representative oxygen electrode traces in the presence of glutamate/malate as substrate

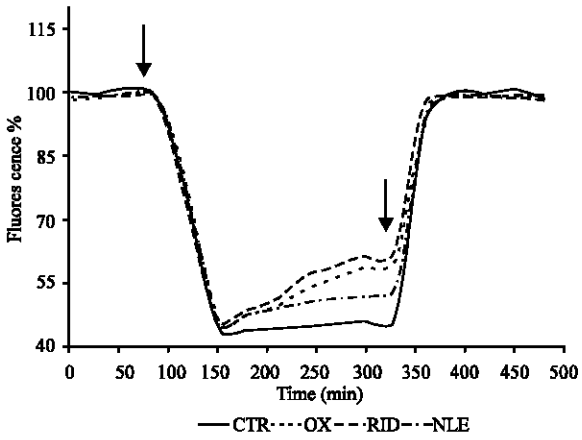

Fig. 2: Mitochondrial membrane potential determination. Where indicated (first arrow) glutamate/malate were added; second arrow shows addiction of FCCP (1 μM). The experiment showed is representative of five separate measurements. Experimental conditions are reported in Methods

Table 1: MTS assay for effects of 40 μM peptides on PC12 cell viability

Peptides	Cell death (% control)
Aβ(25-35)Met	19.5±2.10**,***
Aβ(25-35)Met^{ox}	15.0±0.95*
Aβ(25-35)Met^{Nle}	8.0±1.85

All values indicate means±s.d. of five independent experiments
Significantly different from control: *= p<0.05; **= p<0.001
Significantly different from other peptides: ***= p<0.05

(Fig. 1A) and glutamate/malate (Fig. 1B). As we can see the most evident effects are observed in the experiments performed with glutamate/malate (panel B). In particular, it should be noted that Aβ(25-35)Met-35 has a major effect in comparison with the effects shown by the oxidized peptide, inducing a respiration inhibition (with respect to the control) of approximately 20% against 10% (p<0.05 respect to the control).

When succinate was used as substrate, the results obtained with Aβ(25-35)Met-35, Aβ(25-35)Met-35^{ox} and Aβ(25-35)Met-35^{Nle} are comparable.

Another crucial parameter to investigate the mitochondrial functionality, is the $\Delta\psi_m$ (Mitochondrial transmembrane potential) which decrement appears to occur in cells undergoing apoptosis[21]. To investigate the alteration of $\Delta\psi_m$ during Aβ(25-35) treatment, the fluorescent probe rhodamine 123 was used. The addition of glutamate plus malate to mitochondria suspensions causes quenching of the probe fluorescence, due to rhodamine 123 uptake by mitochondria. As shown in Fig. 2, in mitochondria treated with Aβ(25-35)Met-35 and Aβ(25-35)Met-35^{ox}, the reduction rate of the fluorescence was lower (respectively, 40 and 45%) than that observed

Fig. 3: Cytochrome c released from brain purified mitochondria untreated, lane 2; and after incubation with 40 μM Aβ(25-35)Met-35, lane 3; Aβ(25-35)Met-35^{ox}, lane 4; and Aβ(25-35)Met-35^{Nle}, lane 5. Standard commercial horse heart cytochrome *c* in lane 1.

in control mitochondria and in those treated with Aβ(25-35)Met-35^{Nle}, indicating that the presence of Methionine 35, in Aβ(25-35), induces an higher drop of the $\Delta\psi_m$ and that the Methionine in reduced state induces a larger mitochondrial membrane damage. This effect was not evident in the experiments performed in presence of succinate as substrate (data not shown).

Transfer of cytochrome *c* from the mitochondrial inter-membrane space to the cytosol occurs as an early event in apoptosis[22]. Cytochrome *c* release from mitochondria into the cytosol of PC12 cells was evaluated by SDS-page electrophoresis followed by immunoblotting of the cytosolic fraction, as described in materials and methods. As shown in Fig. 3 the release of the cytochrome c from mitochondria treated with Aβ(25-35)Met-35^{Nle} was comparable with control, while in the mitochondria treated with Aβ(25-35) Met-35^{ox} was only slightly increased respect to the controls. On the contrary, the amount of cytochrome *c* released in the supernatants of the mitochondria incubated with Aβ(25-35)Met-35 was significantly higher respect to that evident in control experiment.

DISCUSSION

It is well documented that Aβ plays a central role in the pathogenesis of AD. The effects of Aβ seems to be localized to amino acid residues 25-35 of the full length peptide[23]. Aβ(25-35) may exist in two different forms: the aggregated and soluble ones. They are both cytotoxic for neuronal cells[24] even if they have different action mechanism due to their different lipophilic properties. The aim of this study was to investigate the toxic mechanisms of the Aβ(25-35) in its soluble form, focusing on the crucial role played by the methionine residue. To this aim, we have used Aβ(25-35)Met-35, Aβ(25-35)Met-35^{ox} and Aβ(25-35)Met-35^{Nle} where the methionine at C-terminal is, respectively in the reduced, oxidated and substituted with norleucine residue.

The data presented here show that Aβ(25-35) induces cell death in PC12 neuronal cells as confirmed by the MTS assay. A proposed model of Aβ(25-35) mediated toxicity includes methionine 35 side chain of AβP because it has been clearly evidenced that Met –35 is the residue in AβP most susceptible to oxidation *in vivo*, resulting in the formation of methionine sulfoxide in biological systems[6]. The oxidation of methionine in peptides and proteins represents an important post-translational modification under conditions of oxidative stress, aging and during pathogenesis of Alzheimer's disease[25].

Present results on cell viability show that Aβ(25-35)Met-35 results more toxic than Aβ(25-35) Met-35^{ox}. Aβ(25-35)Met-35^{Nle} causes no significant effects on cellular vitality. These findings stress out that the intensity of effects changes according to the redox state of methionine 35 and that the substitution of this aminoacid by structurally similar norleucine, abrogates the toxic action of Aβ(25-35).

Because neuronal apoptosis, mitochondrial-mediated, is a supposed pathway of neurodegeneration in Alzheimer's disease, we investigated the role of Aβ(25-35) in this pathway responsible of activation of neuronal cell death.

Three pro-apoptotic events, such as mitochondrial respiration, mitochondrial transmembrane potential ($\Delta\psi_m$) and release of cytochrome *c* were examined in order to evaluate the apoptotic nature of Aβ(25-35) dependent effects. In mammalian cells, mitochondria functionality is an important index of physiological cellular state and mitochondria plays a crucial role in the regulation and execution of cell death pathways[26].

As regards to the mitochondrial respiration we observed that both the reduced and the oxidized peptide show an inhibitory effect on the activity of the complex I of the mitochondrial respiratory chain. In particular Aβ(25-35)Met-35 induces an higher inhibitory effect on the mitochondrial respiration, in comparison with the effects elicited by the Aβ(25-35)Met-35^{ox}.

The study on $\Delta\psi_m$ has further outlined the higher toxic effects elicited by Aβ(25-35)Met-35 peptide with respect to the oxidized form. It is hypothesizing that $\Delta\psi_m$ alteration may be due to a damage in mitochondrial membrane associated with changes in the membrane pore proteins conformation. For example, a reduction in the mitochondrial membrane potential increases the probability that a mitochondrial permeability transition pore will occur in response to a stimulus[27].

The correlation between the $\Delta\psi_m$ alteration and the apoptotic pathway induction has been confirmed by the presence of cytochrome *c* in the cytosolic fraction of AβP

treated PC12 cells. Although the direct relationship between mitochondrial $\Delta\psi_m$ alteration and cytochrome *c* release is still controversial, it is hypothesizing that this release in cytoplasm, triggers apoptosis associated with proteolytic cascade activation. Also in this case, Aβ(25-35) Met-35 had the ability to induce an higher level of cytochrome *c* release, compared with other two peptides tested in this study.

Although we provide evidence that oxidation of Met-35 to Met-35^{ox} does not affect the internalization process of the Aβ(25-35) peptide[28], it significantly affects the amplitude of Aβ(25-35) induced toxicity within the cell. The differences observed between the neurotoxic properties of the Aβ(25-35) and Aβ(25-35)Met-35^{ox} peptides might result from differences in the ability to interact with lipid bilayer of the mitochondrial membrane[29].

In conclusion, on the basis of the results obtained it is suggestive to hypothesise that mitochondrial damage and the following apoptotic events induced by AβP may be responsible for the initial cellular toxicity that occurs during the development of Alzheimer's disease. Because elevated levels of Aβ peptides containing the methionine oxidised to sulfoxide were found in AD brains[7], Aβ peptides oxidized would prolong or even exacerbate the extensive cellular damages found in AD brain.

ACKNOWLEDGMENT

We are grateful to Dr. Beatrice Sampaolese of CNR, ICRM (Rome, Italy) for expert scientific assistance. This study was supported in part by MIUR nr. 2003058409 2003.

REFERENCES

1. Xiao, X.Q., H.Y. Zhang and X.C. Tang, 2002. Huperzine A attenuates amyloid-β peptide fragment 25-35 induces apoptotic in rat cortical neurons via inhibiting reactive oxigen species formation and caspase-3activation. J. Neurosi. Res., 67: 30-36.
2. Qiao, H., R.C. Koya, K. Nakagawa, H. Tanaka, H. Fujita, M. Takimoto and N. Kuzumaky, 2005. Inhibition of Alzheimer's amyloid-β peptide-induced reduction of mitochondrial membrane potential and neurotoxicity by gelsolin. Neurobiol. Aging., 26: 849-855.
3. Casley, C.S, J.M. Land, M.A. Sharpe, J.B. Clark, M.R. Duchen and L. Canevari, 2002. β-amyloid fragment 25-35 causes mitochondrial dysfunction in primary cortical neurons. Neurobiol. Dis., 10: 258-267.
4. Varadarajan, S., S. Yatin, M. Aksenova and D.A. Butterfield, 2000. Alzheimer's amyloid β-peptide associated free radical oxidative stress and neurotoxicity. J. Struct. Biol., 130: 184-208.
5. Smith, M.A., C.A. Rottkamp, A. Nunomura, A.K. Raina and G. Perry, 2000. Oxidative stress in Alzheimer's disease. Biochem. Biophys. Acta, 1502: 139-144.
6. Varadarajan, S., J. Kanski, M. Aksenova, C. Lauderback and D.A. Butterfield, 2001. Different mechanisms of oxidative stress and neurotoxicity for Alzheimer's Aβ(1--42) and Aβ(25-35). J. Am.. Chem. Soc., 123: 5625-5631.
7. Kuo, Y.M., T.A. Kokjohn, T.G. Beach and L.I. Sue, *et al*., 2001. Comparative analysis of amyloid-β chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. J. Biol. Chem., 276: 12991-12998.
8. Pike, C.J., A.J. Walencewicz-Wasserman, J. Kosmoski, D.H. Cribbs, C.G. Glabe and C.W. Cotman, 1995. Structure-activity analyses of β-amyloid peptides: Contributions of the β 25-35 region to aggregation and neurotoxicity. J. Neurochem., 64: 253-265.
9. Preston, M.R., J.D. Estermyer, J. Kelly and P.E. Mason, 1996. Alzheimer's Disease Amyloid β peptide 25-35 is localized in the membrane hydrocarbon core: X-ray diffraction analysis. Biochem. Biophys. Res. Commun., 222: 78-82.
10. Buchet, R., E. Tavitian, D. Ristig, R. Swoboda, U. Stauss and H.U. Gremlich, 1996. Conformation of synthetic β peptides in solid state and in aqueous solution: Relation to toxicity in PC12 cells. Biochem. Biophys. Acta, 1315: 40-46.
11. Dahlgren, K.N., A.M. Manelli, W.B.Jr. Stine, L.K. Baker, G.A. Krafft and M.J. LaDu, 2002. Oligomeric and fibrillar species of amyloid-β peptides differentially affect neuronal viability. J. Biol. Chem., 277: 32046-32053.
12. Kim, H.S., J.H. Lee, J.P. Lee, E.M. Kim and K.A. Chang *et al*., 2002. Amyloid β peptide induces cytochrome *c* release from isolated mitochondria. Neuroreport., 13: 1989-1993.
13. Cardoso, S.M., R.H. Swerdlow and C.R. Oliveira, 2002. Induction of cytochrome c-mediated apoptosis by amyloid β 25-35 requires functional mitochondria. Brain. Res., 931: 117-125.
14. Misiti, F., G.E. Martorana, G. Nocca, E. Di Stasio, B. Giardina and M.E. Clementi, 2004. Methionine 35 oxidation reduces toxic and pro-apoptotic effects of the amyloid β- protein fragment (31-35) on isolated brain mitochondria. Neuroscience, 126: 297-303.

15. Clementi, M.E., G.E. Martorana, M. Pezzotti, B. Giardina and F. Misiti, 2004. Methionine 35 oxidation reduces toxic effects of the amyloid β- protein fragment (31-35) on human red blood cell. Int. J. Biochem. Cell Biol., 36: 2066-2076.
16. Boland, K., M. Behrens, D. Choi, K. Manias and D.H. Perlmutter, 1996. The serpin-enzyme complex receptor recognizes soluble, nontoxic amyloid-β peptide but not aggregated, cytotoxic amyloid-β peptide. J. Biol. Chem., 271: 18032-18044.
17. Wood, S.J., B. Maleeff, T. Hart and R. Wetzel, 1996. Physical, morphological and functional differences between pH 5.8 and 7.4 aggregates of the Alzheimer's amyloid peptide Aβ. J. Mol. Biol., 256: 870-877.
18. Iuvone, T., G. Esposito, R. Esposito, R. Santamaria, M. Di Rosa and A.A. Izzo, 2004. Neuroprotective effect of cannabidiol, a non-psychoactive component from *Cannabis sativa*, on β-amyloid-induced toxicity in PC12 cells. J. Neorochem., 89: 134-141.
19. Cory, A.H., T.C. Owen, J.A. Barltrop and J.G. Cory, 1991. Use of an aqueous soluble tetrazolium/ formazan assay for cell growth assays in culture. Cancer. Commun., 3: 207-212.
20. Scaduto, R.C. and L.W. Grotyohann, 1999. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. J. Biophys., 76: 469-477.
21. Nicholls, D.G. and S.L. Budd, 2000. Mitochondria and neuronal survival. Physiol. Rev., 80: 315-60.
22. Gorman, A.M., S. Orrenius and S. Ceccateli, 1998. Apoptosis in neuronal cells: role of caspases. Neuroreport, 9: 49-55.
23. Mattson, M.P., B. Cheng, D. David, K. Bryant, I. Leiberburg and R.E. Rydel, 1992. β-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J. Neuroscience, 12: 376-389.
24. Pike, C.J., D. Burdick, A.J. Walencewicz, C.G. Glabe and C.W. Cotman, 1993. Neurodegeneration induced by β-amyloid peptides *in vitro*: the role of peptide assembly state. J. Neurosci., 13: 1676-1678.
25. Hong, J. and C. Schoneich, 2001. The metal-catalyzed oxidation of methionine in peptides by fenton systems involves two consecutive one-electron oxidation processes. Free Radic. Biol. Med., 31: 1342-1441.
26. Green, D.R. and J.C. Reed, 1998. Mitochondria and apoptosis. Science, 281: 1309-1312.
27. Hsueh-Meei, H., F. Corinne, X. Hui, Z. Hui and G.E. Gibson, 2004. Mitochondrial function in fibroblasts with aging in culture and/or Alzheimer's disease. Neurobiol. Aging, 26: 839-848.
28. Misiti, F., B. Sampaolese, M. Pezzotti, S. Marini, M. Coletta, L. Ceccarelli, B. Giardina and M.E. Clementi, 2005. Aβ (31-35) peptide induces apoptosis in PC12 cells: Contrast with Aβ (25-35) peptide and examination of underlying mechanisms. Neurochem. Int., 46: 575-583.
29. Watson, A.A., D.P. Fairlie and D.J. Craik, 1998. Solution structure of methionine-oxidized amyloid β-peptide (1-40). Does oxidation affect conformational switching. Biochem., 37: 12700-12706.