Institut für Pharmazie/Pharmazeutische Biologie, Freie Universität Berlin, Germany

Research on an *in vitro* cell system for testing the neurotoxicity of kynurenine pathway metabolites

N. Wszelaki, M. F. Melzig

Received April 11, 2011, accepted May 13, 2011

Prof. Dr. Matthias M. Melzig, Institut für Pharmazie/Pharmazeutische Biologie, Freie Universität Berlin, Königin-Luise-Str. 2+4 D-14195 Berlin, Germany
melzig@zedat.fu-berlin.de

Pharmazie 66: 899–903 (2011) *doi: 10.1691/ph.2011.1560*

Quinolinic acid (QUIN), kynurenine acid (KYNA) and 3-hydroxykynurenine (3-HK) - metabolites of the kynurenine pathway are considered to be associated with many central nervous system diseases. However, in neuroscience research in order to test neurotoxicity or neuroprotection against these compounds only primary cell models are available. In this investigation we aimed to develop a simple, rapid and accurate cellular *in vitro* model using immortalized human neuroblastoma cell lines, namely SK-N-SH and SH-SY5Y differentiated by treatment with various agents. In order to alter the cell response to the neurotoxins, tumor necrosis factor-alpha and retinoic acid (RA) as differentiating agents and modulation of the cellular metabolism through changing the sugar composition from galactose to glucose in media were used. Our results indicated that although RA-differentiation of both cell lines induced the expression of neuronal features, cell vulnerability after exposure to control neurotoxicants (salsolinol, 6-hydroxydopamine) and 3-HK was decreased in comparison to untreated cells and was not influenced after exposure to QUIN and KYNA. Interestingly, the same observations were done in cells grown in galactose containing media.

1. Introduction

Neuronal cell death in the central nervous system can be caused by several neurotoxic agents and may lead to the development of some neurodegenerative disorders including Alzheimer's (AD), Parkinson's (PD) or Huntington's disease (HD). Research on the causes of brain pathologies has revealed a number of features accompanying neurodegeneration. There is emerging evidence for coexistence of oxidative stress, neuroinflammation, imbalanced calcium homeostasis, and mitochondrial dysfunction in a large number of neurodegenerative diseases (Floyd 1999; Lin et al. 2006). Some of these features can be triggered by a wide range of pathological endogenous molecules, including beta-amyloid (Aβ), 6-hydroxy-dopamine (6-OHDA), tumor necrosis factor-alpha (TNF-α) and several metabolites of the kynurenine pathway, namely kynurenine acid (nanomolar concentrations), 3-hydroxy-kynurenine (3-HK), quinolinic acid (QUIN) by a various mechanism of action, which can lead to neuronal cell death (Behan et al. 1999; Mattson et al. 1997; Maury 1995; Okuda et al. 1996; Rozsa et al. 2008; Ungerstedt 1976; Wanpen et al. 2004).

In order to induce neuronal damage *in vitro*, primary cell cultures would be the best system, due to the ability to resemble the *in vivo* state and generate physiologically relevant data. However, the difficulty connected with isolation, maintenance and growth is limiting the application in research facilities. As an alternative for primary cells tumor-derived, immortalized cell lines can be used. For neurotoxicity studies human neuroblastoma cell lines, namely SK-N-SH and SH-SY5Y, which represent immature neurons with high proliferation capacity are frequently used (Biedler et al. 1973). Both neuroblastoma cell lines lack some neurochemical markers that define nerve cells, including neuronal morphology, inhibited cell division, and expression of neuron-specific markers. However, exposure of neuroblastoma cells to various differentiation agents, for instance, retinoic acid (RA) induces a switch from non-neuronal to neuronal phenotype (Wainwright et al. 2001). Furthermore, upon differentiating with RA, neuroblastoma cells express functional NR1 and NR2B subunits of NMDA receptors (Pizzi et al. 2002), which play crucial role in excitotoxicity (Garthwaite and Garthwaite 1987).

The aim of the present study was to review the most frequent used cell models and evaluate a reliable and useful *in vitro* cytotoxic cellular system, which could partly imitate real pathological situations for testing potential neuroprotective agents against several kynurenine pathway intermediates.

2. Investigations and results

In order to develop a neuronal morphology in neuroblastoma cell lines, namely SK-N-SH and SH-SY5Y, and to modulate their responsiveness to neurotoxins, cells were exposed to various neuronal differentiating agents acting through different cell death pathway. It has been previously reported that neuroblastoma cells can be differentiated to more neuron-like cells by adding RA to the culture medium for 5 days (Pahlman et al. 1984). Due to the fact that RA is commonly used in neuroscience research as differentiation factor for neuroblastoma cells, we decided to evaluate, if this system is appropriate to investigate neurotoxicity of several metabolites. In the current investigation RA-differentiating was performed for 6 days. TNF-α was used as another agent with differentiating potential. This was based on the report of Obregon et al. (1997), who postulated that 24 h treatment with 500 U/ml TNF-α might induce neuroblastoma

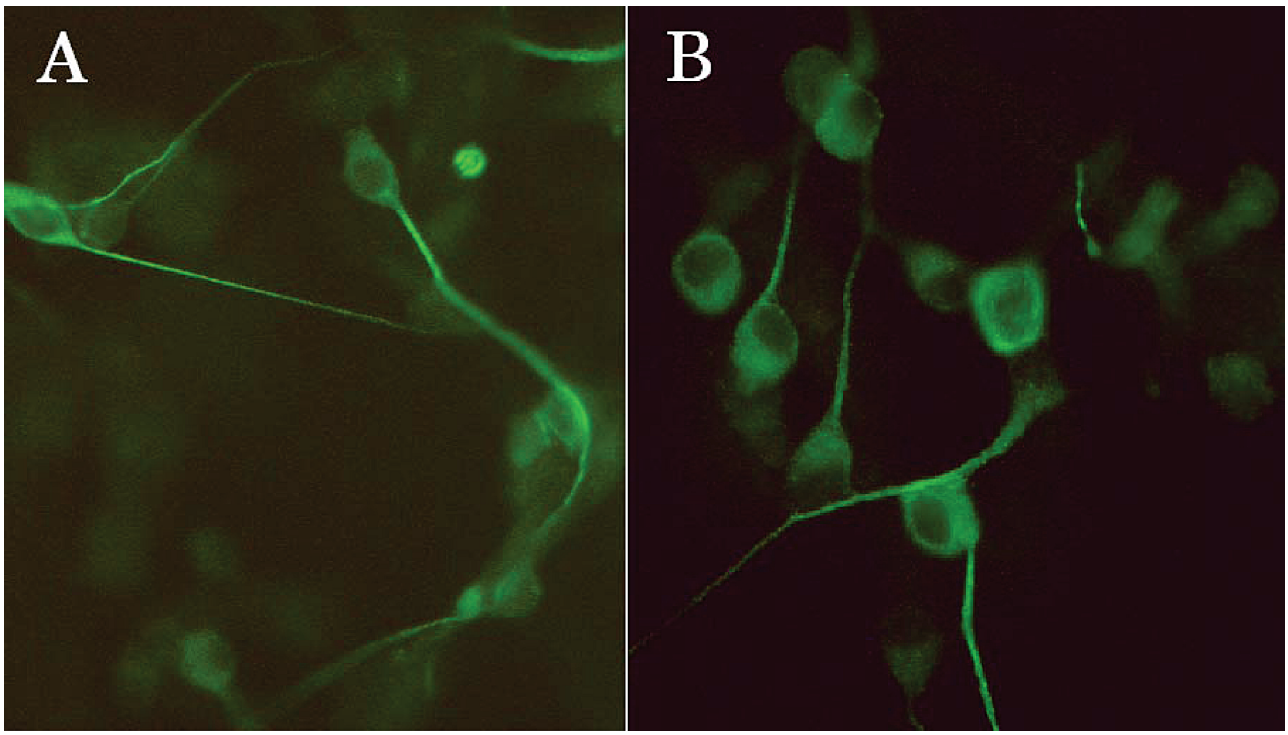


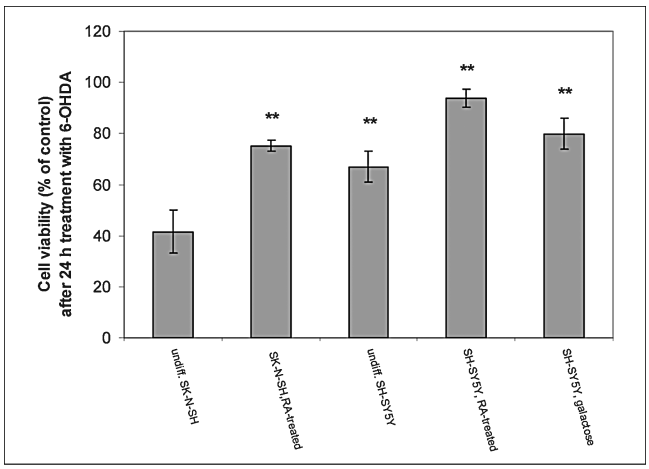
Fig. 1: Immunofluorescence staining for MAP2 protein in SK-N-SH cells (A) and in SH-SY5Y cells treated for 6 days with RA (10 μM). MAP2 immunoreactivity (green) was detected in cells treated with RA. Data were a representative similarly obtained from three independent experiments

cell differentiation. To confirm the neuronal identity of neuroblastoma cell lines after 6 days RA- and 24 h -TNF-α treatment, immunofluorescence staining with microtubule-associated protein MAP2 was performed, which is a specific marker for neurite extension. In the current study, as shown in Fig. 1, we were able to verify previous investigations on expressing MAP2 by RA-differentiated cells (Encinas et al. 2000; Pizzi et al. 2002), however TNF-α treated cells were negative for MAP2, which we report for the first time.

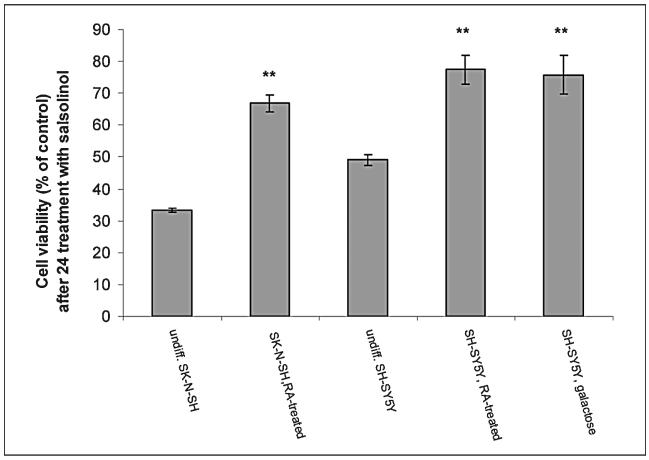
For this reason, only RA-differentiating cells were chosen for further investigations with neurotoxins. Another way of improving the responsiveness of the cell cultures to neurotoxins is to supplement the cell media with galactose and to deprive of glucose. Marroquin et al. (2007) suggested that cells grown in glucose media are more resistant to mitochondrial toxicants than those cells grown in media where glucose had been replaced by galactose as the primary oxidizable substrates. Cells under these conditions are forced to use oxidative phosphorylation and not glycolysis for their energy demand, which make the cells more susceptible to mitochondrial insults. Based on these findings, we created a similar cell system using neuroblastoma cells. Afterwards cells were exposed to molecules reported to cause neuronal cell death via altering mitochondrial function and molecules of the kynurenine pathway. 6-Hydroxydopamine (6-OHDA), an endogenously produced metabolite of dopamine oxidation, is a well-recognized neurotoxin and due to this reason was used in this study as a control. During auto-oxidation of-6-OHDA are formed quinones and ROS (Galindo et al. 2003), which are able to inhibit mitochondrial complex I and IV function and cause apoptosis in experimental models of PD in neuroblastoma cell lines, including SK-N-SH and SH-SY5Y (Shimizu et al. 2002; Von Coelln et al. 2001). To analyze the effects of 6-OHDA and other toxins on differentiated and undifferentiated neuroblastoma cells and grown in galactose media SH-SY5Y cells cell viability assay – MTT was used. Results of MTT assay after exposure of the undifferentiated and differentiated SK-N-SH and SH-SY5Y cells and SH-SY5Y grown in galactose to the tested compounds are presented in Fig. 2.

In the current study, 6-OHDA induced cell death in differentiated and undifferentiated SK-N-SH and SH-SY5Y cells in dose dependent manner. This confirms earlier observations, in which 6-OHDA was used as a neurotoxin towards human neuroblastoma cells (Shimizu et al. 2002; Gomez-Lazaro et al. 2008). As expected, undifferentiated SH-SY5Y cells were more resistant to 6-OHDA exposure in comparison to undifferentiated SK-N-SH cells, where cell viability resulted in an increase from 42% to 67%, respectively. However, the viability in SK-N-SH cells differentiated with RA and treated with 6-OHDA (50 μM) was about 30% higher than 6-OHDA-treated cells that were not subjected to the differentiating agent. Interestingly, comparative growth-response data for 6-OHDA showed that toxicity was less pronounced in media containing galactose. 6-OHDA (50 μM) induced approximately 50% cell death within 24 h in medium lacking galactose, whereas in medium containing galactose the same concentration induced only 20% cell death in SH-SY5Y cells.

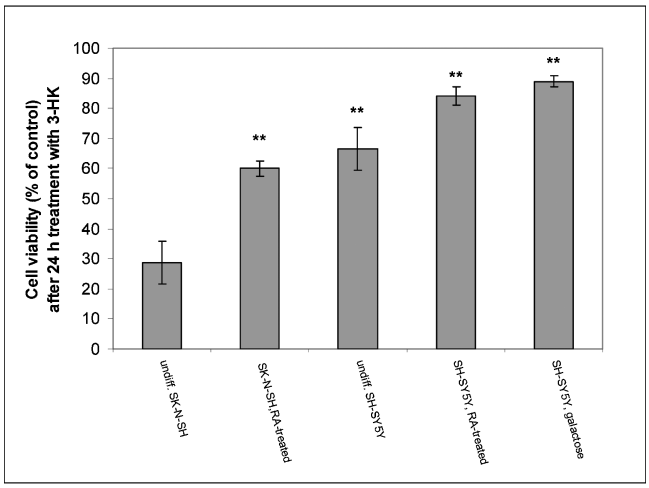
Another toxin used as a control was salsolinol, which has a similar structure to 6-OHDA and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and is also known to induce death of catecholaminergic cells. Salsolinol is reported to impair the cellular energy metabolism via inhibiting the mitochondrial complex II (succinate-Q reductase) activity, which leads to neurotoxicity (Storch et al. 2000). The present work investigates the effects of a 50:50 mixture of *R* and *S* enantiomers of salsolinol. When neuroblastoma cells were cultured with variable amounts of salsolinol (10–500 μM) for 24 h, the cell viability decreased in a concentration-dependent manner. Treatment with salsolinol 500 μM for 24 h reduced cell viability of untreated SK-N-SH cells in about 70% of population. In contrast, RA differentiated SK-N-SH cells after exposure to 500 μM salsolinol decreased the cell number to about 35%. Similar observations were recorded in undifferentiated and differentiated SH-SY5Y neuroblastoma cells, where 500 μM salsolinol caused $49.08 \pm 1.8\%$ and $22.5 \pm 4.5\%$ cell death, respectively. The viability of salsolinol treated SH-SY5Y cells in medium containing galactose was 76%.



(A)



(B)



(C)

Fig. 2: Effect of (A) 6-OHDA (50 μM), (B) salsolinol (500 μM) and (C) 3-HK (400 μM) on cell viability in control SK-N-SH cells, in SK-N-SH cells treated for 6 days with RA (10 μM) and in control SH-SY5Y cells, in SH-SY5Y cells treated for 6 days with RA (10 μM), in SH-SY%Y cells grown in galactose media. The cells were incubated with each neurotoxin for 24 h. Cell viability was assessed by the MTT method and presented as untreated controls. Data points represent the mean ± SEM of three independent experiments performed in triplicates. **: $p < 0.01$ vs undifferentiated SK-N-SH cells

Several intermediates in the kynurenine pathway, an alternative route for the tryptophan metabolism, have been associated with neurodegenerative disorders. Among them, 3-hydroxykynurenine is due to production of hydrogen peroxide (H_2O_2) and superoxide, considered to be an oxidative stress generator, which causes neuronal apoptosis with brain region selectivity and has been linked to many central nervous diseases (Okuda et al. 1998). Another kynurenine pathway compound is QUIN, an NMDA receptor agonist, which can lead to significant lipid peroxydation of the membrane and potentiating of its own toxicity and that of other excitotoxins, including NMDA and glutamate (Guillemin et al. 2005). In addition, KYNA in submicromolar concentrations blocks the NMDA receptors localizing on glutamergic nerve terminals and releases glutamate (Rozsa et al. 2008).

Effects of 3-HK on differentiated and undifferentiated neuroblastoma cells and grown in galactose media SH-SY5Y cells were tested at the range of 1–500 μM. Consistent with previous studies, our results show that 3-HK produces cell death in both undifferentiated human neuroblastoma cell lines (Jeong et al. 2003; Lee et al. 2005). 3-HK induced a significant reduction in cell viability in all cell systems, in a dose-dependent manner, measured after 24 h treatment. However, addition of 400 μM to undifferentiated SK-N-SH cells induced 30% cell viability, whereas in RA-treated SK-N-SH, differentiated and undifferentiated SH-SY5Y cells the same concentration induced, respectively 60% and 67%, 84% cell viability. Also here remarkable differences were noticed between undifferentiated SH-SY5Y cells grown in galactose or glucose media. KYNA and QUIN unlike 3-HK were not found to evoke reduction in cell viability at any tested concentration (0.25–500 μM) in any cell model.

3. Discussion

There is little information on the actions of kynurenine pathway metabolites in widely available immortalized cell cultures. Recent reports indicate that QUIN, 3-HK and KYNA (in submolar concentrations) may play an important role in the development of some neurological disorders and regulation of the CNS (Rozsa et al. 2008). SK-N-SH cells and its subclone SH-SY5Y cells are human neuroblastoma cell lines, which are widely used as a model for *in vitro* neurotoxicity testing. The SK-N-SH cell line contains mainly neuronal (N-type) and Schwannian (S-type) phenotypes, whereas the SH-SY5Y cell line is more homogeneous and consists in greater part of S type cells. In agreement with previous investigations, neuroblastoma cells under treatment with RA undergo differentiation towards neuronal cells and express neuronal phenotype (Fig. 1), but not with TNF-alpha treatment (Encinas 2000 et al.; Pizzi et al. 2002). In this study, we confirmed previously reported neurotoxical effects of 3-HK (Jeong et al. 2003; Lee et al. 2005), however QUIN and KYNA failed to induce cell death in differentiated and undifferentiated neuroblastoma cells compared with control cells. Although, according to Pizzi et al. (2002), SK-N-SH and SH-SY5Y under differentiation with RA express functional NR1 and NR2B subunits of NMDA receptors and despite the fact that QUIN and KYNA have previously been shown to be toxic to primary cells, in this experiment these two kynurenine pathway metabolic intermediates were not found to cause cell loss at none of the tested concentrations. A possible explanation for this action is that primary cells express high heterogeneity in culture and probably contain also microglial cells, which are mostly affected by QUIN toxicity. In addition, neuroblastoma cells represent population of human neuron-like cells, do not contain glial cells and therefore it is not possible to induce neuro-inflammation.

Moreover, 6-OHDA, salsolinol and 3-HK induced toxicity towards RA-differentiated neuroblastoma cells was less pronounced comparing untreated cells. These results indicate that RA-differentiation makes cells more resistant to cytotoxicity as was observed in this and other studies (Cheung et al. 2009;

Mak et al. 2010). Cell toxicity can be influenced by certain constituents of the media in which cells grow. Rodriguez-Enriquez et al. (2001), suggested for instance, that immortalized cells grown in media with glucose derive almost all of their energy from glycolysis and not through mitochondrial oxidative phosphorylation ("Crabtree effect"). The substitution of glucose by galactose proposed by Marroquin made human liver HEPG-2 cells more susceptible to several mitochondrial toxins. However, the observation made by Marroquin et al. (2007) conflicts with what has been detected in our cell cytotoxicity models of human neuroblastoma cells. Neuroblastoma cells under these conditions were found to be more resistant against 6-OHDA, salsolinol and 3-HK induced toxicity. Therefore, galactose-media grown neuroblastoma cells cannot serve as a model for testing neurotoxic agents causing mitochondrial dysfunction. Further investigations are required to understand the processes that occur during the treatment of neuroblastoma cells with galactose.

In summary, neither RA-treatment nor switching media from glucose to galactose were found to increase the cell susceptibility to well-established neurotoxins and several compounds of the kynurenine pathway. On the contrary, data from this assay indicate that RA-differentiated human neuroblastoma cell lines are not a well suited *in vitro* model for neurotoxical research. Moreover, undifferentiated, differentiated or grown in galactose SK-N-SH and SH-SY5Y lines are not appropriate for studying toxicity of QUIN and KYNA effects or neuroprotection against them, probably due to absence of microglial cells in these cell lines.

4. Experimental

4.1. Materials and cell lines

Human neuroblastoma cell line SK-N-SH and its subclone SH-SY5Y were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DMSZ), Braunschweig, Germany. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum superior (FBS), phosphate buffered saline (PBS), L-glutamine- L-alanine, trypsin-EDTA were purchased from Biochrom, Berlin, Germany. 3-hydroxykynurenine, quinolinic acid (QUIN), kynureinic acid, 6-hydroxydopamine, TNF-α, all-trans-retinoic acid (RA), D (+) galactose, (±) salsolinol hydrochloride, triton-X, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma, St. Louis, MO; paraformaldehyde, bovine serum albumin, ethanol, dimethyl sulfoxide (DMSO) were obtained from Roth (Karlsruhe, Germany). The primary antibody used for immunofluorescence staining was anti-microtubule-associated protein 2 (MAP2) mouse antibody (Acris Antibodies, Hiddenhausen, Germany) and the secondary antibody was FITC-conjugated goat anti-mouse antibody from (Abnova Corporation, Taipei, Taiwan).

4.2. Cell culture technique

Human neuroblastoma cell lines SK-N-SH and SH-SY5Y were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 15% fetal bovine serum (FBS) (both Biochrom, Berlin, Germany). All cells were maintained at 37°C in a humidified 5% CO_2/air atmosphere. The cells were transferred to low serum media (1% FBS) 2 h prior to the treatment. Cells were weekly passaged (Trypsin-EDTA, Biochrom, Germany). Cell concentration was measured using CASY cell counter (Schärfe System, Reutlingen, Germany). Tests were carried out in 96-well microplates, and the density of 8×10^4 (SK-N-SH) or 5×10^4 (SH-SY5Y) cells per well was considered in the screening experiment. Cells were incubated 24 h before the drug was added, and the drug remained in contact with the cells for 24 h.

Galactose media: DMEM (F405) deprived of glucose supplemented with 10 mM galactose, 4 mM L-glutamine-L-alanine and 15% FBS. SH-SY5Y cells were grown in galactose media until 10^{th} passage to accustom the cells with new conditions and to switch them from glycolysis to oxidative phosphorylation in order to obtain ATP.

4.3. Induction of differentiation

For differentiation, cells were plated at a density of 3×10^4 (SK-N-SH) or 2×10^4 (SH-SY5Y) cells per well in 96-well plates in 100 µl of medium containing 10% FBS. Twenty-four hours after plating, the cells were incubated in medium with 500 U/ml TNF, 10 µM RA in ethanol or ethanol alone (the final ethanol concentration not exceeding 0.1%). Cells containing only ethanol in medium were treated as a control. The medium was changed every second day, and cultures were allowed to differentiate for 6 days.

4.4. Immunofluorescence staining

For immunostaining, cells were fixed in 4% paraformaldehyde in PBS for 15 min at room temperature, rinsed twice with PBS, and then permeabilized with 0.5% Triton-X in PBS for 40 min. Blocking was done in 5% bovine serum albumin in PBS and incubated for 1 h with gentle rocking. Specimens were incubated with primary antibody: MAP-2 (1:200) overnight at 4 °C. Thereafter, samples were rinsed twice with PBS and incubated with the secondary antibody: FITC-conjugated anti-mouse (1:50) for 1 h at room temperature in the dark to visualize MAP-2. Next, cells were washed with PBS for 5 min and fixed. Stained cells were imaged on an Olympus BX41 epifluorescence microscope equipped with a Nikon – Digital Still DXM 1200 camera controlled by ACT-1 Nikon software (all from Nikon, Tokyo, Japan), using a 40 × Olympus objective with a 1.4 aperture. The fluorescence light source was a 75 W xenon arc lamp shuttered using a custom-made shutter unit so that cells were only illuminated during image acquisition. For detection of FITC, cells were viewed under a filter set with an excitation wavelength of 460–500 nm and an emission wavelength of 510–560 nm.

4.5. Cell viability assay – 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay

For the viability assay, cells were plated at a density of 8×10^4 (SK-N-SH) or 5×10^4 (SH-SY5Y) cells per well on 96-well plates in 100 µl of DMEM containing 10% FBS. One day after plating 3-HK, 6-OHDA, QUIN, KYNA or salsolinol were added at various concentrations to the cells for 24 h, whereas $A\beta_{25-35}$ and TNF-α were added to the cells for 48 h and 72 h, respectively. Cell viability was assessed using the modified MTT assay as first described by Mosmann (1983). Briefly, after the cells had been subjected to appropriate treatments and the medium changed, MTT was added. Metabolic active cells cleaved the yellow tetrazoliumsalt MTT to purple formazan crystals. Cells were incubated for 2 h at 37 °C with MTT solution (5 mg/ml). The supernatant was removed and the formazan formed was dissolved in DMSO. Finally, the absorbance was measured by a multiplate reader at 570 nm test wavelength and at 620 nm reference wavelength (Tecan, Austria). Results were expressed as percentage of control.

References

Behan WM, McDonald M, Darlinton LG, Stone TW (1999) Oxidative stress as a mechanism for quinolinic acid induced hippocampal damage: protection by melatonin and deprenyl. Br J Pharmacol 128: 1754–1760.

Biedler JL, Helson L, Spengler BA (1973) Morphology and growth, tumorigenicity, cytogenetics of humanneuroblastoma cells in continuous culture. Cancer Res 33: 2643–2652.

Cheung YT, Lau WKW, Yu MS, Lai CSW, Yeung SC, So KF, Chang RCC (2009) Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as *in vitro* model in neurotoxicity research. Neurotoxicology 30: 127–135.

Encinas M, Iglesias M, Liu Y, Wang H, Muhaisen A, Cena V, Gallego C, Comella JX (2000) Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor-dependent, human neuron-like cells. J Neurochem 75: 991–1003.

Floyd RA (1999) Neuroinflammatory processes are important in neurodegenerative doseases: an hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development. Free Radical Biol. Med 26: 1346–1355.

Galindo MF, Jordan J, Gonzales-Garcia C, Cena V (2003) Chromaffin cell death induced by 6-hydroxydopamine is independent of mitochondrial swelling and caspase activation. J Neurochem 84: 1066–1073.

Garthwaite G, Garthwaite J (1987) Receptor linked ionic channels mediate N-methyl aspartate neurotoxicity inrat cerebellar slices. Neurosci. Lett. 83: 241–246.

Gomez-Lazaro M, Bonekamp NA, Galindo MF, Jordan J, Schrader M (2008) 6-Hydroxydopamine (6-OHDA) induces Drp1-dependent mitochondrial fragmentation in SH-SY5Y cells Free Radic Biol Med 1960–1969.

Guillemin GJ, Kerr SJ, Brew BJ (2005) Involvement of quinolonic acid in human brain cultures. J Neurovirol 5–6: 375–380.

Jeong JH, Kim HJ, Lee TJ, Kim MK, Park ES, Choi BS (2004) Epigallocatechin 3-gallate attenuates neuronal damage induced by 3-hydroxykynurenine. Toxicology 195: 53–60.

Lee MW, Lee HJ, Bach JH, Park SC, Park JS, Lee KH, Yeo JH, Lee WB, Lee SH, Kim SS (2005) Down-regulated reactive oxygen species by HSP90 in 3-HK-induced SK-N-SH cell death. J Therm Biol 30: 43–49.

Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443: 787–795.

Mak YT, Lam WP, LÜ L, Wong YW, Yew DT (2010) The toxic effect of ketamine on SH-SY5Y neuroblastoma cell line and human neuron. Miscrosc Res Tech 73: 195–201.

Marroquin LD, Hynes J, Dykens JA, Jamieson JD, Will Y (2007) Circumventing the crabtree effect: replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. Toxicol Sci 97: 539–547.

Mattson MP, Barger SW, Furakawa K, Bruce AJ, Wyss-Coray T, Mark RJ, Mucke L (1997) Cellular signalling of TGFβ, TNF-α, βAPP in brain injury responses and Alzheimer's disease. Brain Res Rev 23: 47–61.

Maury CPJ (1995) Biology of disease; molecular pathogenesis of β-amyloidosis in Alzheimer's disease and other cerebral amyloidoses. Lab Invest 72: 4–16.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55–63.

Obregon E, Punzon MC, Gonzales- Nicolas J, Fernandez-Cruz E, Fresno M, MA Munoz-Fernandez (1997) Induction of adhesion/differentation of human neuroblastoma cells by tumor necrosis factor-α requires the expression of an inducible nitric oxide synthase. Eur J Eurosci 9: 1184–1193.

Okuda S, Nishiyama N, Saito H, Katsuki H (1996) Hydrogen peroxide-mediated neuronal cell death induced by an nedogenous neurotoxin, 3-hydroxykynurenine. Proc Natl Acad Sci USA 93: 12553–12558.

Okuda S, Nishiyama N, Saito H, Katsuki H (1998) 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. J Neurochem 70: 299–307.

Pahlman S, Ruusala AI, Abrahamsson L, Mattson MEK, Esscher T (1984) Retinoic acid -induced differentiation of cultured human neuroblastoma cells: a comparison with phorbol ester-induced differentiation. Cell Differ 14: 135–144.

Pizzi M, Boroni F, Bianchetti A, Moraitis C, Sarnico I, Benarese M, Gofii F, Valerio A, Spano PF (2002) Expression of functional NR1/NR2B-type NMDA receptors in neuronally differentiated SK-N-SH human cell line 16: 2342–2350.

Rodriguez-Enriquez S, Juarez O, Rodriguez-Zavala JS, Moreno-Sanchez R (2001) Multisite control of the Crabtree effect in ascites hepatoma cells Eur. J. Biochem 268: 2512–2519.

Rozsa E, Robotka H., Vecsei L, Toldi J (2008) The Janus-face kynurenic acid. J Neural Transm 115: 1087–1091.

Shimizu E, Hashimoto K, Komatsu N, Iyo M (2002) Roles of endogenous glutathione levels on 6-hydroxydopamine-induced apoprotic neuronal cell death in human neuroblastoma SK-N-SH cells. Neuropharmacology 43: 434–443.

Storch A, Kaftan A, Burkhardt K, Schwarz J (2000) 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline salsolinol is toxic to dopaminergic neuroblastoma SH-SY5Y cells via impairment of cellular energy metabolism. Brain Res 855: 67–75.

Ungerstedt U (1976) 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine pathway: the turning syndrome. Pharmacol Ther B 2: 37–40.

Wainwright LJ, La sorella A, Iavarone A (2001) Distinct mechanisms of cell cycle arrest control the decision between differentiation and senescence in human neuroblastoma cells Proc Natl Acad Sci USA 98: 9396–9400.

Wanpen S, Govitrapong P, Shavali S, Sangchot P, Ebadi M (2004) Salsolinol, a dopamine-derived tetrahydroisoquinoline, induces cell death by causing oxidative stress in dopaminergic SH-SY5Y cells, and the said effect is attenuated by metallothionein. Brain Res 1005: 67–76.

von Coelln R, Kugler S, Bahr M, Weller M, Dichgans J, Schulz JB (2001) Rescue from death but not from functional impairment: caspase inhibition protects dopaminergic cells against 6-hydroxydopamine-induced apoptosis but not against the loss of their terminals. J Neurochem 77: 263–273.