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Effect of Cigarette Smoke Exposure Against Oxidative Damage in Scrapie-infected Mice

Hyung-Ok Sohn[1)*], Ja-Young Moon[2)], Heung-Bin Lim[3)], and Dong-Wook Lee[4)]

1) KT&G Central Research Institute, Daejeon, Seoul 305-805, Korea
2) Department of Biochemistry and Health Sciences, College of Natural Sciences, Changwon National University, Changwon, Kyungnam 641-773, Korea
3) Department of Industrial Plant Science & Technology, College of Agriculture, Chungbuk National University, Chungbuk 361-763, Korea
4) Natural Resources Research Institute, Jangheung, Jeonnam, Korea
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ABSTRACT : Although prion diseases, a group of fatal neurodegenerative diseases of human and animals, are presumed to be caused by several mechanisms including abnormal change of prion protein, oxidative stress is still believed to play a central role in development of the diseases. Cigarette smoking has a few beneficial effects on neuronal diseases such as Alzheimer's disease and Parkinson's disease despite of many detrimental effects. In this study, we investigated how chronic cigarette smoking could exert such beneficial effect against oxidative damage. For this study, homogenates of 87V scrapie-infected brain was inoculated on intracerebral system of IM mice through stereotaxic microinjection and biochemical properties concerning with oxidative stress were examined. The scrapie infection decreased the activity of mitochondrial Mn-containing superoxide dismutase by 50% of the control, meanwhile the effects on other antioxidant enzymes including Cu or Zn-containing superoxide dismutase were not significant. Additionally, the infection elevated superoxide level as well as monoamine oxide-B (MAO-B) in the infected brain. Interestingly, many of the detrimental effects were improved in partial or significantly by long-term cigarette smoke exposure (CSE). CSE not only completely prevented the generation of mitochondrial superoxide but also significantly ($p<0.05$) decreased the elevated mitochondrial MAO-B activity in the infected brain. Concomitantly, CSE prevented subsequent protein oxidation and lipid peroxidation caused by scrapie infection; however, it did not affect the activities of antioxidant enzymes. These results suggest that chronic exposure of cigarette smoke contribute to in part preventing the progress of neurodegeneration caused by scrapie infection.

Key words : Prion disease, neurodegeneration, oxidative stress, cigarette smoke exposure

*연락저자 : 305-805 대전광역시 유성구 신성동 302 번지, KT&G 중앙연구원
*Corresponding author : *KT&G Central Research Institute, 302 Shinseong-dong, Yuseong-gu, Daejeon 305-805, Korea (phone: 82-42-866-5599; fax: 82-42-866-5426; e-mail: hosohn@ktng.com)*

Cigarette smoking is known to have an inverse relationship to incidences of Alzheimer's disease (Lee, 1994) and Parkinson's disease (Grandinetti *et al*., 1994). This relationship has led many investigators to propose that some aspect of cigarette smoking may exert a neuroprotective influence with respect to development of these diseases. As prion diseases and AD share many similar properties (Wisniewski, 1984; Price, 1993; Prusiner, 1995), using them as models of dementia has drawn considerable attention (Carp *et al*., 1984). Because scrapie, an archetype of TSE and one type of prion diseases, naturally occurs in sheep and goats, it has been experimentally used in a wide range of animal study. Among scrapie agents 87V strain has a relatively long incubation period in IM mice and displays physiological characteristics analogous to dementia (Kim *et al*., 1990). We already demonstrated that the exposure of cigarette smoke attenuates the neurobiological and histopathological characteristics such as reduction of spatial movement, loss of neurotransmitters, induction of astrocytosis and vacuolation caused by scrapie infection in the brain of IM mice (Sohn *et al*., 2005).

Although the mechanism of prion diseases remains elusive, accumulating evidence has suggested that oxidative impairment is a pivotal event in the disease (Lee *et al*., 1999; Guentchev *et al*., 2000; Milhavet and Lehmann., 2002). In scrapie-infected animals, several studies have reported that oxidative stress may be involved in neurodegeneration. They showed the increase of oxidative stress, alteration of antioxidant defense system, and mitochondrial dysfunction in the brain of scrapie-infected mice (Lee *et al*., 1999 Guentchev *et al*., 2000 Kim *et al*., 2001). Milhavet *et al*. (2000) recently reported that total SOD activity was significantly reduced in scrapie infection, and it is likely that this diminution could in part be attributed to the decreased SOD-like activity of PrP. Wong *et al*. (2001) observed the alterations of brain metals, especially copper of metals levels in scrapie-infected brain. These changes may play a pivotal role in the pathogenesis of prion diseases, as they lead to loss of antioxidant function associated with PrP, which may promote the diversity of prion diseases.

In this study, we investigated how chronic cigarette smoking could exert such beneficial effect against oxidative damage in scrapie infected animals model. For this study, homogenates of 87V scrapie-infected brain was inoculated on intracerebral system of IM mice through stereotaxic microinjection and biochemical properties concerning with oxidative stress were examined.

MATERIALS AND METHODS

Animals and inoculation of 87V scrapie strain

Male IM mice were used and their original breeding stock was provided by Dr. Alan Dickinson (AFRC & MRC Institute, Edinburgh, U.K). The mice were divided into four groups: control (C), scrapie-injected (P), control with exposure to cigarette smoke (CS), and scrapie-injected with exposure to cigarette smoke (PS). Twenty and thirty mice were used for each control (C and CS) and each scrapie-infected group (P and PS), respectively. Five mice were housed in each cage and supplied with water and food *ad libitum* in a clean conventional animal facility (22 ± 2 ℃, 50-60 % relative humidity, and 12 h light/dark cycle).

For the scrapie-infected groups (P and PS), 6-week-old mice were injected with the 87V strain as a scrapie agent. For control groups (C and CS), the homogenates of normal mouse

brain were prepared as described previously (Carp *et al*., 1984). Mice were inoculated intracerebrally in the left hemisphere with 5 uL of 1 % brain homogenate in phosphate-buffered saline pH 7.4 under general anesthesia.

Cigarette smoke exposure

The mice of CS and PS groups were exposed to diluted mainstream of cigarette smoke (1:5) generated from 15 cigarettes (tar and nicotine contents in a cigarette was 11mg and 1.1 mg, respectively) for 10 minutes a day in a round polycarbonate chamber (D37 x H 13 cm). Cigarettes were smoked following the ISO standard. The mice were acclimated for 4 weeks prior to the start of cigarette smoke exposure. The exposure was continued for 5 consecutive days in a week until all experiments were completed.

Tissue preparation and subcellular fractionation for antioxidant enzymes assay

After the scrapie incubation of 273 days, at least five to seven mice were sacrificed for biochemical study. Animals were anesthetized with 16.5 % urethane and then whole brain was quickly removed and chopped on ice. The tissue samples homogenized in 4 volumes of 10 mM HEPES buffer (pH 7.4) containing 150 mM KCl using a homogenizer with a Teflon pestle. Mitochondria and cytosolic fractions were isolated from the homogenates by differential centrifugation (Laganiere and Yu, 1988). The homogenate was centrifuged at 1,000 x *g* for 10 min to remove nuclei and debris. The supernatant was centrifuged at 10,000 x *g* for 20 min. The resultant supernatant was subsequently centrifuged at 100,000 x *g* for 1 hr to yield the cytosolic fraction. The 10,000 x *g* pellet corresponding to the mitochondrial fraction, was resuspended with theHEPES buffer. Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Aliquotes of cytosol and mitochondrial fractions were stored at -70℃ until use. Whole brain homogenates were used for the analysis of protein carbonyl content.

Cytosolic fractions were used for the enzymatic assay of catalase, Cu, Zn-superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione-*S*-transferase, as well as the analysis of thiol compounds. Mitochondria fractions were sonicated at 38 W for 30 s on ice and suspension for the enzymatic assay of Mn-superoxide dismutase and monoamine oxidase.

Superoxide dismutase assay

The activity of superoxide dismutases (superoxide: superoxide oxidoreductase, E.C. 1.15.1.1, SOD) in cytosolic and mitochondrial fractions was measured by monitoring the inhibition of the cytochrome c reduction at 550 nm by the reaction of xanthine with xanthine oxidase to generate superoxide radicals (McCord *et al*., 1972). The unit (U) of SOD activity was defined as the amount of enzyme that inhibits the rate of ferricytochrome c reduction by 50 %. Results were expressed units/mg protein/min.

Catalase assay

Catalase (H_2O_2: H_2O_2 oxidoreductase, E.C. 1.11.1.6, CAT) activity was assayed based on the direct measurement of decomposition of hydrogen peroxide at 240 nm spectrophotometically (Abei, 1983); 1 U is the amount of enzyme that decomposes 1 μM H_2O_2/min.

Glutathione peroxidase assay

Glutathione peroxidase (glutathione: H_2O_2 oxidoreductase, E.C. 1.11.1.9, GSH-Px) activity

was measured with the coupled-enzyme system using cumene hydroperoxide as a substrate (Flohe and Gunzler, 1984). The optical density changes at 340 nm were spectrophotometrically monitored at 25°C; 1 mU is defined as the amount of the enzyme which oxidizes 1 nM GSH/min. Determination of GSH-Px activity was achieved by the equation: (A340 min/6,220 mol/L^{-1} cm^{-1}) x assay dilution factor = mU/mL, , where A340 = change in absorbance per minutes at 340 nm, 6200 mol/L^{-1} • cm^{-1}= the molar extinction coefficient of NADPH. Results for GSH-Px assay was then adjusted to give data as mU/mg protein. One GSH-Px unit consumes 1 μmol NADPH per minute at 25°C and pH 7.6.

Glutathione reductase assay

Glutathione reductase (NADPH: oxidized-glutathione oxidoreductase, E.C.1.6.4.2, GSH-Red) activity was assayed by measuring NADPH oxidation at 340 nm (Racker, 1955). Determination of GR activity was based on the equation: (A340 min/6200 mol/L^{-1} • cm^{-1}) x dilution factor = mU/ml, where A340 = change in absorbance per minutes at 340 nm, 6,200 mol/L^{-1} • cm^{-1} = the molar extinction coefficient of NADPH. One GSH-Red unit reduces 1 μmol of oxidized glutathione (GSSG) per min at 25 °C and pH 7.6.

Glutathione-S-transferase assay

Glutathione-S-transferase (GST) activity towards 1-chloro-2,4-dinitro- benzene (CDNB) was measured in the cytosolic fraction as described by Habig *et al.* (1974).

Thiol compounds determination

Contents of sulfhydryl compounds in brain cytosol were measured at 412 nm according to the procedure of Sedlak and Lindsay (1968) using 5,5'-dithiobis-2(2-nitrobenzoic acid) (DTNB) as a substrate. Extinction coefficient was $91cm^{-1}mM^{-1}$.

Superoxide generation assay

The rate of superoxide generation in mitochondria was assayed based on the inhibitory effect of superoxide dismutase on the superoxide-induced oxidation of epinephrine to adrenochrome by the method of Nohl and Hegner (1978). Results were expressed as nmoles superoxide/mg protein/min.

Protein oxidation assay (Protein carbonyls quantification)

Protein carbonyl content was measured by the method of Levine *et al.* (1990). Protein was precipitated with 20 % trichloroacetic acid (TCA). After centrifuging at 11,000 × *g* and 4 °C for 15min, the supernatant was removed. The pellet was resuspended in 0.5L of 102,4-dinitrophenylhydrazine (DNPH)/2HCl (50℃). Samples were holded in a dark place for 1h by vortexing in each 10 min. The samples were precipitated with 0.5of 20 % TCA, and centrifuged at 11,000 × *g* and 4℃ for 3The same procedure was repeated with 10% TCA for three times. Precipitate was dissolved in 2of NaOH at 37 °C. Absorbance was recorded at 360Protein carbonyl levels were expressed as nmol carbonyl/mg protein using ε_{max} $=22{,}000M^{-1}cm^{-1}$.

Lipid peroxidation assay

The content of free malondialdehyde (MDA) in brain homogenates was determined at 270 nm by HPLC on an aminophase (Lichrospher, 250 x 4 mm) column with acetonitrile/0.03 M Tris buffer, pH 7.4 (1:9, v/v) (Esterbauer, *et al.*, 1984). Results were expressed as nmoles MDA/mg protein

Monoamine oxidase assay

Total activity of monoamine oxidase (MAO) in brain mitochondria was measured by fluorimetric

assay using kynuramine as a substrate (Morinan and Garratt, 1985). Briefly, aliquots (0.1 mL) of mitochondrial suspension were preincubated at 37 °C for 5 min with 0.87 ml of 10 mM phosphate buffer (pH 7.2). The reaction was started by the addition of 0.3 ml of 0.4 M perchloric acid. One milliliter of aliquots of the supernatant obtained after centrifugation of the reaction mixture at 11,600 x g for 15 s was transferred to test tubes containing 2 mL of 1.0 M sodium hydroxide. After mixing, the 4-hydroxyquinoline fluorescence was measured at an excitation wavelength of 315 nm and an emission wavelength of 380 nm using a Perkin- Elmer fluorescence spectrophotometer. MAO-B activity was assayed by the procedure of Rajesh *et al.* (1987). The reaction mixture contained 0.8 mL of 0.1 M phosphate buffer (pH 7.4), 0.05 ml of 30 mM sodium azide, and 0.1 mL of sample (100-250 μg protein). The reaction was started by addition of 0.05 mL of 10 mM benzylamine, incubated for 30 min at 37 °C, and stopped by addition of 0.5 mL of the hydrogen peroxide measuring solution (which contained 0.5 M phosphate/citrate buffer (pH 4.0), 1.8 mM 2,2'-azino-bis(3-ethylbenzenthiazoline-6-sulfonic acid) and 5 units of horseradish peroxidase). After 5 sec, 0.25 mL of 0.75 M hydrochloric acid containing 5 % SDS was added, and the coloured product was measured spectrophotometically at 414 nm. Results were expressed as nmoles H_2O_2/mg protein/min for MAO-B and arbitrary units of fluorescence intensity for MAO-T.

Statistical Analysis

Statistical analysis was done by one-way ANOVA with a post hoc Duncan test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Prion disease was successfully induced in 87V scrapie strain-infected IM mice. Decrease of physical activities and a slight reduction of body weights were observed in the infected mice. Histopathological findings showed pathological changes including the presence of amyloid plaques and PrP^{Sc}, vacuolation, and astrocytosis in the brain of scrapie-infected mice (Sohn *et al.*, 2005).

Because of the potential importance of free radicals in the induction of neuronal damage, we compared levels of antioxidant enzymes and thiol content in brain cytosolic and mitochondrial fractions. As shown in Table 1, the activity of glutathione peroxidase and the content of cytosolic sulfhydryl compounds responsible for scavenging reactive oxygen species were not influenced significantly by either CSE or scrapie infection. Glutathione reductase activity was slightly decreased in the cigarette smoke-exposed group, but the difference was not significant. However, activities of catalase ($p<0.05$) and glutathione-*S*- transferase ($p<0.01$) increased significantly in the mice infected with scrapie agent. Interestingly, mitochondrial Mn-containing superoxide dismutase (Mn-SOD) activity in the brains of mice infected with 87V scrapie strain was decreased by 50 % ($p<0.05$), although Cu,Zn-containing superoxide dismutase (Cu,Zn-SOD) showed no change. This result suggests that Mn-SOD rather than Cu, or Zn-SOD is more susceptible to infection with this scrapie strain. The decline in the activity of Mn-SOD caused by scrapie infection was partially attenuated by long-term exposure to cigarette smoke.

The scrapie-injected mice yielded two fold higher rate of superoxide generation when compared to controls. Although cigarette smoke contains various free radicals, superoxide generation was not changed in the mitochondria

of cigarette smoke exposed mice brain (CS). **Significantly different from control ($p<0.01$).

Table 1. Effect of exposure to cigarette smoke on antioxidant enzymes and thiol compounds in the brains of control (C), scrapie-injected mice (P), control group exposed to cigarette smoke (CS), and scrapie-injected group exposed to cigarette smoke (PS)

Antioxidants	C	P	CS	PS
Cu,Zn-SOD	3.9 ± 0.4	4.0 ± 0.4	3.8 ± 0.2	4.0 ± 0.2
Mn-SOD	2.0 ± 0.1	1.0 ± 0.1*	2.1 ± 0.3	1.3 ± 0.6*
Catalase	0.15±0.03	0.20 ± 0.01*	0.14 ± 0.04	0.16 ± 0.06
Glutathione peroxidase	30.4 ± 2.3	31.0 ± 4.2	30.3 ± 3.0	29.9 ± 3.9
Glutathione reductase	27.7 ± 3.7	25.5 ± 4.9	27.9 ± 3.8	28.8 ± 7.5
Glutathione-S-transferase	124 ± 10	158 ± 18*	138 ± 13	153 ± 19*
Total - SH	1.8 ± 0.9	2.0 ± 0.2	2.1 ± 0.2	2.0 ± 0.3

Values are the mean ± S. D. of 7 mice. Activities of catalase and glutathione-*S*-transferase are depicted as mmoles/min/mg protein, glutathione peroxidase and glutathione reductase as mU/mg protein (nmoles/mg protein/min) and total-SH as nmoles/g tissue. SOD activity are defined as units/mg protein/min.
*Significantly different from control ($p<0.05$).

Furthermore, the elevated level of superoxide generation in the mitochondria of scrapie-injected mice was significantly attenuated by cigarette smoke exposure (Fig. 1).

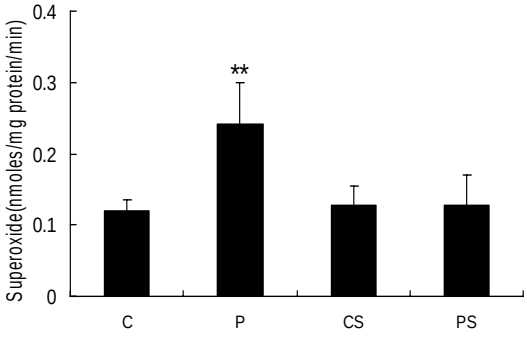

Fig. 1. Generation of superoxide superoxide in mitochondria of the brain from control (C), scrapie-injected mice (P), control group exposed to cigarette smoke(CS), and scrapie-injected group exposed to cigarette smoke (PS). Data are expressed as mean S. D. of 5-7 mice.

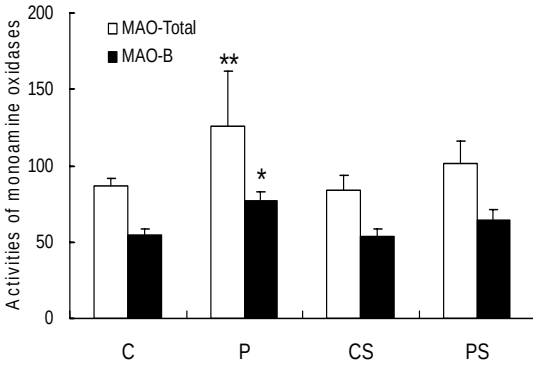

Fig. 2. The change in activity of monoamine oxidases in brain mitochondria from control (C), scrapie-injected mice (P), control group exposed to cigarette smoke (CS), and scrapie-injected group exposed to cigarette smoke (PS). MAO activity was measured in mitochondrial fractions obtained from the homogenates of entire brain. The data in nmoles hydrogen peroxide produced per mg protein per min for MAO-B and arbitrary units of fluorescence intensity for

MAO-Total, are mean S. D. of 5-7 mice. *Significantly different from control ($p<0.05$).

Monoamine oxidase (MAOs) is another source of hydrogen peroxide production in brain and MAO-B is a responsible enzyme for dopamine oxidation (Gotz *et al.*, 1994; Saura *et al.*, 1994). The activities of these enzymes were significantly increased following scrapie infection (MAO-B; $p<0.01$, MAO-T; $p<0.05$). However, CSE significantly attenuated the increase of MAO-B activity caused by scrapie infection ($p<0.05$, Fig. 2).

Protein oxidation, one of brain biomarker of oxidative stress, is known to be increased in several neurodegenerative diseases such as AD, PD, and CJD (Butterfield and Kanski, 2001). We assayed protein carbonyls, protein oxidation marker in entire brain homogenates of the mice. The content of protein carbonyls compounds was significantly increased by 27% in brain homogenate of scrapie-injected mice compared with controls. CSE significantly attenuated protein oxidation caused by scrapie infection (Fig. 3).

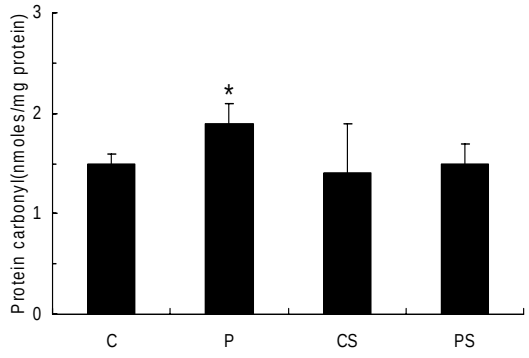

Fig. 3. Contents of protein carbonyl levels in brain homogenates of control (C), scrapie-injected mice (P), control group exposed to cigarette smoke (CS), and scrapie-injected group exposed to cigarette smoke (PS). Data are expressed as mean ± S. D. of 5-7 mice per group. *Significantly different from control ($p<0.05$).

To ascertain the presence of oxidative stress in the brains of scrapie- injected mice, we measured the level of MDA, an established marker for cellular oxidative damage (lipid peroxidation). The levels of MDA were significantly elevated in the brain of scrapie-injected mice but not in scrapie-injected mice, that were exposed to cigarette smoke (Fig. 4). These results indicate that CSE attenuated oxidative stress caused by scrapie infection.

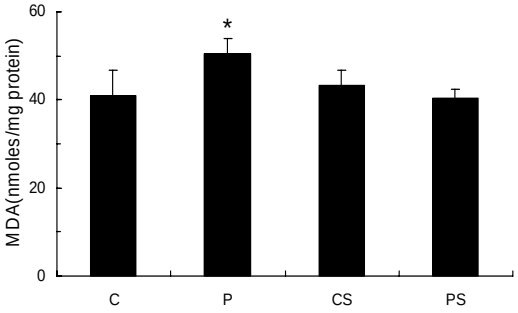

Fig. 4. Level of MDA in brain homogenates of control (C), scrapie-injected mice (P), control group exposed to cigarette smoke(CS), and scrapie-injected group exposed to cigarette smoke (PS). Data were expressed as mean S. D. of 5-7 mice. *Significantly different from control ($p<0.05$).

There is increasing evidence for the involvement of free radicals and oxidative stress in the pathogenesis of prion diseases (Milhavet and Lehmann, 2002). Cigarette smoke is composed of several thousand compounds and one or more of the components may have antioxidant properties. Several *in vitro* and *in vivo* studies have shown that the beneficial effects of nicotine and some substances in both PD and AD may be, partly, due to antioxidant mechanisms (Linert *et al.*, 1999; Cormier *et al.*, 2003). Similar effects were manifested in scrapie

infected mice exposed to cigarette smoke. In the present study, long-term exposure of cigarette smoke significantly attenuated ROS generation and oxidative stress caused by scrapie infection; the levels of MDA and protein carbonyl compounds were declined to normal conditions. Although further investigations are necessary to identify what kind of substances may be responsible, present results suggest the possibility that a substance(s) in cigarette smoke which are trapped into the physiological fluid at lung may circulate into the central nervous system and produce the biologically active species that protect neuronal functions against oxidative stress, in addition to plenty of oxidative substances.

These findings suggest that chronic exposure of cigarette smoke may partly contribute to suppression in the progress of neurodegeneration caused by scrapie infection. Further studies are necessary to establish the beneficial actionof cigarette smoke exposure against neurological diseases in mechanistic aspects.

CONCLUSINS

Scrapie infection caused to increase of catalase and MAO-B activities but to decrease of sulfhydryl compounds in the brain of mice. Elevations of protein oxidation and lipid peroxidation could be direct evidence of free radical damage in the scrapie infected mice brain. Scrapie infection dramatically increased superoxide in mitochondria of the mice brain, where it abnormally decreased Mn-SOD activity in mitochondria of scrapie infected mice. Taken together, it seems that elevated oxygen free radical generation and lowered scavenging activity in mitochondria might cause free radical damage to the brain. Such deleterious changes in mitochondria may contribute to the neurodegeneration in prion disease. We have demonstrated that long-term exposure of cigarette smoke protects neurons against oxidative damage caused by scrapie infection. Cigarette smoke exposure not only prevented the generation of mitochondrial superoxide but also significantly ($p<0.05$) decreased the elevated mitochondrial MAO-B activity in the infected brain. Concomitantly, CSE prevented subsequent protein oxidation and lipid peroxidation caused by scrapie infection; however, it did not affect the activities of antioxidant enzymes. These results suggest that chronic exposure of cigarette smoke contribute to in part preventing the progress of neurodegeneration caused by scrapie infection.

REFERENCES

Abe, M., Reiter, R. J., Orhii, P. H., Hara, M., Poeggeler, B., and Barrow-Walden, L. R. (1994) Inhibitory effect of melatonin on cataract formation in newborn rats: evidence of an antioxidative role for melatonin. J. Pineal Res. 17, 94-100.

Carp, R. I., Callahan, S. M., Sersen, E. A. and Moretz, R. C. (1984) Preclinical changes in weight of scrapie infected mice as a function of scrapie agent-mouse strain combination. *Intervirol*. 21: 61-69.

Cormier, A., Morin, C., Zini, R., Tillement, J. P., and Lagrue, G. (2003) Nicotine protects rat brain mitochondria against experimental injuries. Neuropharmacology 44(5), 642-652.

Esterbauer, H., Lang, J., Zadravec, S., and Slater, T. F. (1984) Detection of malonaldehyde by high-performance liquid chromatography. Methods Enzymol. 105, 319-28.

Grandinetti A., Morens D. M., Reed D. and MacEachern D. (1994) Prospective study of cigarette smoking and the risk of developing

idiopathic Parkinson's disease. *Am. J. Epidemiol.* 139(12): 1129-1138.

Guentchev, M., Voigtlander, T., Haberler, C., Groschup, M. H., and Budka, H. (2000) Evidence for oxidative stress in experimental prion disease. Neurobiol. Dis. 7, 270-273.

Habig, W. H., Pabast, M. J., and Jakoby, W. B. (1974) Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Kim, Y. S., Carp, R. I., Callahan, S. M. and Wisniewski, H. M. (1990) Incubation period and histopathological changes in mice infected stereotaxically in different brain areas with the 87V scrapie strain. *Acta Neuropathol.* 80: 388-392.

Kim, J. I., Choi, S. I., Kim, N. H, Jin, J. K., Choi, E. K, Carp, R. I., and Kim, Y. S. (2001) Oxidative stress and neurodegeneration in prion diseases. Ann N Y Acad Sci. 928, 182-186.

Laganiere, S. and Yu, B. P. (1988) Anti-lipoperoxidation action of food restriction. Biochem. Biophys. Res. Commun. 145, 1185-1191.

Lee, P. N. (1994) Smoking and Alzheimer's disease: A review of the epidemiological evidence. *Neuroepidemiol.* 13: 131-144.

Lee, D. W., Sohn, H. O., Lim, H. B., Lee, Y. G., Kim, Y. S., Carp, R. I., and Wisniewski, H. M. (1999) Alteration of free radical metabolism in the brain of mice infected with scrapie agent. Free Radic Res. 30(6), 499-507.

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent I., Lenz, A. G.. et al. (1990) Determination of carbonyl content in oxidatively modified proteins. Meth. Enzymol. 186, 464-478.

Linert, W., Bridge, M. H., Huber, M., Bjugstad, K. B., Grossman,S., and Arendash, G. W. (1997) In vitro and in vivo studies investigating possible antioxidant actions of nicotine: relevance to Parkinson's and Alzheimer's diseases. Biochim. Biophys. Acta.1454(2), 143-152.

Lowry, O. H., Rosebrough, H. J., Farr, A. B., and Randall, R. J. (1951) Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193, 265-275.

McCord, J. R., Colby, M. D., and Fridovich, I. (1972) Superoxide dismutase, enzymatic function for erythrocuprein (hemocuprein). J. Biol. Chem. 231, 6049-6055.

Milhavet, O. and Lehmann, S. (2002) Oxidative stress and the prion protein in transmissible spongiform encephalopathies. Brain Res. Rev. 38(3), 328-339.

Milhavet, O., McMahon, H. E., Rachidi, W., Nishida, N., Katamine, S., Mange, A., Arlotto, M., Casanova, D., Riondel, J., Favier, A., and Lehmann, S. (2000) Prion infection impairs the cellular response to oxidative stress. Proc. Natl Acad. Sci. USA. 97, 13937-13942.

Morinan, A. and Garratt, H. M. (1985) An improved fluorometric assay for brain monoamine oxidase. J. Pharmacol. Method. 13, 213-223.

Nohl, H. and Hegner, D. (1978) Do mitochondria produce oxygen radicals in vivo? Eur. J. Biochem. 82, 563-567.

Prusiner, S. B. (1995) The prion disease. *Scientific American* 272:30-37.

Rajesh, N., Kalaria, R. N., Michell, M. J., and Harik, S. (1987) Correlation of MPTP neurotoxicity with blood-brain barrier monoamine oxidase activity. Proc. Natl. Acad. Sci. USA. 84, 13521-3525.

Saura, J., Richards, J. G., and Mahy, N. (1994) Age-related changes on MAO in C57BL mouse tissues: a quantitative radioautographic study. J. Neural. Transm. (Suppl) 41, 89-94.

Sohn, H.O., Hyun, H.C., Shin, H. J., Han, J. H.,

Park, C. H., Moon, J. Y., Lim, H. B. and Lee, D. W. (2005) Cigarette smoke attenuates histopathological and neurobiological changes caused by 87V scrapie agent infection in IM mice. *J. Korean Soc. Tob. Sci.* 27(2): 212-218.

Wisniewski, H. M., Merz, G.. S. and Carp, R. I. (1984) Senile dementia of the Alzheimer's type : possibility of an infectious ethiologyin genetically susceptible individuals. *Acta Neurol. Scand.* Suppl. 69:91-99.

Wong, B. S., Brown, D. R., Pan, T., Whiteman, M., Liu, T., Bu, X., Li, R., Gambetti, P., Olesik, J., Rubenstein, R., Sy, M. S. (2001) Oxidative impairment in scrapie-infected mice is associated with brain metals perturbations and altered antioxidant activities. *J. Neurochem. 79(3),* 689-698.