Journal of the Korean Society of
Tobacco Science Vol. 27, No. 2, 212~218 (2005)
Printed in Republic of Korea

Cigarette Smoke Attenuates Histopathological and Neurobiological Changes Caused by 87V Scrapie Agent Infection in IM Mice

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(Received November 23, 2005)

ABSTRACT : Cigarette smoking has been known to have a few beneficial effects on some neuronal diseases such as Alzheimer's disease(AD), Parkinson's disease(PD) and prion disease by scrapie agent shows many similar properties with AD. In this respect, we investigated what biological effects are exerted by cigarette smoke exposure(CSE) in the brain of mouse infected by 87V scrapie. The scrapie agent was inoculated through stereotaxic microinjection of the homogenates of the scrapie agent infected brain into the intracerebral system in the IM mice. The inoculation into mice typically exhibits neurochemical, physiological and histopathological characteristics of prion disease: loss of neurotransmitters and induction of astrocytosis and vacuolation in brain as well as reduction of spatial movement and loss of body weight. CSE led to alleviated the loss of body weight and also improved spatial movement of the infected mice. Most interestingly, CSE attenuated astrocytosis and vacuolation caused by scrapie infection in the brain. In addition, decreased levels of dopamine in striatal and hypothalamic regions as well as serotonin level in hippocampus caused by scrapie infection were also attenuated by exposure to cigarette smoke. These findings suggest that cigarette smoke, by its inhibition of astrocytosis and vacuolation followed by its restoration of levels of some neurotransmitters, may partly contribute to suppression in the progress of neurodegeneration caused by scrapie infection.

***Key words* : cigarette smoke exposure, scrapie agent, neurodegeneration**

Cigarette smoking is known to have an inverse relationship to incidences of AD (Lee, 1994) or PD (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.

Infection by the scrapie agent is accom- panied by conversion of a 33 to 37 kDa cellular protein referred to as PrP^c (cellular isoform of the prion

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protein) to an altered form known as PrP^{sc} that is more resistant to proteolysis and forms fibrils characteristic of all transmissible spongeform encephalopathies (TSE). Aggregated PrP^{sc} is a major component of the amyloid deposits in diseases induced by unconventional agents (Bendheim *et al.*, 1984). TSE known as prion diseases occur in both animals and humans and are characterized by several typical histopathological properties: accumulation of abnormal prion protein (PrP^{sc}), vacuolation, astrocytosis, and neuron loss and in some case, amyloid deposition in the brain. All of these changes are also accompanied by dementia (Wisniewski *et al.*, 1984; Diedrich *et al.*, 1991). 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; Vorbrodt *et al.*, 1990). 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; Diedrich *et al.*, 1991; Price *et al.*, 1993). Considering that most neurodegenerative diseases including AD commonly occur at approximately three quarters of total life span, the features of 87V scrapie strain have an advantage in animal model for dementia study.

We demonstrate that exposure of cigarette smoke attenuates or improves 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.

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 according to 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.

Measurement of spatial movement

Behavioral signs of the mice were observed daily and their body weights were measured weekly. Spatial movement of the mice in open field was observed at 10 AM for 5 minutes five times a day by using a video track for 6 days

from day 265 to day 270 after scrapie infection. All animals were sacrificed at day 273 after scrapie infection.

Biochemical assay

For the evaluation of astrocytosis, glial fibrillary acidic protein (GFAP) was detected by immunohistochemical analysis (Diedrich *et al.*, 1991). The contents of dopamine and serotonin in hippocampus, cerebellum, striatum, hypothalamus, and the cortex in brains were determined using HPLC equipped with an electrochemical detector (Saller and Salama, 1984). Activities of acetylcholine esterase and choline acetyltransferase in brain homogenates were assayed by the methods prescribed by Ellman *et al.* (1961) and Fonnum (1975), respectively.

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

To ascertain the attenuation of pathophysiological changes in scrapie-induced prion disease by cigarette smoke exposure, body weight loss in the experimental groups was first examined. During the experimental period, mice in the C and CS groups did not show any discernible signs, body weight loss or death rate from those in normal mice. Compared with the control group, body weight of the mice in the scrapie injected groups more severely decreased from day 260 after scrapie infection (Fig. 1). However, body weight loss of mice in the PS group was somewhat attenuated compared to that in the P group, implying that cigarette smoke exposure alleviates the loss of body weight caused by scrapie agent.

Since alteration of spatial movement could be an index of brain defects, behavior of mice in open field was observed using a video track. As

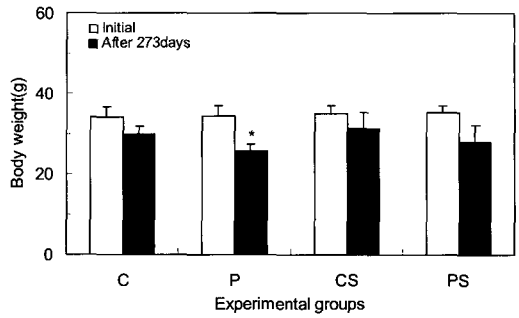

Fig. 1. Effect of exposure to cigarette smoke on body weight of control and scrapie infected mice. The values of the body weight were expressed as the mean ± SD of 7-10 mice.
* Significantly different from their initial body weight ($P<0.05$). C: control group, P: scrapie-injected group, CS: control group exposed to cigarette smoke, PS: scrapie-injected group exposed to cigarette smoke.

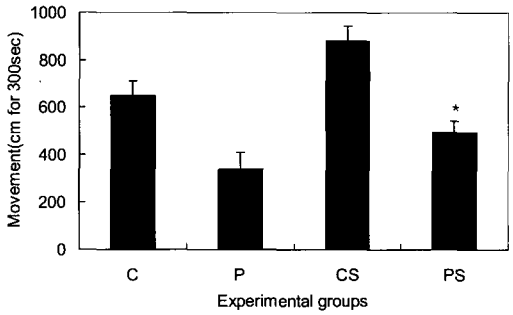

Fig. 2. Effect of exposure to cigarette smoke on the spatial movement of control and scrapie infected mice. The movement of mice was observed at 10 AM for 5 min for 6 days from the 265th day after scrapie infection using a video track. This procedure was repeated from day 265 to day 270 after injection. *Significantly different from P ($P<0.05$). Abbreviations are the same as those in Fig. 1.

expected, spatial movement in P group at day 269 after scrapie injection was significantly diminished ($p<0.05$) compared with that in the control group (Fig. 2). The mice in PS group showed more spatial movement than those of P

one, which suggests that cigarette smoke exposure restored the spatial movement decreased by scrapie infection, although this stimulating effect was marginally observed in CS group.

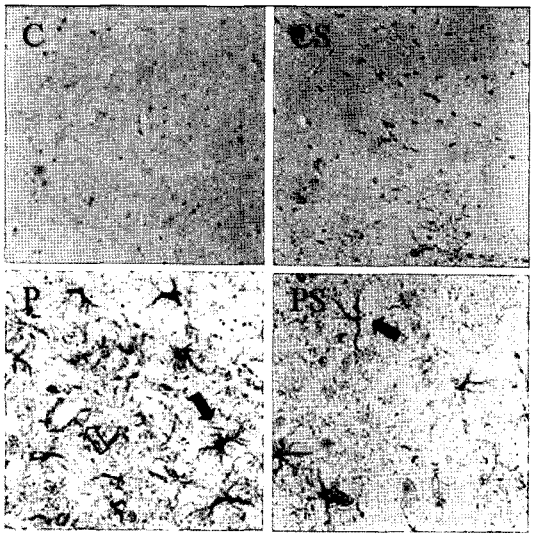

Fig. 3. Immunohistochemical localization of GFAP and vacuoles in the cerebral cortex from control and scrapie-infected mice. Solid and open arrows indicate GFAP and vacuoles, respectively. Abbreviations are the same as those in Fig. 1.

To evaluate the relationship between the improvement of spatial movement and the changes of histopathological characteristics typical to prion disease induced by cigarette smoke exposure, vacuolation and astrocytosis in the brain tissue sectioned from the 4 experimental groups were examined. As shown in Fig. 3, marked increases of vacuolation and astrocyte-specific marker GFAP were shown in the cerebral cortex of the scrapie-infected mice, whereas in PS group these phenomena were dramatically decreased. These results suggest that cigarette smoke exposure attenuates the vacuolation and astrocytosis induced by scrapie infection in the mouse brain. These findings also suggest that astrocytes may play a significant role in the pathogenic process associated with PrP^{Sc} production and accumulation and that some component(s) in cigarette smoke might be involved in the inhibitory effect of replication of the scrapie agent in the astrocytes.

For further study of brain defect in scrapie infected mice, the content of neurotransmitters in various regions of the mouse brain from the experimental group of mice was measured. As shown in Table 1, injection of scrapie agent to mouse led to decrease in the contents of

Table 1. Effect of exposure to cigarette smoke on the level of dopamine in various regions of the brain from the control and scrapie-infected mice

Regions	C	P	CS	PS
Striatum	30.16 ± 3.40	15.77 ± 1.64*	24.85 ± 9.83	23.79 ± 2.74#
Hippocampus	0.28 ± 0.24	0.15 ± 0.01	0.43 ± 0.41	0.13 ± 0.49
Hypothalamus	3.56 ± 0.50	1.08 ± 0.53*	4.90 ± 0.19	3.91 ± 1.88#
Cerebellum	0.44 ± 0.62	0.36 ± 0.32	0.35 ± 0.42	0.56 ± 0.87
Cortex	0.79 ± 0.58	0.87 ± 0.59	0.91 ± 0.56	0.89 ± 0.64

Values are the mean ± SD, n = 5-7 per group. Data are expressed as μg per g tissue.
* Significantly different from control ($P<0.05$).
# Significantly different from scrapie-infected group ($P<0.05$).
Abbreviations are the same as those in Fig. 1.

dopamine in all brain regions except for the cortex in the P group. Cigarette smoke exposure partially or completely restored the dopamine level decreased by scrapie infection to the control level in the brain regions except for hippocampus. In the hippocampus, cigarette smoke exposure had no indication of restoration of the dopamine content reduced by scrapie infection. Exposure to cigarette smoke itself resulted in regiospecific response to dopamine contents in the brain regions of mice: decrease in the striatum and cerebellum, increase in the hippocampus, hypothalamus, and cortex. The contents of serotonin in the brain regions of IM mouse after scrapie-infection, cigarette smoke exposure, or both exposures were also measured (Table 2). Scrapie-infection to the mouse resulted in a remarkable decrease of serotonin in all the brain regions. The decrease was specifically prominent in the hippocampus and hypothalamus, showing only 49% and 43% of the control level, respectively. Exposure of the scrapie-infected mice to cigarette smoke somewhat reduced the decrease of the serotonin in the hippocampus and hypothalamus. Exposure to cigarette smoke itself resulted in regiospecific response to serotonin contents in brain regions of mice: decrease in the striatum and cerebellum, increase in the hypothalamus and cortex. The depletion of dopamine and serotonin by the scrapie infection varied depending on the region of the brain. Namely, dopamine level was decreased by 48% in the striatum and 70% in the hypothalamus in scrapie infected mice compared to the control mice, whereas, serotonin was decreased in the striatal, hypothalamic, cerebral cortex and hippocampal regions. These results are consistent with those obtained from experiments using hamsters by Bareggi *et al.* (2003).

The activities of enzymes that are involved in biosynthesis and catabolism of neurotransmitter acetylcholine were examined. As shown in Table 3, even though it is statistically insignificant there was a little decline in AChE activity, the enzyme which metabolizes acetylcholine (ACh), in the scrapie infected brain homogenates. In contrast, the activity of acetylcholine esterase was not changed in the brain regions by exposure to cigarette smoke. In addition, the activity of choline acetyltransferase, the enzyme responsible for manufacturing ACh, was not changed in the brain homogenates either by scrapie infection or by exposure to cigarette smoke. These data indicate that the scrapie agent does not damage intrinsic cholinergic neurons.

To our knowledge, this is the first report which shows that cigarette smoke alleviates the

Table 2. Effect of exposure to cigarette smoke on the level of serotonin in various regions of the brain from the control and scrapie-infected mice

Regions	C	P	CS	PS
Striatum	0.73 ± 0.11	0.55 ± 0.12	0.45 ± 0.30	0.57 ± 0.24
Hippocampus	0.37 ± 0.06	0.18 ± 0.03*	0.35 ± 0.02	0.21 ± 0.02
Hypothalamus	0.77 ± 0.22	0.33 ± 0.03*	0.97 ± 0.21	0.50 ± 0.29
Cerebellum	0.10 ± 0.12	0.07 ± 0.03	0.07 ± 0.04	0.05 ± 0.01
Cortex	0.58 ± 0.09	0.42 ± 0.05	0.62 ± 0.03	0.40 ± 0.11

Values are the mean ± SD, n = 5-7 per group. Data are expressed as μg per g tissue.
*Significantly different from control ($P<0.05$).
Abbreviations are the same as those in Fig. 1.

Table 3. Effect of exposure to cigarette smoke on acetylcholine esterase and choline acetyltransferase in the brain homogenates from the control and scrapie-infected mice

Enzymes	C	P	CS	PS
ChAT	57 ± 4	56 ± 1	56 ± 1	62 ± 7
AChE	60 ± 5	57 ± 1	60 ± 6	59 ± 8

Values are the mean ± SD of 7 mice. Activities of ChAT and AchE are defined as nmoles/mg protein/hr and nmoles/mg protein/min, respectively.
ChAT: Choline acetyltransferase; AChE: Acetylcholine esterase.
Abbreviations are the same as those in Fig. 1.

symptom of prion disease induced by scrapie-agent infection in mouse. Several hypotheses can be discussed with respect to the mechanism of the beneficial effect of cigarette smoke in scrapie infected mice. Direct inhibitory effect of cigarette smoke on the conversion of normal prion protein (PrP^{C}) to the abnormal form (PrP^{Sc}) is one possibility. Some component(s) of cigarette smoke may interfere with the processing and replication of scrapie agent by reducing susceptibility of a certain cell population or by altering the action of a sub-cellular component. Cigarette smoke exposure attenuates the vacuolation and astrocytosis induced by scrapie-infection in the mouse brain. These findings indicate that astrocytes may play a significant role in the pathogenic process associated with PrP^{Sc} production and accumulation and suggest that cigarette smoke might be involved in the inhibitory effect of replication of the scrapie agent in the astrocytes. For the clear elucidation of this hypothesis, further detailed studies of the two pathological events are necessary. Another possibility is that components of cigarette smoke may enhance neurotransmitter's action in the brain by stimulating the release of neurotransmitters or by affecting the density of neurotransmitter receptor binding sites. For instance, nicotine, a component present in cigarette smoke, increases the level of dopamine (Behmand and Harik, 1992), increases the density of nicotine binding sites in human brain (Maureen *et al.*, 1988), and affects the behavioral responses in an animal model (Ksir and Benson, 1983) and in AD patients (Sahakian *et al.*, 1989; van Duijn and Hoffman, 1991). It has been reported that chronic nicotine treatment prevented neuronal cell loss in the neocortex of an animal model of AD (Sjak-Shie *et al.*, 1991). Such facts suggest that nicotine may have a cytoprotective effect. It is necessary, however, to study the possible protective effects of other components of cigarette smoke and their metabolites.

CONCLUSIONS

In this study, we demonstrated that long term exposure of mice to cigarette smoke retards a number of processes seen in scrapie agent host interactions. Cigarette smoke or cigarette smoking may also lead to improvement of brain function in patients with AD. The fact that the clinical manifestations of neurological diseases with a variety of causes can be improved by cigarette smoke complicates the search for the mechanism(s) of the beneficial effect of cigarette smoke. Further studies are necessary to establish the beneficial action of cigarette smoke exposure against neurological diseases in mechanistic aspects.

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