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DNA damages with Fpg/Endo Ⅲ FLARE Assay in cynomolgus monkeys exposed to stainless steel welding fume

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용접흄 흡입노출 영장류에서 Fpg/Endo Ⅲ FLARE Assay를 이용한 DNA 손상 및 회복

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선박제조업을 비롯한 운송업 및 건축업 등의 다양한 분야에서 용접기술이 이용되어 옴에 따라 용접근로자들에 대한 산업보건학적 관심이 높아지고 있다. 노출정도가 다양하기는 하지만 용접흄은 6가 크롬을 비롯한 금속화합물과 유해가스, 화학물질 등을 복합적으로 포함하고 있는 스테인레스 스틸 용접흄에 대한 유전독성영향을 평가하기 위하여 흡입챔버를 이용, 실험동물인 영장류에 스테인레스 스틸 용접흄을 노출시키고 혈액 내 lymphocytes에 생성된 용접흄 노출농도 및 시간별 DNA 손상정도 및 그 회복효소를 측정함으로써, 유해성이 완전하게 확인되지 않은 용접흄에 노출되어 나타날 수 있는 암을 비롯한 심각한 건강영향을 예방하기 위한 각 지표들을 찾아 그 유용성을 비교하고자 하였다.

영장류를 노출시키기 위해 robotic arm을 장치한 영장류 흡입노출 시스템을 개발하였으며, 이 노출 시스템을 이용하여 수컷 영장류 6마리에 대해 용접흄 노출시험을 실시하였는데 실험군은 대조군 2, 저농도 (31 ㎎/㎥) 노출군 2, 고농도 (63 ㎎/㎥) 노출군 2마리로 구성하였고, 1일 2시간씩 일주일에 5일 동안 용접흄에 노출시켰다. 노출 농도는 지속적으로 모니터링 하였고, 노출과정 중에 영장류의 혈액을 채취하여 lymphocytes를 분리, 단세포 DNA 손상을 선별하기 위해 DNA 손상회복 효소인 E. coli formamidopyrimidine-DNA glycosylase (Fpg)와 endonuclease Ⅲ (Thymine Glycol-DNA glycosylase) 투여와 Comet asaay (single cell gel electrophoresis, 단세포겔전기영동기법)를 결합시켜 이용하는 Fpg/Endo Ⅲ FLARE 분석법을 사용하였다.

Fpg enzyme에 의한 olive tail moment값의 변화는 16주 노출군부터 노출부검(34주)군 까지 노출농도가 높아짐에 따른 olive tail moment 기하평균 값의 양 반응관계를 보

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기는 어렵지만, 고농도군의 경우 27주 노출군에서 가장 높은 olive tail moment 값을 보이고 이후 차츰 감소하였다. 한편 16주에서 22주까지의 노출기간에서는 대조군에 비해 노출군에서 DNA손상정도(olive tail moment값)는 모두 유의하게 높았으나, 6, 12, 18, 25, 31, 33, 35주간 노출하였을 때는 다른 결과를 보였다.

각 실험군의 Fpg enzyme에 의한 tail length값의 분포를 살펴볼 때, 저농도군 및 고농도군에서 27주간 노출하였을 때 가장 높은 tail length 값을 보이고 이후 차츰 감소하는 경향을 보였다. 또한 16, 22주간 노출하였을 때 대조군에 비해 노출군에서 tail length 값이 유의하게 높았으나, 20주간에서만 양 반응관계가 관찰되었고, 다른 주간에서는 양 반응 및 기간 반응관계를 나타내지는 않았다.

Endo Ⅲ enzyme에 의한 olive tail moment값의 변화는 기간별 노출군에서 대조군에 비해 높은 DNA손상정도(olive tail moment값)를 나타내는 결과들이 있었지만, 10, 12, 16, 22, 25, 31주간 노출하였을 때 등 상당수 노출기간에서 반응관계를 나타내지는 않았다.

각 실험군의 Endo Ⅲ enzyme에 의한 tail length값의 분포를 살펴볼 때, 18, 20, 27, 33주간 노출하였을 때 대조군에 비해 노출군에서 tail length 값이 조금 높았지만, 양 반응 및 기간 반응관계를 보이지 않았고 수치의 크기가 불규칙하게 변화하였다.

즉, DNA에 있어 산화된 pyrimidine을 형성하여 손상된 부위의 염기를 제거함으로써 AP site (abasic site)를 만들고 이들이 Comet assay를 통해 break로 전환된 것을 포함한 DNA손상을 측정하기 위하여 endonuclease Ⅲ (Endo Ⅲ)를 첨가시킨 Endo Ⅲ FLARE 분석법을 실시한 결과, 본 연구에서 나타난 결과는 용접흄 노출 영장류에서 Olive tail moment 및 tail length 공히 노출량 및 노출기간 반응관계를 볼 수 없었다. Endo Ⅲ FLARE 분석법을 통한 산화적 DNA 손상지표는 영장류에 적용하기에는 적응반응현상으로 대조군과 유의한 차이도 관찰할 수 없었고 더욱이 역으로 대조군에서의 자연발생적 수치가 더 높아질 수 있어 용접흄 노출 영장류의 모니터링 지표로 사용하기에는 제한점이 있었다.

Key Words : welding fume, cynomolgus monkey, 8-oxodG, Single Cell Gell Electrophoresis (comet assay), lymphocytes, DNA repair, Fpg/Endo Ⅲ FLARE assay

Ⅰ. Introduction

It is being researched about biomarkers for early diagnosis and prevention of occupational diseases with many kinds of chemicals or compounds exposures in workplaces. It is reported that the limits of these biomarkers with regarded to monitoring of workers or animals through chromosome aberration test, micronucleus test and the sister chromatid exchange test and so on (Jelmert et. al., 1994).

Recently, it is continuously introduced the reports of genetic research with molecular biological methods to increase the accuracy of the biomarkers in screening the genotoxic effects.

It was reported the limited evidence of carcinogenic effect of welding fumes to human and in vitro tests, but not known the biochemical mechanism.

Several metal compounds are introduced oxidative DNA damages, DNA base excision and chromosomal aberrations by ROS (reactive oxygen species) through Fenton-type reaction induced by superoxide in metabolism (Itoh et. al., 1995; Kortenkamp et. al., 1996). Welding fumes are compounds of several metal (e.g. Cr (VI)) with anticipation these mechanisms.

It is the main tool of oxidative DNA repair biomarkers that the analysis of 8-hydroxyguanine(8-OHGua/8-OHdG) formed in DNA double helix (Kasai & Nishimura, 1984), and reported that the 8-OHGua is introduced by radiation, reactive oxygen induced chemicals either in vivo or in vitro, and induce GC-TA transversion in DNA polymerase reaction (Kasai et al., 1986; Shibutani et al., 1991). The GC-TA transversion is the results of cell malignant transformation resulted from the codon 12 of ras gene, glycine to GTT (Kamiya et. al., 1992).

Single cell gel electrophoresis assay (comet assay) is a method to detect DNA damage from DNA chain excision and alkali labile sites, not chromosomal aberration, so it is possible to detect DNA damage from undivisible tissues (Fairbairn et. al., 1995). It was introduced by Ostling & Johanson (1984) to detect directly DNA damage in each cellular level, made more sensitive by Singh (1988).

In alkali condition, it can detect base modification, alkali-labile abasic site (AP site) formed when the damage site was inactively excised with DNA repair enzymes. And reported that it can measured the DNA damage in specific sites with FLARE (Fragment Length Analysis with Repair Enzyme) assay, the improved method of comet assay by treatment of endonuclease Ⅲ or formamidopyrimidine DNA glycosylase (Fpg) (Collins et. al., 1993).

In this study, FLARE assay with Fpg and endonuclease Ⅲ treatment was used to investigate DNA damage and its repair enzyme activity of lymphocytes from cynomolgus monkeys exposed to welding fumes. And analysed the utility of each biomarkers to prevent health risks (e.g. cancer) occurred from welding fume.

Ⅱ. Materials and Methods

1) Animals and welding fume exposure

The welding fumes were generated with an automatic fume generator with robotic arm, and two chambers to inflow and expose high and low dose welding fumes organized with fume collector system to sample the exposed fumes. The welding electrodes were used MMA-SS (manual metal arc-stainless steel) (KST 308, 26 x 300 mm, Korea Welding Electrode Co. LTD, Seoul, Korea). The welding fumes in the chamber were sampled using a personal sampler (MSA 484107, Pittsburgh, PA, USA) with a flow rate of 2 liters/min. The welding fume particulate captured on the membrane filters (pore size 0.8 ㎛, 37 mm diameter, Millipore AAWP 03700, Bedford, MA, USA) was analyzed for its metal composition with an inductively coupled plasma analyzer (Thermojeralash, IRIS, Houston, TX, USA) using NIOSH 7300 method. The gaseous fumes, O_3, NO_2 and nitrous fumes were measured using Dräger tubes (Cat No. 6733181, CH 31001 and CH 30001, respectively). The gaseous fumes were sampled by stroking a gas detector pump (6400000, Dräger, Lübeck, Germany), following the manufacturer's guidances 1h after the welding-fume exposure began.

An Anderson sampler (AN-200, Shibata, Tokyo, Japan) was used to measure the mass median aerodynamic diameters of the welding fumes. The flow rate was 28.3 liters/min and the sampling time 5 min.

From three to four-year-old male Macaca fascicularis (Cynomolgus Monkey) (Yunnan National Laboratory Primate Center, China) were acclimated to this chamber for 3 months and assigned with Path/Tox System(Version 4.2.2, Xybion Medical Systems Corporation, USA) to an unexposed group (2 monkeys) and exposed group with low- and high-dose (2 monkeys each group). Exposed group to welding fumes with a time weighted average (TWA) total suspended particulate (TSP) concentration of 31.36 ± 2.75 ㎎/㎥ (low dose), and 62.45 ± 2.70 ㎎/㎥ (high dose) for 2 h/day in inhalation chamber for 34 weeks and recovered for 22 weeks. The exposure concentration was established according to the reports that pneumoconiosis were not occurred with exposed low dose and high dose for 90 days, but low level inflammation in lung (Yu et. al., 2001, 2003, 2004; Sung et. al., 2004), and dose-dependent increase of Mn concentration in brain, lung, liver after 60 days welding fume exposure (Yu et. al., 2003).

2) Chemicals

Ficoll-paque plus (Amersham Biosciences, Brown Deer, WI, USA) was used to extract lymphocytes. FLARE analysis kit (Trevigen, Gaithersburg, MD, USA) with Fpg and Endo Ⅲ was used to analyse DNA damage in single cells. Normal melting agarose (Bio-Rad, Richmond, CA, USA) and low melting agarose (Amresco Co., USA) were used in FLARE assay.

3) FLARE (Fragment Length Analysis with Repair Enzyme) assay

FLARE assay was performed according to the method of Tice et. al. (2000). The single cell suspensions in a 0.5 % low melting point agarose were pipetted immediately onto a conventional glass microscope slide precoated with a 1 % normal melting agarose in PBS. The slides were then immersed in a lysing solution(1 % Triton X-100, 10 % DMSO, 2.5 M NaCl, 0.1 M EDTA, and 10 mM Tris, pH 10) at 4 ℃ for 1h and subjected to alkaline conditions (>pH 13). For FLARE assay, the Fpg or Endonuclease Ⅲ were pipetted onto these slides and incubated at 37 ℃ for 30 min. Electrophoresis was performed at 25 V and 100 mA for 20 min in the dark. The ethidium bromide-stained electropherograms were examined under an epifluorescence microscope equipped with a computer including the image analysis software Komet 5.0 (Kinetic Imaging, Ltd., Liverpool, UK). Fifty randomly captured cells from each animal were analyzed at each sampling time. The Olive tail moment was used to quantitatively measure the extent of DNA damage.

4) Statistical analysis

All results are expressed as the geometric means (GeoMean). One way ANOVA test and Tukey test (α=0.05) were used with SigmaStat 3.11 to compare genotoxic parameters obtained from the two dose groups with those obtained from the unexposed control monkeys.

Ⅲ. Result

1. MMA-SS welding-fume characterization

The MMA-SS welding fumes consisted of mainly Fe, Mn, Cr and Ni. The metal concentrations and gaseous fractions in the welding fumes are shown in Table 1.

2. Genotoxic responses of welding-fume exposure with FLARE assay

The geomean values of measured Olive tail moment with Fpg enzyme were not changed dose-dependently, but the highest value in 27 weeks high concentration group. These values of exposure groups from 16 to 22 week were significantly higher than control group in these exposure durations, but different results in 6, 12, 18, 25, 31, 35 weeks exposure group.

Tail length with Fpg enzyme was the highest in 27 week low & high concentration groups, but most of these have no dose or duration dependency.

Olive tail moment with Endonuclease Ⅲ enzyme of exposed groups were higher than control group, except 10, 12, 16, 22, 25, 31 weeks. Tail length values of exposure groups with Endonuclease Ⅲ enzyme were higher than control group in 18, 20, 27, 33 weeks exposure, but not dose response and changes irregularly.

Table 2 and 3 refer that inter-group comparison of all DNA damage including oxidative damage in Fpg sensitive sites such as altered purines (8-oxoguanine and formamidopyrimidine) by Fpg FLARE assay, and Endo Ⅲ sensitive sites, oxidized pyrimidine (thymidine glycol), by Endo Ⅲ/FLARE assay in controls and welders.

These reflect the oxidative DNA damage level in specific site, such as recovery of DNA adducts (e.g. formamidopyrimidine or 8-oxoguanine, etc.) formation.

Ⅳ. Discussion

It is well known that chemicals, radiation, metal compounds can make reactive oxygen species, and the ROS induce protein or DNA damage (Asami et. al., 1998). Consequently, it have been regarded that DNA base damage may induce 8-oxodG, a type of oxidative DNA damages, is the important biomarker of carcinogenesis (Kasai, 1997; Collins, 1993). Also, analysis of 8-OHdG level in DNA can measure the risk of carcinogenesis by oxidative stress from the metal compounds contained carcinogenic Cr (VI).

The metallic ingredients of welding fumes are Fe, Mn or Cr, and the Cr (VI), 20~60 % of Cr, is classified as human carcinogen in case of inhalation exposure.

SCGE (comet assay) is a outstanding method that very fast, simple, sensitive, cheap and confidentially detected DNA-strand break, DNA alkali-labile site of each cells induced by DNA damage level or intercellular distribution. It applies widely that not only molecular DNA damage but also epidemiological method in human populations (Collins et. al., 1997). The comet tail indicates the relaxed loop of super-coiled DNA, and relative tail intensity indicates the number of DNA segments.

It was performed (Yu et. al., 2004) that genetic toxicity measured with alkaline (pH>13) SCGE analysis with rats exposed the welding fume stainless steel manual metal arc welding. The exposed groups

Table 1. Concentrations of welding fume, heavy metals and feed ingredients (mean ± S.E.).

		Low dose (㎎/㎥)	High dose (㎎/㎥)
Welding fume		31.36±2.75	62.45±2.70
Metal	Fe	1.84±0.22	3.99±0.46
	Cr	1.41±0.16	3.03±0.29
	Mn	0.90±0.11	1.95±0.20
	Ni	0.15±0.02	0.34±0.05
	Zn	0.03±0.01	0.03±0.02
Gas (ppm)	O_3	0.01	0.05
	NO_2	0.3	0.4
	Nitrous fumes	3.8	8.2

for 1 and 15 day were dose-response Olive tail moment, but low-dose exposed group was higher than high-dose group. We presented that it also need DNA damage repair enzyme activity tests with SCGE for detailed analysis of DNA damage by welding fume.

It was reported that the alkaline (pH>13) SCGE assay is very effective to detect base oxidation, DNA single strand breakage (SSB), but the DNA lesion by any genotoxicant is fast repaired physiologically, so it has no lethal or mutational lesion and impossible to lead segments in DNA chain. Alkaline SCGE assay had alkali-labile AP sites by genotoxicant, and these sites reflect

Table 2. Olive tail moment values of DNA isolated from lymphocytes of Cynomolgus Monkeys exposed with welding fume(50 cells per slide), represented by geometric mean.

	Cont-buf	Low-buf	High-buf	Cont-fpg	Low-fpg	High-fpg	Cont-endo	Low-endo	High-endo
Group period	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean
E2	3.85	1.78	2.00	2.80	3.28	1.51	2.59	2.37*	2.02*
E4	4.08	3.21	1.69	1.22	1.06	1.81	1.62	1.95	2.41
E6	1.30	4.28	1.27	2.59	0.94*	0.89*	3.85	1.32*	1.45*
E8	0.70	1.41	2.29	1.14	1.61	2.15	0.98	1.30	2.18
E10	6.15	6.13	3.48	5.10	5.87	3.77	5.63	6.55	3.59
E12	1.91	1.92	1.20	2.54	2.18	1.11	3.50	2.63	1.61
E14	1.06	0.88	1.02	1.75	2.10	1.97	1.23	3.15*	1.49*
E16	1.53	0.73	0.75	3.58	3.99*	4.22*	2.69	1.78	1.64
E18	0.95	1.29	0.77	2.71	0.91*	2.47*	1.62	1.98	1.84
E20	0.49	0.56	0.77	1.18	1.75*	3.70*	0.59	0.78	1.60
E22	1.44	0.85	0.52	2.56	3.62*	2.12*	1.83	1.44	0.89
E25	3.80	2.89	1.61	7.31	2.32*	2.25*	6.07	3.15*	4.78*
E27	0.74	0.67	1.30	9.88	4.61*	13.03*	1.05	1.83*	2.35*
E29	0.47	0.76	0.85	4.86	5.34	5.62	1.21	1.30	1.39
E31	0.78	0.59	0.56	5.71	3.96	2.90	1.28	1.03	1.22
E33	0.90	1.52	1.95	8.05	5.04*	5.89*	1.89	3.59*	2.61*
E34(EA)	1.26	1.15	0.64	6.97	1.69*	2.17*	1.44	1.36	1.23
R2	1.26	0.90	1.04	2.32	2.39	5.28	1.96	1.41	1.64
R5	1.93	1.98	1.50	3.43	2.96	5.52	3.18	2.21	2.83
R7	1.40	1.92	1.12	3.34	5.45	2.09	2.61	3.97	3.41
R9	1.74	0.72	0.70	2.54	2.76*	1.07*	3.68	0.83*	0.84*
R11	1.63	1.61	1.65	8.72	4.59*	4.21*	2.85	1.87*	1.61*
R13	0.38	1.00	3.40	2.66	4.20	2.43	2.09	2.94	3.10
R15	1.48	6.60	1.50	3.27	5.24*	4.72*	3.41	5.47*	2.27*
R20	0.78	1.20	0.59	4.12	2.92*	6.36*	2.12	1.59*	3.37*
R22(RA)	1.29	0.96	1.33	3.64	3.13	3.80	1.71	2.14*	2.55*

*, $p<0.05$, significant different between control and low dose, One-way ANOVA & Tukey Test
unit : a.u. (arbitrary unit)
En : n weeks after welding fume exposure
EA : autopsy group after welding fume exposure for 34 weeks
Rn : n weeks after recovery
RA : autopsy group after recovery for 22 weeks

segments in alkali electrophoresis buffer. DNA segments in alkaline SCGE assay is also temporarily present when a cell repaired DNA damage by base excision, nucleotide excision, so the high level of segments in SCGE assay is represent either high DNA damage level or some repair activities (Collins et. al., 1997). It is applied that the study with cellular repair activity with DNA segments for reveal or amplify the genotoxic effects, and that measuring the repair activity with DNA repair enzymes or inhibition of DNA damage by

Table 3. Tail length values of DNA isolated from lymphocytes of Cynomolgus Monkeys exposed with welding fume(50 cells per slide), represented by geometric mean.

	Cont-buf	Low-buf	High-buf	Cont-fpg	Low-fpg	High-fpg	Cont-endo	Low-endo	High-endo
Group period	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean	GeoMean
E2	39.65	22.03	25.20	34.73	30.65*	19.15*	36.42	32.00	22.84
E4	24.10	33.51	17.66	17.14	13.97*	18.18*	21.15	13.30*	7.58*
E6	18.58	31.12	15.51	22.82	11.88*	11.56*	38.61	14.32*	15.01*
E8	11.67	15.42	19.14	15.51	22.25	22.73	14.53	17.24	21.91
E10	42.55	42.19	27.51	39.78	42.68	31.21	38.81	43.42	28.80
E12	22.61	21.01	14.01	25.34	22.23	15.60	29.38	22.93	16.36
E14	14.11	11.83	13.15	19.90	22.56	20.22	14.81	28.71*	15.36*
E16	18.24	13.41	12.69	30.00	34.07*	33.92*	25.89	19.46	17.86
E18	13.53	17.11	11.79	24.87	13.73*	24.94*	17.12	19.92	18.20
E20	11.10	10.38	12.32	14.49	17.46*	29.79*	10.87	13.34	18.92
E22	18.99	12.45	9.98	26.60	33.29*	21.73*	20.87	17.61	13.19
E25	32.90	26.04	18.19	48.49	27.63	22.10	42.91	29.43*	36.39*
E27	13.81	12.92	15.84	77.51	41.13*	87.69*	15.87	19.84*	26.40*
E29	10.48	11.01	10.80	45343	39.45	41.12	15.42	15.61	15.25
E31	11.96	10.81	10.30	38.78	36.48	27.19	15.47	13.37	15.61
E33	13.10	17.55	19.69	57.80	41.72*	41.02*	19.63	28.37	21.39
E34(EA)	15.55	15.15	11.80	45.11	18.85*	21.49*	16.38	17.20	16.15
R2	15.74	13.49	13.63	22.93	22.89*	35.99	20.25	17.17	19.23
R5	19.33	22.62	17.11	27.95	24.48*	41.34*	27.07	21.54*	26.09*
R7	16.74	21.08	16.51	29.80	37.71	21.29	25.57	29.15	28.68
R9	16.98	11.62	11.60	21.13	22.89*	13.40*	28.49	14.25*	13.18*
R11	17.05	14.17	14.58	53.26	35.74*	30.18*	26.41	18.11	16.08
R13	9.66	14.70	24.22	29.53	31.41	26.93	23.97	25.30	26.24
R15	14.99	46.03	15.72	28.92	33.95*	34.02*	25.59	35.65*	19.27*
R20	12.12	14.33	10.97	28.32	22.27*	48.13*	19.61	16.18*	24.41*
R22(RA)	15.27	11.36	14.97	28.43	25.30	28.82	17.96	19.06	20.72

*, $p<0.05$, significant different between control and low dose, One-way ANOVA & Tukey Test
unit : ㎛ (micrometer)
En : n weeks after welding fume exposure
EA : autopsy group after welding fume exposure for 34 weeks
Rn : n weeks after recovery
RA : autopsy group after recovery for 22 weeks

antioxidants (Jekinson et. al., 1999), and quantitative measurements of specific oxidative base (Collins et. al., 1993; Covallo et. al., 2003).

The geomean values of measured Olive tail moment with Fpg enzyme were not changed dose-dependently, but the highest value in 27 weeks high concentration group. These values of exposure groups from 16 to 22 week were significantly higher than control group in these exposure durations, but different results in 6, 12, 18, 25, 31, 35 weeks exposure group.

Tail length with Fpg enzyme was the highest in 27 week low & high concentration groups, but most of these have no dose or duration dependency.

Olive tail moment with Endonuclease Ⅲ enzyme of exposed groups were higher than control group, except 10, 12, 16, 22, 25, 31 weeks. Tail length values of exposure groups with Endonuclease Ⅲ enzyme were higher than control group in 18, 20, 27, 33 weeks exposure, but not dose response and changes irregularly.

Table 2 and 3 refer that inter-group comparison of all DNA damage including oxidative damage in Fpg sensitive sites such as altered purines (8-oxoguanine and formamidopyrimidine) by Fpg FLARE assay, and Endo Ⅲ sensitive sites, oxidized pyrimidine (thymidine glycol), by Endo Ⅲ/FLARE assay in controls and welders.

These reflect the oxidative DNA damage level in specific site, such as recovery of DNA adducts (e.g. formamidopyrimidine or 8-oxoguanine, etc.) formation.

In this study, it can deduct that the Olive tail moment in 27 week exposure reflected either DNA damage and its repair, but reduced mean value after 27 week and repair group are caused by reducing the DNA damage and its repair. These DNA damage with welding fume and forecasted change modality of these base excision repair are different to the results of Maeng et. al. (2003), in rat's lung with Cr (VI) exposure, a simple metal compound. They reported that 8-OHdG repair activity by Cr (VI) exposure was significantly reduced in early exposure phase and returned to control group level after 3 weeks, reflected that the changes of 8-OHdG repair activity are more effective to 8-OHdG level changes by Cr (VI) exposure. These differences indicate that Cr (VI) is a significantly important in welding fumes, but the other gaseous or metal ingredients are participated in DNA damage and repair enzyme activities with longer exposure duration.

Phoa & Epe (2002) was measured oxidative DNA damages with nitric oxide (NO) generated to cultured mammalian fibroblasts endogeneously or exogeneously, investigated the effects of DNA damages by H_2O_2 to repair enzymes, and reported that NO induced cellular DNA damages inefficiently and protected the DNA damages by H_2O_2, but flexibly inhibited the repair of oxidative DNA base modification. It supposed that the activity of NO to DNA damaged induced by welding fume is very little but helps the repair enzyme inhibition.

Also, according to Abalea et. al. (1998), it could determine 7 DNA base oxidation products with analysis of induced oxidative DNA damage and repair of DNA base lesion with addition of ferric nitrilotriacetate (Fe-NTA) to in vitro primary cultured hepatocyte. In these, the oxidized-purine such as 8-oxo-guanine, xanthine, fapy-adenine, 2-oxo-adenine, and DNA base repair activity have dose-response with time. Therefore, it reported that the DNA repair pathway is activated in addition to Fe in hepatocyte, but it is not sufficient to protect the accumulation of high mutagenic DNA oxidants by Fe in genomic DNA.

According to Asmuss et. al. (2000), it is known that carcinogenic metal compound such as Ni, Cd, As, Co inhibits base excision repair or nucleotide excision repair process in very low (nontoxic) concentrations. Therefore, it must be considered that the exposure concentration, oxidative status of these metal compounds in target site is important to form a DNA oxidative damage or its repair (Maeng et. al., 2003). It is supposed that these opposite results are caused by exposure concentration factors in view of the fact that very high concentrations (low dose group 65.6 ㎎/㎥, high dose group 116.8 ㎎/㎥) exposed to animals in early study.

Also, it can be considered as adaptive response, the adaptive response is a protective effect in occurred to exposure higher concentration of it or chemicals with similar action mechanisms after exposure to low dose radiation or genotoxicant, it was reported (Yarosh et. al., 1984) the formation of adaptation related protein such as DNA methyltransferase in animal and plant cells after reported this phenomenon with E. coli in 1977 (Samson & Cairns, 1977). Ikushima (1989) reported that Chinese hamster V79 cell adapted with radioactive thymidine has adaptive effect to high dose X-ray exposure since then, the cell adapted with γ-ray has resistance to mitomycin C and UV and induced sister chromatid exchange and reduced micronucleus formation. Pant et. al. (2003) explained the inhibition of neoplastic transformation related adaptation to low dose with two mechanisms, upregulation of antioxidant glutathione and extremely increase DNA repair capabilities (super repair).

Therefore, it is considered that the measured values in welding fume exposed cynomolgus monkey group lower than control group are a adaptive response or stochastic effect (Scott, 2004) being studied and reported generally interested in radioactive rays.

In Fpg/Endo Ⅲ FLARE analysis, it was investigated the DNA damage level indirectly to measure the breaks amounts repaired oxidative DNA damage site with DNA repair enzymes, and it comes to the conclusion that it is difficult to explain the super repair related with adaptive response mechanism mentioned Pant et. al. (2003), and could not substitute directly to welding fume because of its complex hazards and difference with radioactive rays. It could be explained more easily to link the existing research results (Asmuss et. al., 2000; Hartwig, 1998) of DNA damage repair enzyme reduction with exposure to the metal in low concentration. And then, it could be assumed that the adaptive response of 8-oxodG in welding fume exposed cynomolgus monkey is rather caused upregulation of antioxidant glutathione reduction than increase of repair enzyme with exposure of low concentration.

Also, Potts et. al. (2001) reported that oxidative DNA damage in alveolar epithelial cell adapted with cadmium was inhibited, the results of increase thiol-containing antioxidants such as metallothionein, glutathione adapted in low concentration. But the repairing ratio of entirely oxidative damage in cells adapted with cadmium was lower than not adapted cells. They were reported that the cadmium adaptation was significantly injured to the repairing of Fpg and Endo Ⅲ specific sites by comet assay with lesion specific enzyme (Fpg/Endo Ⅲ). And these results fell in with this study results.

From the above findings, FLARE assay was found out as limited as a biological tool for DNA damage induced by welding fume exposure in cynomolgus monkeys.

Ⅴ. Conclusion

It has been known that stainless steel welding fume is associated with the increase of lung fibrosis, pneumoconiosis and lung cancer. To clarify whether a kind of DNA damage from reactive oxygen species are involved in its mechanism of potential health hazards, we measured the DNA damage through Fpg/Endo Ⅲ FLARE(Fragment Length Analysis with Repair Enzyme) assay with cynomolgus monkeys.

Accordingly, to investigate the DNA damage from reactive oxygen species (ROS) after welding fume-exposure, six cynomolgus monkeys were acclimated for a month and assigned to 3 dose groups: unexposed, low dose (31.36 ㎎/㎥), and high dose (62.45 ㎎/㎥) total suspended particulates. The primates were exposed to manual metal arc-stainless steel (MMA-SS) welding fume for 2 hrs per day in an inhalation chamber system that is equipped with an automatic fume generator.

During the exposure, the lymphocytes were extracted from cynomolgus monkeys along the time schedule.

We used the method (fragment length analysis with repair enzyme, FLARE) in combination with oxidative base damage-specific enzymes, foramidopyrimidine-DNA glycosylase (Fpg) and thymine glycol-DNA glycosylase (Endo Ⅲ), to estimate oxidative DNA damage in the same animals. As for Fpg FLARE assay, we found the 27 week high concentration group was the highest level of Olive tail moment and tail length. As for Endo Ⅲ/FLARE assay, no statistically significant increase in site specific base damage between the control and welding fume exposed group. No statistically significant effect was seen on the level of all DNA damage determinations, both direct (DNA strand breakage and alkali-labile lesions) and enzyme-combined (base damage) in the control or in the exposed group.

From the above findings, FLARE assay was suggested as being available as a biological tool for DNA damage induced by welding fume exposure in cynomolgus monkeys. However, further investigations were necessary because of its lack of correlation to exposure duration and for clarification of its mechanisms.

REFERENCES

Anderson D, Yu T-W, Phillips BJ, Schmerzer P (1994). The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. Mutation Research. 307, 261~271.

Asami S, Hirano T., Yamaguchi R, Tsurudome Y, Itoh T, Kasai H (1998). Effects of forced and spontaneous exercise on 8-hydroxydeoxyguanosine levels in rat organs. Biochemical and Biophysical Research Communications. 243, 678~682.

Asmuss M, Mullenders LH, Eker A, Hartwig A (2000). Differential effects of toxic metal compounds on the activities of Fpg and XPA, two zinc finger proteins involved in DNA repair. Carcinogenesis. 21, 2097~2104.

Collins AR, Dobson V, Dusinska M, Kennedy G, Stetina R (1997). The comet assay: what can it really tell us? Mutation Research. 375, 183~193.

Collins AR, Duthie SJ, Dobson VL (1993). Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis. 14, 1733~1735.

Covallo O, Ursini CL, Setini A, Chianese C, Piegari P, Perniconi B,

Iavicoli S (2003). Evaluation of oxidative damage and inhibition of DNA repair in an in vitro study of nickel exposure. Toxicology in Vitro. 17, 603~607.

Fairbairn DW, Olive PL, O'Neill KL (1995). The comet assay: A comprehensive review. Mutation Research. 339, 37~59.

Gedik CM, Ewen SWB, Collins AR (1992). Single cell gel electrophoresis applied to the analysis of UV-C damage and its repair in human cells. International Journal of Radiation Biology. 62, 313~320.

Halliwell B, Dizdaroglu M (1982). The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. Free Radical Research Communications. 16, 75~87.

Hartwig A (1998). Carcinogenicity of metal compounds: possible role of DNA repair inhibition. Toxicology Letters. 102~103, 235~239.

Henriksson H, Tj?lve H (2000) Manganese taken up into the CNS via the olfactory pathway in rats affects astrocytes. Toxicological Sciences. 55, 392~398

Ikushima T (1989). Radioadaptive response: characterization of a cytogenetic repair induced by low-level ionizing radiation in cultured Chinese hamster cells. Mutation Research Letters. 227, 241~246.

Itoh M, Nakamura M, Suzuki T, Kawaki K, Horitsu H, Takamizawa K (1995). Mechanism of chromium (VI) toxicity in Escherichia coli: is hydrogen peroxide essential in Cr (VI) toxicity? Journal of Biochemistry (Tokyo). 117, 780~786.

Jekinson AM, collins AR, Duthie SJ, Wahle KWJ, Duthie GG (1999). The effect of increased intakes of polyunsaturated fatty acids and vitamin E on DNA damage in human lymphocytes. The FASEB Journal. 13, 2138~2142.

Jelmert O, Hansteen I-L, Langard S (1994). Chromosome damage in lymphocytes of stainless steel welders related to past and current exposure to manual metal arc welding fumes. Mutation Research. 320, 223~233.

Kamiya H, Miura K, Ishikawa H, Inoue H, Nishimura S, Ohtsuka E (1992). c-Ha-ras containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent positions. Cancer Research. 52, 3483~3485.

Kasai H (1997). Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutation Research. 387, 147~163.

Kasai H, and Nishimura S. (1984). Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Research. 12: 2137~2145.

Kasai, H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H (1986). Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis. 7, 1849~1851.

Kortenkamp A, Casadevall M, Faux SP, Jenner A, Shayer RO, Woodbridge N, O'Brien, P (1996). A role for molecular oxygen in the formation of DNA damage during the reduction of the carcinogen chromium (VI) by glutathione. Archives of Biochemistry and Biophysics. 329, 199~207.

Maeng SH, Chung HW, Yu IJ, Kim HY, Lim CH, Kim KJ, Kim SJ, Ootsuyama Y, Kasai H (2003). Changes of 8-OH-dG levels in DNA and its base excision repair activity in rat lungs after inhalation exposure to hexavalent chromium. Mutation Research. 539, 109~116.

McKelvey-Martin VJ, Green MHL, Scchmezer P, Pool-Zobel BL, De Meo M, Collins A (1993). The single cell gel electrophoresis assay (comet assay): a European review. Mutation Research. 288, 47~63.

NIOSH manual of analytical methods (1999). Method No. 7300. National Institute for Occupational Health, Cincinnati.

Ostling O, Johanson KJ (1984). Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochemical and Biophysical Research Communications. 123, 291~298.

Pant MC, Liao XY, Lu Q, Molloi S, Elmore E, Redpath JL (2003). Mechanisms of suppression of neoplastic transformation in vitro by low doses of low LET radiation. Carcinogenesis. 24, 1961~1965.

Potts RJ, Bespalov IA, Wallace SS, Melamede RJ, Hart BA (2001). Inhibition of oxidative DNA repair in cadmium-adaptive alveolar epithelial cells and the potential involvement of metallothionein. Toxicology. 161, 25~38.

Samson L, Cairns J (1977). A new pathway for DNA repair in Escherichia coli. Nature. 267, 281~283.

Scott BR. (2004). A biological-based model that links genomic instability, bystander effects, and adaptive response. Mutation Research. 568, 129~143.

Shibutani S, Takeshita M, Grollan AP (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature. 349, 431~434.

Singh NP, McCoy MT, Tice RR, Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research. 175, 184~191.

Yarborough A, Zhang Y-J, Hsu T-M and Santella RM (1996). Immunoperoxidase detection of 8-hydroxydeoxyguanosine in Aflatoxin B1-treated rat liver and human oral mucosal cells. Cancer Research. 56, 683~688.

Yarosh DB, Rice M, Day RS 3rd, Foote RS, Mitra S. (1984). O6-Methylguanine-DNA methyltransferase in human cells. Mutation Research. 131, 27~36.

Yin B, Whyatt RM, Perera FP, Randall MC, Jedrychowski W, Cooper Y, Santella RM (1995). Determination of 8-hydroxydeoxyguanosine by immunoaffinity chromatography-monoclonal antibody-based ELISA. Free Radical Biology and Medicine. 18, 1023~1032.

Yu IJ, Kim KJ, Chang HK, Song KS, Han KT, Han JH, Maeng SH, Chung YH, Park SH, Chung KH, Han JS, Chung H (2000). Pattern of deposition of stainless steel welding fume particles inhaled into the respiratory systems of Sprague-Dawley rats exposed to a novel welding fume generating system. Toxicology Letters. 116, 103~111.

Yu IJ, Park JD, Park ES, Song KS, Han KT, Han JH, Chung YH, Cho BS, Chung HK, Cho MH (2003). Manganese distribution in brains of Sprague-Dawley rats after 60 days of stainless steel welding-fume exposure. NeuroToxicology. 24, 777~785.

Yu IJ, Song KS, Chang HK, Han JH, Chung YH, Han KT, Chung KH, Chung HK (2003). Recovery from manual metal arc-stainless steel welding-fume exposure induced lung fibrosis in Sprague-Dawley rats. Toxicology Letters. 143, 247~259.

Yu IJ, Song KS, Maeng SH, Kim SJ, Sung JH, Han JH, Chung YH, Cho MH, Chung KH, Han KT, Hyun JS, Kim KJ (2004). Inflammatory and genotoxic responses during 30-day welding-fume exposure period. Toxicology Letters. 154, 105~115.