Exposure to Ambient PM2.5 and PM 10 and Health Effects

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ABSTRACT

We collected ambient PM2.5 and PM10 particulate samples in Chiang Mai and Lamphun provinces, using Mini Volume Air Sampler. Daily levels of the particulate matter were measured, and screened for cytotoxicity and apoptosis induction in cultured lung cells and macrophages. The results should yield a better understanding of how exposure to ambient particulate matter might involve in short-term and long-term respiratory and health problems. Current knowledge about particulate matter and respiratory allergies will be presented and reported to scientists, regulators and industry workers in a workshop at the end of the research period. The results from our study concerning the health impacts of ambient particulate matter will strengthen the basis for preventive strategies, and may help the government implement policies that will improve the air quality in Chiang Mai and Lumphun provinces.

Key words: Air pollution, PM 2.5, PM10 toxicity

INTRODUCTION

Air pollutants have been recognized as a major problem for human health. Adverse health effects of exposure to airborne particles have been described in numerous epidemiological studies. PM10 and PM 2.5 are airborne particles with an aerodynamic diameter of less than 10 and 2.5 μm, respectively. The PM 2.5 fraction are called "fine particles", and those particles between 10 and 2.5 μm are currently named "coarse particles". PM 2.5 and PM10 are considered as respirable particulate matter and have been found to be associated with acute and chronic adverse health effects (Dockery et al., 1993; Schwart et al., 1996). Airborne particulate matter (PM) is associated with pulmonary diseases, including cancer (Schwartz, 1996; Becker et al., 2002). The WHO Working Group stated that there was strong evidence to conclude that fine particles (<2.5 μm, PM2.5) are more hazardous than larger ones (coarse particles) in terms of mortality and cardiovascular and respiratory endpoints. However, the coarse fraction of PM10 is still innocuous (WHO, 2003). Much evidence shows that PM causes damage to DNA, protein and lipid (Harrison and Yin, 2000; Daya et al., 2003). PM is known to damage DNA and protein and

causes apoptosis through mitochondria-regulated death pathway (Knaapen et al., 2003). Epidemiological studies have associated the increase in respiratory disorders with high levels of ambient particulate matter (Kunzli et al., 2000).

It is reported that cancer incidence rates, especially lung cancer in Chiang Mai, are much higher than in other places of Thailand (Vatanasapt et al., 1993). The particulate matter level in Chiang Mai air has been reported to vary from 17.73 - 211.58 μg/m^3 (Matsushita et al., 1987). If these particles were mostly PM 2.5, such levels would be much higher than the USEPA 24 hour PM 2.5 standard of 65 μg/m^3. In addition, organic extracts of Chiang Mai airborne particulate matter have demonstrated genotoxicity (Vinitketkumnuen et al., 2002). Thus there is ample reason to be concerned about air quality in Chiang Mai and Lamphun.

The objective of this work was to collect ambient particulate matter, PM10 and PM2.5, from 5 different locations in Chiang Mai and Lamphun provinces. The sampling locations were chosen with respect to various air quality conditions in order to obtain a representative sample for the Chiang Mai-Lamphun area. The distances between sampling sites were less than 30 km. We expected that the selected monitoring sites could represent air pollution levels over a large area of Chiang Mai and Lamphun provinces. *In vitro* toxicity of PM2.5 and PM10 collected were evaluated in order to verify the toxicity of ambient particulate material. In addition, we investigated the indoor air concentration of particulate matter in residential house and its relationship with ambient pollution level.

MATERIALS AND METHODS

Collection of PM

Airborne PM was collected, using mini-volume air samplers (AIRmetrics Minivol portable air samplers, USA, www.airmetrics.com). Based on the impaction, particles were collected according to fine particle (<2.5 μm) and a coarse one (<10 μm). Particle samples were collected onto filter (47mm fiber-film filters (type T60A20, Pallflex, USA) in each air sampler, one for PM10 and another for PM2.5.

Sampling locations

From June 2004 to May 2005, PM 2.5 data were taken at 2 different indoor and outdoor sampling sites. Site A was in residential house of Chiang Mai Rajabhat University and Site B was in the Bioassay Research Laboratory, Faculty of Medicine, Chiang Mai University. These two sites are impacted with a heavy-traffic road nearby.

From May to October 2005, PM 2.5 and PM 10 outdoor data were taken at 5 different sampling sites. Site 1 was a rural area with limited direct automotive traffic exposure (Mae Rim district, Chiang Mai province), Site 2 was a typical commercial area impacted with busy streets which experience year-round heavy traffic (Waroros Market, Chiang Mai province), Site 3 was located in an area of high incidence of

lung cancer and impacted by the local traffic sources (Sarapee district, Chiang Mai province), Site 4 was located about 4 km east of the Lumphun industrialized area (Ban Klang, Lamphun province) and Site 5 was in an urban area with local traffic emission (Kai Kaew community, Lamphun province).

Preparation and extraction of filters

The fiber-coated filter was cut into small pieces and placed in a beaker with approximately 200 ml of dichloromethane. The filters were then sonicated in an ultrasonic bath for 15 minutes. The extract was filtered through Whatman No. 41 filter paper into a round-bottom flask. Anhydrous sodium sulfate was put into the funnel before filtering in order to remove water from the sample. The sonication was repeated two more times with ~100 ml of dichloromethane. The beakers and funnels were rinsed with dichloromethane after subsequent filtration. The extract was evaporated by using a vacuum rotary evaporator to nearly dryness at 35°C. In order to obtain enough extractable material from filter, one-month's samples were mixed together according to the sampling time. This tends to compensate for the fluctuations in airborne particulate matter over the course of a month's sampling. The residue was quantitatively resolved in culture media to make the highest concentration and passed through a Millipore filter membrane to get a sterile solution for use in the cytotoxicity test. Extracts were stored in the dark at 4°C in sealed vials prior to the assays.

Cell lines

Human cell line A549 (alveolar type II-derived cell line) and MH-S alveolar macrophages were obtained from the American Tissue Type Collection. A549 and MH-S were grown on F-12K/DMEM (1:1) and RPMI-1640 medium respectively, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and10 μg/mL streptomycin in a humidified atmosphere at 37°C and 5% CO_2.

Cell viability

Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay. Cells ($1x10^4$ cells/well) were seeded in a 96-well plate for a final volume of 100 μl of culture medium supplemented with 10% FBS. On the next day, cells were treated with various concentrations of PM2.5 and PM10 extracts for 3 days. Twenty micro liters of filter sterile stock MTT solution (5mg/ml in PBS) was added to each well and cultures were incubated for 4 hr at 37°C. After supernatant was removed, 100 μl of DMSO was added to completely dissolve crystalline material. An absorbance was measured by ELISA plate reader at 540 nm.

Analysis of DNA fragmentation

Cells were seeded in a six-well plate ($1x10^6$ cells/well) and treated with PM extracts for 18-h. At the end of the incubation, the cells were pelleted and lysed in 100 μl of lysis buffer (1 M Tris-HCl buffer pH 7.4, 1.5 M EDTA pH 8.0 and 10% Triton X-100) for 15 min at 4°C. After the lysate was centrifuged at 15000 rpm for

5 min, the supernatant was incubated at 37°C for 1 h with 5 µl of 10 mg/ml RNase A and followed by incubating with 4 µl of 10 mg/ml Proteinase K at 50°C for 30 min. DNA was precipitated with 5 M NaCl (20 µl) and cold isopropanol (1 ml) at -20°C overnight. After being centrifuged at 15000 rpm for 15 min, the supernatant was removed and washed with 1 ml of cold 70% ethanol. After centrifuge at 15000 rpm for 15 min again, the supernatant was removed and evaporated. The remaining DNA was dissolved in TE buffer. Fragmented DNA was electrophoresed on 1.5% agarose gel and visualized by ethidium bromide staining.

RESULTS AND DISCUSSION

Particulate matter levels in indoor and outdoor air

Indoor and outdoor PM 2.5 concentrations observed at the two sampling sites (Site A and Site B) are shown in Table 1. During winter months (October 2004 to March 2005), particulate matter concentrations were higher than those collected during the summer months (June to September), level in November was low due to the incidence of rain in Chiang Mai. The indoor PM 2.5 levels more closely follow the outdoor PM levels in all seasons. The levels at all sites, both indoor and outdoor, express a similar pattern of gradual increase during the winter. The relationships between the indoor and outdoor air concentrations of PM2.5 were clear. The indoor PM levels in sampling sites were sometimes higher than outdoor level; these may be due to individual behavior patterns and indoor activities at the site. Given these findings and the fact that people spend 80-90% of their time indoors, future studies are needed in order to clarify the characteristics of indoor air particulate matter pollutants and their adverse health effects.

Table 1: Monthly average of PM 2.5 concentrations (µg/m^3) in indoor and outdoor ambient air from July 2004 to May 2005.

Month	Outdoor		Indoor	
	Mean + S.D.			
	Site A	Site B	Site A	Site B
June 2004	15.24 ± 4.53	ND	16.35 ± 4.86	13.08 ± 4.27
July 2004	12.64 ± 3.79	ND	13.61 ± 3.34	9.93 ± 2.71
August 2004	14.79 ± 5.29	15.09 ± 4.54	16.04 ± 4.69	11.56 ± 2.48
September 2004	16.03 ± 5.72	17.14 ± 8.54	16.82 ± 6.77	12.48 ± 5.09
October 2004	44.04 ± 9.93	46.13 ± 12.90	38.74 ± 10.67	31.62 ± 9.83
November 2004	29.69 ± 9.87	30.10 ± 10.13	24.15 ± 7.26	21.62 ± 7.20
December 2004	47.97 ± 14.39	52.90 ± 18.38	41.48 ± 13.97	40.46 ± 17.65
January 2005	45.86 ± 9.82	56.97 ± 13.39	38.58 ± 8.11	44.52 ± 11.17
February 2005	76.97 ± 20.48	69.97 ± 20.92	57.85 ± 13.55	58.52 ± 17.94
March 2005	64.43 ± 22.06	65.31 ± 20.07	43.25 ± 15.55	47.05 ± 15.21

Month	Outdoor		Indoor	
	Mean + S.D.			
	Site A	Site B	Site A	Site B
April 2005	42.57 ± 18.31	40.32 ± 17.89	35.44 ± 13.42	27.52 ± 10.33
May 2005	ND	ND	17.79 ± 4.40	15.73 ± 5.22

N.D = not determined, site A = Chiang Mai Rajabhat University, Site B= Faculty of Medicine, Chiang Mai University

Particulate matter levels in Chiang Mai-Lamphun air

Monthly average 24-hour PM2.5 and PM10 concentrations from 5 sampling sites are shown in Table 2 (PM2.5) and Table 3 (PM10). PM levels at all sites were in the acceptable level of USEPA standard PM10 (150 μg/m^3) and PM 2.5 (65 μg/m^3) for a 24 hr. period. The findings confirmed our previous report that PM levels in Chiang Mai ambient air during the summer months were usually within the recommendation standard level (Vinitketkumnuen et al., 2002). PM levels at site # 2 and #3 seemed to be higher than at the other sites. These two sites are directly impacted by local traffic congestion and other sources at the sites. These findings suggest that local contributions to pollution may influence the PM levels in the ambient air.

Table 2: Monthly average of PM 2.5 concentrations in Chiang Mai and Lamphun ambient air from May to October 2005.

Time	Twenty-four hour PM 2.5 level, μg/m^3 Mean ± SD				
	Site 1	Site 2	Site 3	Site 4	Site 5
May 2005	19.52 + 9.56	N.D	N.D	14.17 + 4.00	17.78 + 5.14
June 2005	9.84 + 2.44	20.53 + 3.78	18.14 + 3.10	6.81 + 1.87	10.31 + 2.52
July 2005	10.90 + 3.57	25.44 + 4.62	25.11 + 9.45	8.65 + 3.88	15.38 + 6.67
August 2005	13.21 + 3.22	32.58 + 5.30	27.68 + 9.92	12.77 + 4.12	18.15 + 4.73
September 2005	12.51 + 8.07	28.89 + 9.02	21.83 + 9.67	12.01 + 8.04	18.92 + 13.96
October, 2005	21.05 + 8.13	37.19 + 9.14	31.31 + 11.04	26.13 + 9.49	29.93 + 11.83

N.D = not determined
Site 1: rural area (Mae Rim district, Chiang Mai province),
Site 2: typical commercial area (Waroros market, Chiang Mai province),
Site 3: high incidence of lung cancer area (Sarapee district, Chiang Mai province),
Site 4: industrialized area (Ban Klang, Lamphun province)
Site 5: urban area (Kai Kaew community, Lamphun province).

Table 3: Monthly average of PM10 concentrations in Chiang Mai and Lamphun ambient air from May to October 2005.

Time	Twenty-four hour PM10 level, μg/m^3 Mean ± SD				
	Site 1	Site 2	Site 3	Site 4	Site 5
May 2005	42.64 + 9.56	N.D.	N.D.	28.10 + 9.52	35.00 + 9.47
June 2005	24.18 + 5.94	54.47 + 8.14	37.57 + 6.37	14.43 + 3.49	18.24 + 5.71
July 2005	21.00 + 6.04	57.11 + 9.84	39.54 + 11.00	18.90 + 7.12	26.38 + 12.30
August 2005	30.34 + 7.65	78.59 + 23.02	46.70 + 13.35	26.61 + 8.87	29.79 + 6.53
September 2005	22.71 + 10.36	64.11 + 16.95	40.21 + 12.60	21.56 + 11.41	30.19 + 19.79
October, 2005	34.41 + 13.22	83.14 + 16.07	55.47 + 17.06	39.25 + 12.19	44.01 + 15.59

N.D = not determined
Site 1: rural area (Mae Rim district, Chiang Mai province),
Site 2: typical commercial area (Waroros market, Chiang Mai province),
Site 3: high incidence of lung cancer area (Sarapee district, Chiang Mai province),
Site 4: industrialized area (Ban Klang, Lamphun province)
Site 5: urban area (Kai Kaew community, Lamphun province).

Cytotoxicity test

MTT assay was performed with various concentrations of PM2.5 and PM10 in human lung cells and alveolar macrophage. PM2.5 and PM10 extracts were toxic to both A549 and MH-S. About 40% of cytotoxicity of PM2.5 and PM 10 extracts to A549 was observed especially in July samples from all sampling sites (Fig. 1). Cytotoxicity of PM2.5 and PM10 extracts to MH-S was dose-independent (Fig. 2). In Mae Rim area, both PM2.5 and PM10 extracts in August samples significantly induced MH-S cell death with IC50 25 and 50 μg/ml, respectively (Fig. 2). Their cytotoxicities are in dose-dependent manner.

Analysis of DNA fragmentation

To detect apoptosis induction, DNA fragmentation assay was performed. DNA laddering pattern was occasionally found in the MH-S cell treated with PM2.5 extracts, collected in May from Bann Klang, Lamphun province (Fig. 3). During June and July, all samples did not cause DNA damage (data not shown).

Alveolar macrophage and lung cells are critically important in processing inhaled airborne particles. Alveolar macrophages are one of the most potent producers of inflammatory mediators in the lung. Our previous report indicated that Comet DNA damage was induced by extractable Chiang Mai airborne organic matter (Vinitketkumnuen et al., 2002). In this study, the extractable matter from Chiang Mai-Lamphun PM2.5 or PM10 affected MH-S cell viability by MTT assay and DNA fragmentation was also detected. In MTT assay, both PM2.5 and PM 10 extracts showed cytotoxicity to lung A549 and alveolar macrophages. Our data indicated that about 40% cytotoxicity was observed for 72 hr- exposure to PM2.5 or PM10 extracts, up to 100 μg/ml, for both cell lines. Particles from all sites showed larger effect on MH-S than on A549 lung cells. PM2.5 collected in May from Bann Klang site

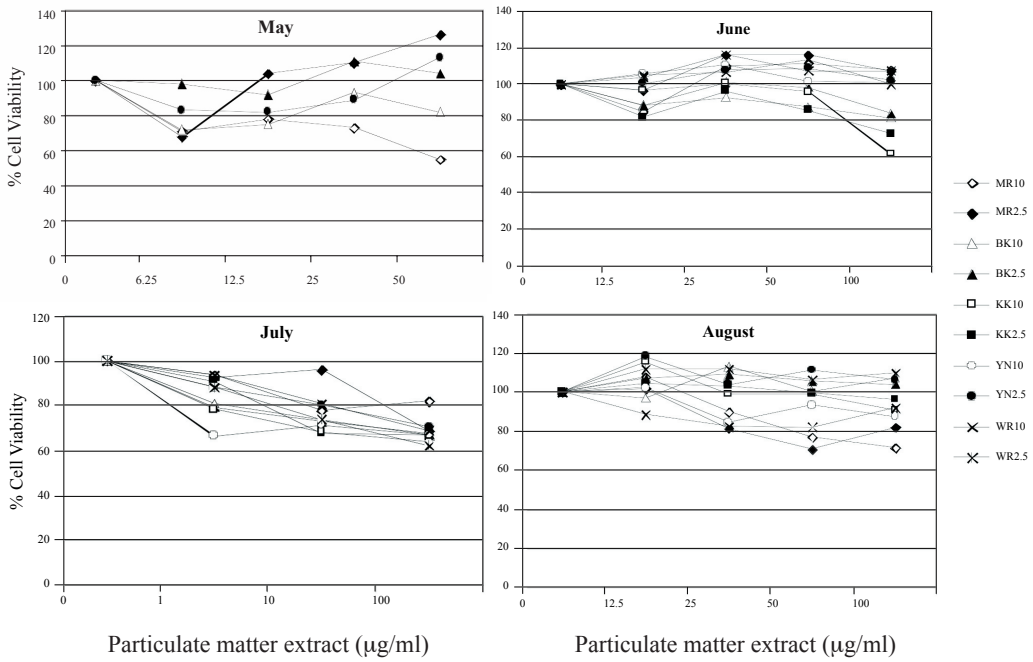

Fig.1. Cell viability of human lung cells (A549) exposed to various concentrations of particulate matter extracts. A549 ($1x10^4$ cells) were treated with PM extracts for 3 days. Cell viability was assessed by MTT assay. Values are means of Mae Rim PM10 (MR10), PM2.5 (MR2.5); Bann Klang PM10 (BK10), PM2.5 (BK2.5); Kai Kaew PM10 (KK10), PM2.5 (KK2.5); Sarapee PM10 (YN10), PM2.5 (YN2.5) and Waroros PM10 (WR10), PM2.5 (WR2.5).

was more toxic than PM10 from the same site. This cytotoxicity could be related to apoptosis induction in the cell treated with PM2.5 (Fig. 3). DNA laddering patterns in PM 2.5 were clearly found, compared to PM10-treated cells. It was reported that PM2.5 might induce more free radicals which can induce DNA damage and apoptosis. This may be due to a different ratio of heavy metals in PM due to different chemical components, especially heavy metals, between PM2.5 and PM10. The percentage of heavy metals (As, Cr and Cd) was reported to be much higher in the constituents of fine particle (PM2.5) than coarse particle (PM10) (Dreher et al., 1998). The different compositions of heavy metals between PM 2.5 and PM 10 in our study may be one of the underlying causes of different toxicity in A549 lung cell and MH-S. The connections between exposure to particulate matter and its adverse effects on human health have been well investigated (Pope, 2000; WHO, 2000; Grigg, 2002). Especially, PM2.5 influences severity of allergic airways disease in mice (Gavett et al., 2003). The PM levels in this study are similar in magnitude to those in our previous study report in the year 1998-1999 (Vinitketkumnuen et al., 2002). These results were preliminary, and further examinations with more samples collected in the remaining months are continuing.

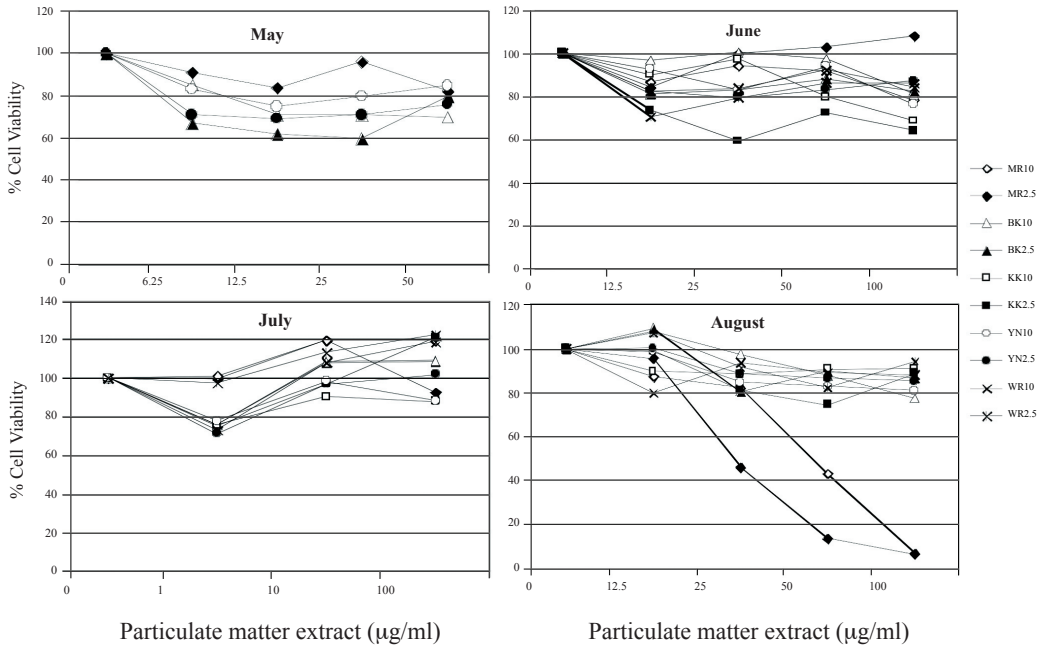

Fig.2. Cell viability of alveolar macrophages (MH-S) exposed to various concentrations of particulate matter extracts. MH-S (1x104 cells) were treated with PM extracts for 18-h. Cell viability was assessed by MTT assay. Values are means of Mae Rim PM10 (MR10), PM2.5 (MR2.5); Bann Klang PM10 (BK10), PM2.5 (BK2.5); Kai Kaew PM10 (KK10), PM2.5 (KK2.5); Sarapee PM10 (YN10), PM2.5 (YN2.5) and Waroros PM10 (WR10), PM2.5 (WR2.5).

In conclusion, we found that PM2.5 and PM10 in Chiang Mai-Lamphun ambient air collected in May to October 2005 were cytotoxic to lung cells and alveolar macrophages, and samples collected in May at Bann Klang, Lamphun province induced apoptosis of alveolar macrophages.

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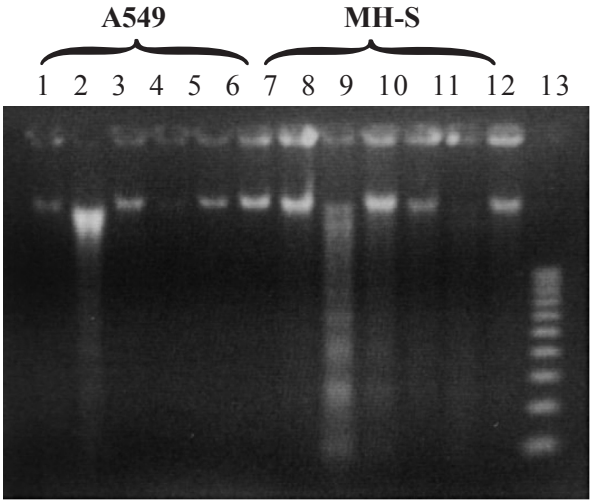

Lane 1: Negative control
Lane 2: Camptothecin 4.5 M
Lane 3: BK2.5 100 μg/ml
Lane 4: BK2.5 100 μg/ml
Lane 5: BK 10 100 μg/ml
Lane 6: KK10 100 μg/ml
Lane 7: Negative control
Lane 8: Camptothecin 4.5 M
Lane 9: BK2.5 100 μg/ml
Lane 10: MR2.5 100 μg/ml
Lane 11: BK10 100 μg/ml
Lane 12: KK10 100 μg/ml
Lane 13: Molecular weight marker

Fig.3. DNA fragmentation induced by PM extracts from May samples in alveolar macrophages (MH-S) and human lung cells (A549). After the cells were cultured for 18 h without or with various concentrations of PM extracts, the fragmented DNA was isolated, electrophoresed on 1.5% agarose gel, and then visualized by ethidium bromide staining.

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