Original Article

Effects of PM2.5 on zebrafish embryonic development

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ABSTRACT

Background and Objectives: Particulate matter 2.5 (PM2.5) has been recognized as an important factor which may cause human respiratory tract related cancer and affect the growth of embryos. In this experiment, the toxicity of PM2.5 on zebrafish's embryos was explored. **Methods:** Zebrafish animal models were used in the experiment. The zebrafish embryos were exposed to PM2.5 solution of different concentrations, and the aggregation rate, the hatching rate and the malformation rate as the changes in the concentration of PM2.5 were observed. **Result:** The results showed that when the concentration of PM2.5 was 0-250 μg/L, it had no significant effect ($P > 0.05$) on the embryo aggregation rate, the hatching rate or the malformation rate. When the concentration of PM2.5 was 500-2500 μg/L, it had a significant effect ($P < 0.05$) on the embryo aggregation rate, the hatching rate and the malformation rate, and PM2.5 concentration was positively correlated with the embryo aggregation rate, but negatively correlated with the embryo hatching rate. **Conclusion:** The experiment shows PM2.5 has clear toxic effects on zebrafish embryos, which may provide a basis for further research on PM2.5 pathogenesis.

Key words: PM2.5, model, animal, agglutination rate, teratogenic rate

INTRODUCTION

Particulate matter 2.5 (PM2.5), also known as granules, is fine particles, which has an aerodynamic diameter of less than or equal to ambient air of 2.5 micron particles. As the industry is highly developed, environmental pollution is increasingly serious, and PM2.5 is becoming the number one killer of human health. Long-term exposure to high concentrations of PM2.5 may cause cardiovascular diseases, respiratory diseases and lung cancer; PM2.5 can enter the bloodstream and go through the placental barrier into the fetus, causing delayed fetal cardiovascular growth, teratogenic malformation and even stillbirth or abortion.[1,2]

Zebrafish (*Danio rerio*) are a kind of small tropical fish, with large output, which are easy to feed. They have become one of the standard animal models for ecotoxicity test. The survival rate and teratogenic rate of Zebrafish embryos can sensitively reflect teratogenic toxic pollutants.[3,4] To investigate the impact of PM2.5 on embryonic growth, zebrafish's fertilized embryos were directly exposed to PM2.5 fine particulate matter solution of different concentrations to observe embryonic growth.[5]

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MATERIALS AND METHODS

PM2.5 collection and standardization process

Collection of particulate matter 2.5

PM2.5 on the cutter was taken from the membrane [Figure 1] provided by Environmental Station in Wuxi City, and six concentrations of PM2.5 were prepared, 526$\mu g/m^3$, 315$\mu g/m^3$, 286$\mu g/m^3$, 256$\mu g/m^3$, 213$\mu g/m^3$ and 438$\mu g/m^3$, as shown in Table 1.PM2.5 adsorbed on the membrane was made into fine sea salt and dissolved in the solution of 60mg/L, with the volume of 1L. Then 1L 2500μg/L of liquor was made. A blank filter was employed, and 200mL blank control solution was made following the steps above.

PM2.5 working solution preparation

Zebrafish embryos were exposed to PM2.5 working solution of different concentrations, as shown in Table 2.

Experiment organisms

The zebrafish were a gift from Medicine College of Nankai University, with regular feeding of adult zebra fish. Before the test, the zebra fish were domesticated in the test system for more than 4 weeks. Healthy and sexually mature zebra fish were chosen 2 days before exposure, with the male and female ratio of 1: 2. Zebrafish were put into a special breeding tank with the water temperature of 25 ± 3°C, and illumination time/dark time of 14/10, followed by natural mating and spawning. The fertilized eggs were collected and embryos were washed quickly with embryonic nutrient solution under a unified configuration temperature of 25 ± 3°C. The normal split fertilized eggs were selected for experiments under a microscope.[6,7]

Experiment instruments and reagents

An Olympus microscope and digital camera, a 24-well cell culture plate, NaCl, and the culture medium were purchased from Sigma-Aldrich, with the purity ω≥99.5%; configuration zebrafish breeding fine sea salt solution was purchased from the flower market.[6,8]

Experiment methods

When testing, the fertilized eggs were put in a 24-well culture plate, and 10 parallel samples were set for each working concentration, with one normal control (using the normal embryo culture medium) and two 24-well culture plates; 8 working concentrations and one blank were arranged in parallel. The distribution of plates is shown in Figure 2 and Figure 3. The fertilized eggs were exposed to PM2.5 according to a predetermined program of working solution of different concentrations. The embryonic growth was observed by naked eyes under a microscope.[6]

RESULTS AND ANALYSIS

The aggregation rate of Zebrafish embryos

The fertilized egg agglutination rate of Zebrafish embryos was observed from No. 1 to No. 9 PM2.5 working solution as the culture time. Figure 4 shows the agglutination [Figure 4a] and normal growth [Figure 4b] of the embryo.

According to the PM2.5 concentration, three groups were set up, low concentration group, moderate concentration group and high concentration group, with the PM2.5 concentration of 0-30μg/L, 62.5-250μg/L, and 500-2500μg/L, respectively, as shown in Tables 3 and 4.

Table 1: PM2.5 cutter membrane concentration and number

No.	Concentrations (μg/m³)	quality (μg)
Blank	0	0
12	438	438
18	315	315
34	286	286
75	256	256
76	526	526
78	213	213

Table 2: PM2.5 working solution preparation

No.	Concentrations (μg/L)	Volume (mL)
1	2500	200
2	1250	200
3	500	200
4	250	200
5	125	200
6	62.5	200
7	30	200
8	15	200

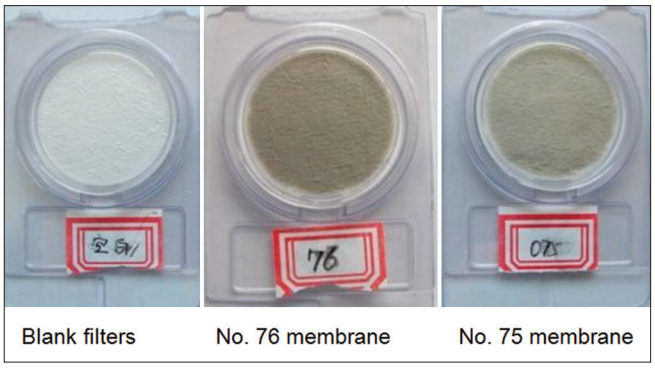

Figure 1: White filter membrane and sample collection

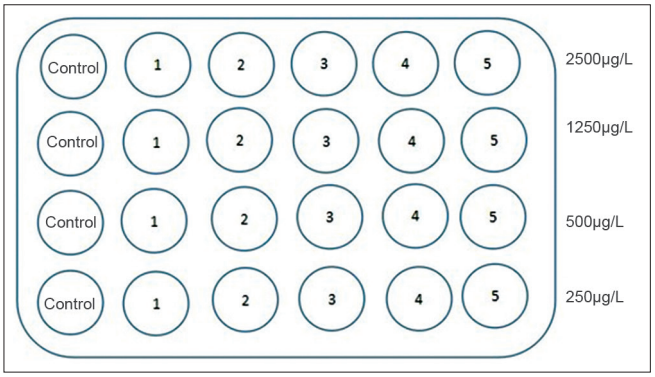

Figure 2: A schematic view of the distribution of plate 1

It was found that among low concentration group, moderate concentration group and high concentration group, p value <0.05 revealed by one-way analysis of variance (ANOVA), indicating that low, moderate and high concentrations of PM2.5 had a significant effect on the aggregated rate of zebrafish embryos.

As shown by Tables 3, it was found that the embryo aggregation was not observed in low concentration group and moderate concentration group, indicating that PM2.5 had no effect on the embryo aggregation rate when PM2.5 concentration was 0-250μg/L. For the high concentration group, p value was 0.052 for the embryo aggregation rate, which was close to the statistical significance. When PM2.5 was 500-2500μg/L, the PM2.5 concentration had a certain effect on the embryo aggregation rate. Along with Figure 5, it was considered that within that range of PM2.5 concentration, there was a positive correlation with the aggregation rate.

72h hatching test of zebrafish embryos

The number of embryos hatch survivedin the cell culture plate wells after 72h was observed and statistically analyzed, with the results shown in Table 5 and Figure 6 below.[9]

PM2.5 reagents were divided into three groups: low concentration group, moderate concentration group and high concentration group, with the concentration range of 0-30μg/L, 62.5-250μg/L, and 500-2500μg/L, respectively. ANOVA was used to analyze the difference among different groups[8], with the results shown in Table 6.

ANOVA indicated p<0.05 when low concentration group, moderate concentration group and high concentration group

Table 3: Embryo aggregation of different periods

No.	Concentrations (μg/L)	2 h	4 h	8 h	16 h	24 h	36 h	Comparison
1	2500	0/10	0/10	0/10	3/10	4/10	6/10	0/10
2	1250	0/10	0/10	0/10	1/10	2/10	4/10	0/10
3	500	0/10	0/10	0/10	0/10	0/10	2/10	0/10
4	250	0/10	0/10	0/10	0/10	0/10	0/10	0/10
5	125	0/10	0/10	0/10	0/10	0/10	0/10	0/10
6	62.5	0/10	0/10	0/10	0/10	0/10	0/10	0/10
7	30	0/10	0/10	0/10	0/10	0/10	0/10	0/10
8	15	0/10	0/10	0/10	0/10	0/10	0/10	0/10
9	0	0/10	0/10	0/10	0/10	0/10	0/10	0/10

Table 4: Analysis on the difference in embryos aggregation rates among 3 different PM2.5 concentration groups

Time (h)	Low concentration group	Moderate concentration group	High concentration group
16	0	0	4
24	0	0	6
36	0	0	12

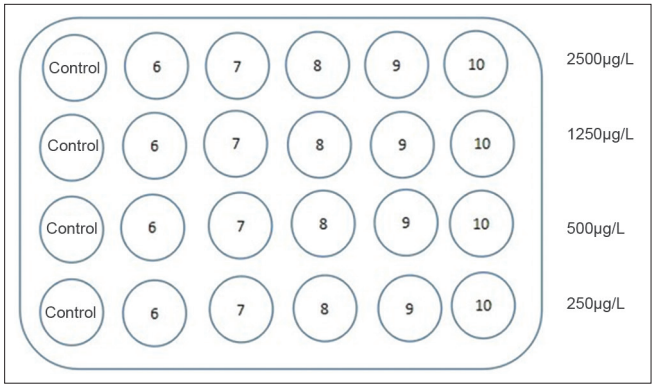

Figure 3: A schematic view of the distribution of plate 2

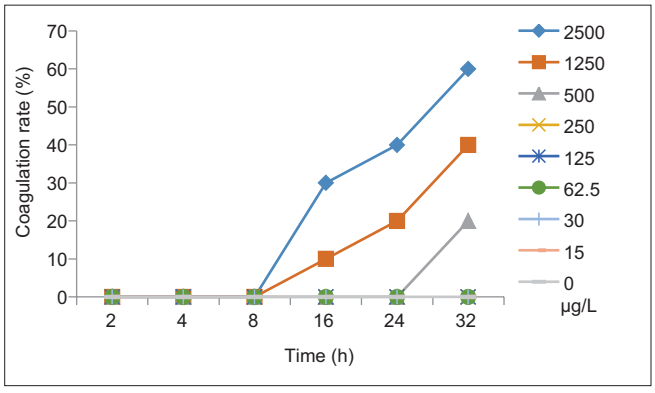

Figure 5: Embryonic agglutination rates and different PM2.5 concentrations

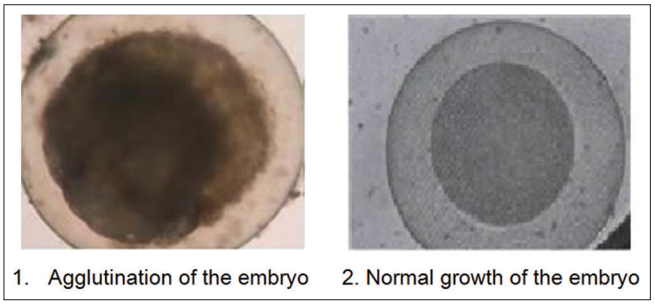

Figure 4: Normal embryos and comparison chart of aggregation

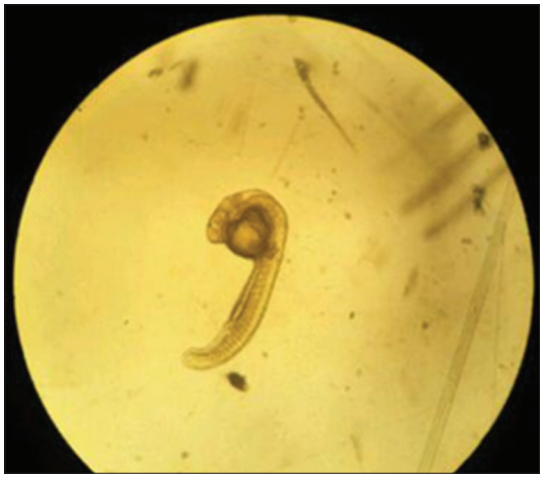

Figure 6: Well-developed juveniles

were compared, indicating remarkable influence of PM2.5 on the zebrafish embryo hatchability.

ANOVA was used to analyze the influence of PM2.5 on zebrafish embryo hatchability in different groups. The p value was >0.05 for low concentration group and moderate concentration group, indicating that when the concentration of PM2.5 was 0 to 250μg/L, the PM2.5 concentration increase had no effect on zebrafish embryo hatchability. For the high concentration group, the p value was <0.05, demonstrating that when the PM2.5 concentration was 500 to 2500μg/L, the PM2.5 concentration had obvious influence on zebrafish embryo hatchability. Along with Figure 7, it was considered that in this range there was negative correlation between PM2.5 concentrations and aggregation rates.

The Zebrafish embryo deformity rate within 72h

The quantity of embryo deformity in the cell culture plate was observed for 72h, and the result is shown in Figures 7 and 8. Figure 9 indicated that when the PM2.5 concentration was 500μg/L, the deformity rate was increased as the concentration rise [Table 7].

Table 5: 72h embryo hatching rates

No.	Concentrations (μg/L)	24 h	36 h	72 h	Comparison
1	2500	0/10	4/10	3/10	9/10
2	1250	0/10	6/10	5/10	9/10
3	500	0/10	8/10	7/10	10/10
4	250	0/10	9/10	9/10	10/10
5	125	0/10	10/10	9/10	10/10
6	62.5	0/10	10/10	10/10	9/10
7	30	0/10	10/10	9/10	10/10
8	15	0/10	9/10	9/10	9/10
9	0	0/10	10/10	10/10	10/10

Table 6: Difference between different concentration groups

Time (h)	Low concentration group	Moderate concentration group	High concentration group
24h	0	0	0
36h	29	29	18
72h	28	28	15

Table 7: The embryo deformity rate within 72h

No.	Concentrations (μg/L)	72 h	Comparison
1	2500	2/10	0/10
2	1250	2/10	0/10
3	500	1/10	0/10
4	250	0/10	0/10
5	125	0/10	0/10
6	62.5	0/10	0/10
7	30	0/10	0/10
8	15	0/10	0/10
9	0	0/10	0/10

DISCUSSION

PM2.5 is the top problem in environment pollution, which negatively influences human life and health. The

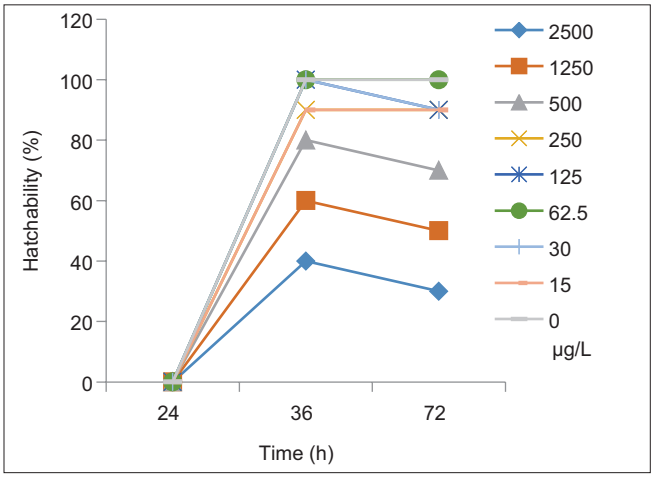

Figure 7: Correlation between hatchability and PM2.5 concentrations

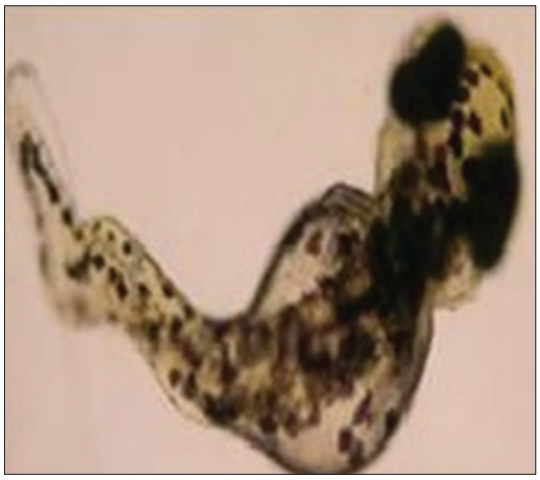

Figure 8: Embryo deformity

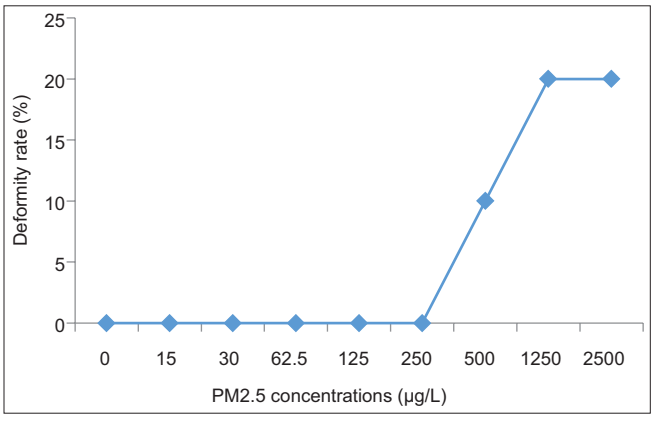

Figure 9: Correlation between deformity rates and PM2.5 concentrations after 72 culture

pathogenic mechanism is a hotspot. In this study, we observed and evaluated the toxic effects of PM2.5 of different concentrations on embryonic development of animal model zebrafish, which may be significant for further investigation on PM2.5 pathogenic mechanism.[6,7,8]

The research showed that PM2.5 had heavy toxic effects on embryonic development, and embryo aggregation of zebrafish as well as teratogenic effects in a dose-dependent manner. The minimum toxic concentration of PM2.5was500μg/L. When the toxic concentration of PM2.5 was reached, the embryo aggregation rate was increased obviously; hatchability showed dramatic decline with PM2.5 concentration rise within 72h; fetal abnormality was caused at 72h, and deformity phenotype was tail curved, with curvature of the spine. The above indices were positively correlated with PM2.5 concentration. The result also showed that when the PM2.5 concentration was 0-250μg/L, the concentration change had no remarkable effect on the embryo aggregation rate, hatchability, and the aberration rate ($P<0.5$). When the PM2.5 concentration was 500-2500μg/L, the concentration change had remarkable effects on the embryo aggregation rate, hatchability, and the aberration rate ($P<0.05$), The PM2.5 concentration was positively correlated with the embryo aggregation rate, while negatively correlated with hatchability.[1,6]

Hence, PM2.5 had obvious toxic effects on zebrafish embryonic development, revealed by zebrafish models. The harm of PM2.5 can be observed directly and quickly, providing basis to the research on PM2.5 pathogenesis.

CONCLUSION

The experiment shows that PM2.5 has definite toxic effects on Zebrafish embryogenesis and may lead to various abnormalities. It may have significant effects on the embryo aggregation rate, the hatching rate and the aberration rate once reaching a certain concentration. Our results provide a basic research for studying the pathogenic mechanism of PM2.5.

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