Comparison of the Effects of Nanometer Titanium Dioxide with Two Crystal Forms on Rabbits Blood Routine Index and Organ Coefficient in the Instillation of Non-exposure Bronchus Toxic Contamination

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Abstract:
Purpose: to compare the effect of nanometer rutile TiO_2 and nanometer anatase TiO_2 with same dose and size on rabbit's important organ coefficient and blood routine.

Method: divided 30 rabbits randomly into 5 groups, namely the saline control group, micron rutile TiO_2, micron anatase TiO_2, nanometer rutile TiO_2, nanometer anatase TiO_2. Dosed each rabbit with 2.5 $ml \cdot kg^{-1} \cdot bw^{-1}$ with one-time non-exposed pipe drip dye, and put them to death after 20 days. Determined the size, shape, main viscera quality, organic coefficient and blood routine (19 indicators) of nanometer TiO_2 with different crystals.

Results: rabbit lung viscera coefficient in the nanometer rutile TiO_2 exposed group is obviously higher than that of micron anatase TiO_2 ($p < 0.05$).

Rabbit blood routine: nanometer rutile TiO_2 has the highest mean corpuscular-hemoglobin concentration, while the other three groups have significant differences in the mean corpuscular-hemoglobin concentration, $p < 0.05$. For the white blood cell count (WBC), micron anatase TiO_2 group has the highest, while compared with the micron anatase TiO_2 group, the normal saline control group, micron rutile TiO_2 group and nanometer anatase TiO_2 group all have significant differences, $p < 0.05$.

Conclusion: nanometer rutile TiO_2 will increase rabbit lung viscera coefficient and mean corpuscular hemoglobin concentration. Toxic effect of nanometer rutile TiO_2 and nanometer anatase TiO_2 on rabbit are different.

Keywords: *Nanometer rutile TiO_2, Nanometer anatase TiO_2, Blood routine, Toxicology, Organ coefficient.*

Introduction

In the recent years, represented by nanometer, nanometer titanium dioxide (TiO_2) has its extensive applications in many fields such as chemical engineering, health care, environmental protection, cosmetics, semiconductor, photoelectric sensor and ultraviolet absorbent [1, 4, 7, 10, 12, 16], and has become a new kind of inorganic functional materials.

According to its crystal shape, TiO_2 can be divided into three kinds – brookite, anatase and rutile; According to the surface properties, it can be divided into hydrophilic type, lipophilic

type and without surface treatment nanometer TiO_2. These nanometer materials feature a high specific surface area, surface atom number, surface energy and surface tension. As the particle size decreases sharply, the small size effect, surface effect, quantum size effect and macroscopic quantum tunneling effect will cause the thermal, magnetic, light, sensitive properties as well as surface stability of the nano-particle to be different from the conventional particles [2, 5, 8, 11, 13, 15]. The interaction between nano-particles and life is unknown territory. On one hand, there are many new phenomena and new issues, new laws. On the other hand, life itself is a series of complex and orderly biochemical or physical processes happening on a nanoscale [6].

Since nanometer TiO_2 has been used in this study, its different crystal forms, particle sizes and surface properties can largely influence the experimental results [3]. In the process of animal experiments, different species of animals have different reactions to drugs or poison; nanometer titanium dioxide toxicity on different kinds of animals will be different; compared with micrometer particles of the same chemicals, nanometer particles' pro-inflammatory and tumorigenicity may be bigger. Therefore, currently the toxicology research of nanometer titanium dioxide has no clear conclusion. However, many studies have shown that nanometer titanium dioxide has certain security risks, so we need to enhance precaution awareness of nanometer titanium dioxide toxicity [14].

Animal blood is closely related to the body's immune function. Some of the routine biochemical indicators in blood are the important parameters for the body health and disease resistance [9]. Mice, guinea pigs, rats and rabbits are the most commonly used experimental animals in scientific research, but different species of animals response to a drug or toxicant differently. However, toxicity data of effect of nanometer titanium dioxide with different crystal forms on rabbit is still blank. Hence, this research selected micro rutile TiO_2 (wj-TiO_2), micro anatase TiO_2 (wr-TiO_2), nanometer TiO_2 (nj-TiO_2), rutile TiO_2 (nr-TiO_2) to contaminate rabbit organ in a one-time non-exposed manner, and then measured rabbit weight, viscera coefficient and 19 blood routines. We also studied and compared the damage mechanism of nanometer titanium dioxide with different crystal forms on rabbit body.

Materials and methods

Material source and powder suspension preparation

The finely-grinded surface-untreated wr-TiO_2 was 45 microns. Surface-untreated rutile type titanium dioxide of 45 microns was purchased from Anhui Kona New Materials Co., Ltd. with primary particle size ≤ 30 nm, specific surface area ≥ 80 $m^2 \cdot g^{-1}$ and nanometer TiO_2 mass fraction ≥ 99.9%. The surface-untreated rutile type nanometer TiO_2 was bought from Hangzhou Wanjing New Material Co., Ltd. with primary particle size ≤ 30 nm, specific surface area ≥ 80 $m^2 \cdot g^{-1}$ and nanometer TiO_2 mass fraction ≥ 99.9%. After high pressure sterilization we prepared all kinds of TiO_2 with normal saline respectively to the concentration of 20 mg/ml of suspension, and then sterilized the suspension under high temperature.

Experimental animals

30 rabbits were provided by Xinjiang Medical University Experimental Animal Center, with the weight of 1.3-2.5 kg. After weighing weight, these rabbits were divided in a random number method into 5 groups, namely normal saline control group (sldz), wj-TiO_2 group, wr-TiO_2 group, nj-TiO_2 group, and nr-TiO_2 group, each group with 6 rabbits. The environmental temperature was maintained at 10°C, and illumination time – from 9:00 to 16:00. All rabbits were fed freely on pellet feed.

Contamination and blood sample collection

It took 4 kinds of TiO_2 powders to prepare the suspension of 20 mg/ml with normal saline. All rabbits were injected with suspension of 2.5 $ml \cdot kg^{-1} \cdot bw^{-1}$, one time through the respiratory tract, while the control group were injected with normal saline of 2.5 $ml \cdot kg^{-1} \cdot bw^{-1}$. All groups would be fed in the same conditions for 20 days.

Blood routine

At the end of 20 days of observation period, the rabbits were kept fasting for 12 h. and forbidden to drink water for 1h before the experiment, then they were given general anesthesia with urethane. The blood was drawn from the jugular vein into a heparin sodium anticoagulant pipe for inspection. We inspected the following features using Mindray BC-2300 automated hematology analyzer:

1. Leukocyte including white cell count (WBC), lymphocytes number (Lymph#), intermediate cells number (Mid#), neutrophilic-granulocyte number (Gran#), lymphocytes percentage (Lymph%), neutrophilic-granulocyte percentage (Gran%);
2. Red blood cell including hemoglobin (HGB), red blood cell number (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width coefficient of variation (RDW-CV), red blood cell distribution width standard deviation (RDW-SD);
3. Platelet including platelet number (PLT), platelet distribution width (PDW), platelet hematocrit (PCT).

Organ coefficient calculation

Animal organs' weight, shape, color are all important indicators of animal health. We can generally determine the nature and extent of visceral organ lesions according to the organ weight, organ coefficients. Therefore, organ weight and organ coefficient of experimental animal are the fundamental data commonly used in experiments. We executed the rabbits with gas embolism, then dissected the rabbits for heart, liver, lung and kidney organ, and gently dried them with filter paper, weighed them with electronic balance to calculate the organ coefficients according to the following formula:

Organ coefficient (%) = (organ green weight (g) /Weight (g))*100%

Statistical analysis

The experimental data were expressed as $\overline{x} \pm s$. We adopted SPSS21.0 software for the statistical analysis, homogeneity test of variance and one-way analysis of variance (One way ANOVA) for comparison between multi-groups. When conducting comparisons between any two groups, if the variance is equal, adopt SNK method for inspection; if not, adopt Games-Howell for inspection. Difference with $p < 0.05$ has its statistical significance.

Result and analysis

Rabbit weight change 20 days after the contamination

It can be seen from Fig. 1, before and after the contamination, wj-TiO_2 group, wr-TiO_2 group, sldz group and nj-TiO_2 group have no obvious change in weight. Compared with sldz group and wj-TiO_2 group, nj-TiO_2 group has no difference, which has no statistical significance ($p > 0.05$). 20 days after the contamination, nr-TiO_2 group's weight loss was greater than that of other groups; yet compared with sldz group and wr-TiO_2 group, it has no statistical significance ($p > 0.05$).

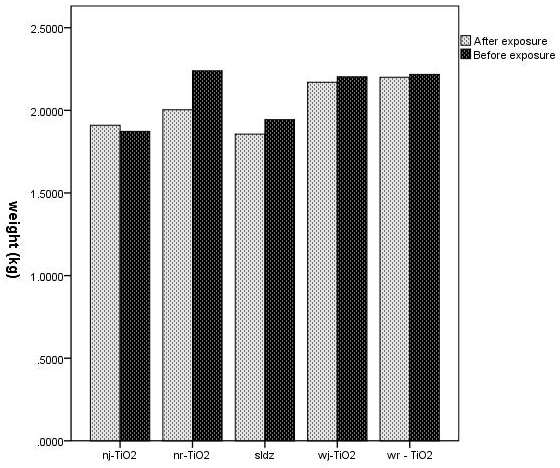

Fig. 1 Rabbit weight change before and 20 days after the contamination

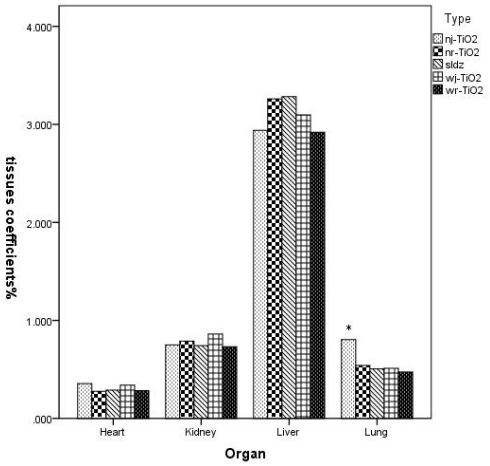

Fig. 2 Effect of TiO_2 with two crystal forms on rabbits' organ coefficient

Infected rabbits' main organ coefficient change

It can be seen from Fig. 2 that 20 days after the contamination, compared with the normal saline control group and other nanometer and micrometer groups with different crystallines, nj-TiO_2 group and nr-TiO_2 group had no significant difference in the rabbits heart, liver and kidney organ coefficient, from statistical point of view ($p > 0.05$). Sign * signifies the comparison between nanometer rutile TiO_2 group and micrometer anatase TiO_2 without significant difference ($p > 0.05$). However, nj-TiO_2 lung viscera coefficient is significantly higher than that of other groups, and is significantly different from that of wr-TiO_2 ($p > 0.05$).

Blood routine changes of nanometer TiO_2 infected rabbits

As can be seen from Table 1, 21 days after the one-time contamination, in the blood routine:

1) Rabbits in the nj-TiO_2 group has the highest mean corpuscular-hemoglobin concentration, and has significant difference than the concentration of nr-TiO_2 group and wr-TiO_2 group ($p < 0.05$);
2) wr-TiO_2 group has the highest white blood count (WBC), and has significant difference compared with sldz group, nr-TiO_2 group and wj-TiO_2 group ($p > 0.05$);
3) wr-TiO_2 group has the highest neutrophilic-granulocyte number (Gran#), and has significant difference compared with sldz group, wj-TiO_2 group and nr-TiO_2 group ($p > 0.05$);
4) In the hemoglobin concentration (MCHC), nj-TiO2 group has significant difference than nr-TiO_2 group and wr-TiO_2 group ($p > 0.05$);
5) Compare the rabbit nj-TiO_2 group, nr-TiO_2 group, wr-TiO_2 group, and wj-TiO_2 group with normal saline control group, and between groups, and there has no significant difference in lymphocytes percentage (Lymph%), intermediate cells percentage (Mid%), neutrophilic-granulocyte percentage (Gran%), lymphocytes number (Lymph#), intermediate cells number (Mid#), red blood cell including hemoglobin (HGB), red blood cell number (RBC), hematocrit (HCT%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell distribution width coefficient of variation (RDW-CV%), red blood cell distribution width standard deviation (RDW-SD), platelet including platelet number (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet hematocrit (PCT) ($p > 0.05$).

Table 1. Blood routine test results and comparison ($\bar{x} \pm s$)

	num	duei	2nj	3nr	4wj	5wr
WBC/109/L	5	11.72 ± 4.086	14.70 ± 6.011	12.23 ± 5.425	11.10 ± 1.778	19.78 ± 6.771❶,❸,❹
Lymph%	5	48.9333 ± 6.96946	38.0000 ± 2.51197	40.6750 ± 4.47018	47.3000 ± 0.70711	37.8750 ± 14.52730
Mid%	5	6.5727 ± 2.30940	9.100 ± 2.19317	12.0500 ± 3.29292	10.8000 ± 1.41421	12.2000 ± 2.35372
Gran%	5	30.1060 ± 8.90169	52.9000 ± 4.23202	47.2750 ± 4.26878	41.9000 ± 2.12132	49.9250 ± 12.88109
HGB/L	5	149.80 ± 38.127	139.33 ± 9.292	155.25 ± 26.850	129.50 ± 9.849	151.00 ± 16.553
RBC 1012/L	5	6.6180 ± 1.42363	6.3067 ± 0.52624	7.0950 ± 0.75496	0 5.8875 ± 0.55752	7.0250 ± 0.87417
HCT%	5	44.42000 ± 11.222611	40.33333 ± 3.139002	46.57500 ± 6.695956	38.37500 ± 3.010399	45.45000 ± 4.351628
MCV/L	5	66.780 ± 3.0744	63.733 ± 1.1719	65.550 ± 3.6701	65.350 ± 2.3868	64.975 ± 3.2745
MCH/L	5	22.440 ± 0.9839	21.967 ± 0.6506	21.775 ± 1.8080	21.975 ± 1.0874	21.475 ± 0.6602
MCHC	5	336.80 ± 7.190	345.00 ± 4.359❷,❸	332.00 ± 11.225	337.00 ± 4.082	331.25 ± 7.274
RDW-CV%	5	13.5800 ± 2.0315	13.2000 ± 0.7211	13.4500 ± 0.8544	13.4500 ± 0.9469	13.4750 ± 0.8180
RDW-SD	5	26.340 ± 4.2677	24.567 ± 0.4041	26.175 ± 1.4221	25.250 ± 1.7786	25.075 ± 1.6581
PLT109	5	353.00 ± 148.465	359.67 ± 160.039	433.75 ± 128.857	421.75 ± 92.554	337.25 ± 79.223
MPV	5	9.120 ± 0.5848	8.867 ± 0.3512	8.900 ± 0.3651	8.725 ± 0.2062	8.900 ± 0.4967
PDW	5	14.520 ± 0.4324	14.400 ± 0.1732	14.475 ± 0.1708	14.375 ± 0.2062	14.350 ± 0.3109
PCT%	5	0.00316 ± 0.001205	0.00318 ± 0.001430	0.00386 ± 0.001192	0.00367 ± 0.000750	0.00298 ± 0.000623
Lymph#109	5	6.7000 ± 2.5515	5.6333 ± 2.4826	4.9000 ± 1.83485	5.1500 ± 1.3435	8.2000 ± 5.4326
Mid#109	5	1.3667 ± 0.47258	1.4333 ± 0.9238	1.5750 ± 1.1843	1.1500 ± 0.2121	2.3000 ± 0.5228
Gran#109	5	5.4667 ± 2.0133	7.6333 ± 2.6502	5.7500 ± 2.57358	4.6000 ± 1.41421	9.275 ± 1.21209❶,❸,❹

❶ means the comparison with sldz group; ❷ represents the comparison with wr-TiO_2 group, $p < 0.05$; ❸ signifies the comparison with nr-TiO_2 group, $p < 0.05$; ❹ denotes the comparison with wj-TiO_2 group, $p < 0.05$.

Conclusions

Organ weight and organ coefficient (organ weight ratio) are important indicators for the assessment of the toxic action of test substance in a toxicology research, which is of referential importance to confirm the target organ of the nanometer material's toxic effect. The decrease in the organ coefficient means atrophy and degeneration of organs; the rise in the organ coefficient means possible congestion, edema and hypertrophy of organs. The organ

weight can comprehensively reflect the poisoning effect of animal bodies. No significant difference ($p > 0.05$) was seen in the rabbits' body weight among different group before and after the contamination. Compared with the control group, the contaminated groups' heart, liver and kidney organ coefficients have no significant difference ($p > 0.05$). The lung organ coefficient in the nanometer rutile titanium dioxide contaminated group is significantly higher ($p < 0.05$) than that of other groups, indicating that the lung hypertrophy caused by the non-exposure intra-trachea instilled contamination with 2.5 $ml \cdot kg^{-1} \cdot bw^{-1}$ in the nj-TiO_2 group is much more serious than that in the nr-TiO_2 group, wj-TiO_2 group and wr-TiO_2 group.

Animal blood routine index is divided into 3 broad categories, namely white blood cells count and their classification, red blood cells and platelets. The primary change in the organism is the increase or decrease of white blood cell. Neutrophil granulocyte and monocytes have the function of phagocytosis; red blood cells are the most important innate immunocyte in the blood circulation, with the function of antigen recognition, adhesion, concentration, antigen, removal of circulating immune complexes. It participates in the regulation of immunity of organism, and has complete self-regulatory system. Hemoglobin is a king of protein existing in the red blood cell which is responsible for carrying oxygen in the blood.

The experimental results showed that:

1) nj-TiO_2 group with the non-exposure intra-trachea instilled contaminated with 2.5 $ml \cdot kg^{-1} \cdot bw^{-1}$ has far higher mean corpuscular hemoglobin concentration than that of nr-TiO_2 group, wr-TiO_2 group; whether nj-TiO_2 group has the ability to increase the oxygen content of rabbit and supply the organs with oxygen still needs further studies;
2) White blood cells count and neutrophil granulocyte number in the wr-TiO_2 group are far higher than that in the wr-TiO_2 group, nr-TiO_2 group and the control group; yet there is no big difference between the white blood cells count and neutrophil granulocyte of nj-TiO_2 group and nr-TiO_2 group ($p > 0.05$) indicating that nj-TiO_2 group and nr-TiO_2 group have no obvious effect on the white blood cells count and neutrophil granulocyte;
3) The platelet count in the nj-TiO_2 group and nr-TiO_2 group have little difference than the control group suggesting that nr-TiO_2 and nj-TiO_2 do not affect the blood coagulation in the rabbits;
4) The lung organ coefficient in the nj-TiO_2 group is much higher than that in the nr-TiO_2 group. Further experimental studies are needed for the root of the problem.

To sum up, when TiO_2 is made into nanometer particles with different crystalline, its physical and chemical properties may be changed, leading to changes in its metabolism and toxicity mechanism. The toxicity of nanometer materials may be subject to the size, shape, crystal form, surface properties, electrical property, and therefore further studies and discussion are needed for its mechanism.

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