Acute Toxicity and Genotoxicity of Perchlorate Based on Flow Cytometry

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Abstract: Perchlorate is a highly diffusive persistent toxic pollutant, which is widely used in many fields such as missile fuel, fireworks, paint and so on. Perchlorate can induce histopathological changes in the thyroid gland, and at the same time lead to changes in thyroid hormone levels, thereby affecting metabolic activity. Flow cytometry (FCM) is a technique that uses laser beams to stimulate single-line flow of cells to detect their scattered light and fluorescence, thus completing cell analysis and separation. The toxicity of perchlorate was studied in depth and its treatment was studied. The detection technology was comprehensively summarized, and the principle of flow cell usage was described in detail. It is very likely to have toxicity by analyzing the perchlorate concentration. In this study, the acute toxicity and genotoxicity of perchlorate were investigated by the organ coefficient test of each group after small perchlorate exposure. Provide reference for future use of perchlorate, pollution prevention and treatment.

1. Introduction

Perchlorate is a salt formed by perchloric acid and contains tetrahedral perchlorate ion ClO-4, in which the oxidation value of chlorine is + 7. At present, perchlorate has spread to many areas of nature along with air, groundwater, fireworks, aerospace and other ways [1]. Flow cytometry is to suspend the sample cells in liquid and measure them rapidly through the measurement area one by one during the flow process. It is characterized by simultaneous determination of multiple parameters of each cell. It is easy to migrate in the environment, and can pollute groundwater, surface water and drinking water sources; it can also be absorbed and enriched by plants through soil and water, and enter the human body through the food chain, interfering with the synthesis and secretion of thyroxine, thus affecting the normal metabolism of the human body, hindering the normal growth and development of the human body [2]. In addition, the storage life of ammonium perchlorate is limited, and the manufacturer will periodically eliminate the original stock, which results in a large number of untreated perchlorates being discharged into the environment at one time. Galvn-Hidalgo et al. poisoned embryos and tadpoles of Xenopus laevis with ammonium perchlorate, sodium perchlorate and ammonium chloride. It was found that the first two substances could delay the metamorphosis of Xenopus laevis, slow down the growth of its hind limbs and cause histological changes of thyroid gland. When the concentration was high, feminization effect could also occur, leading to imbalance of sex ratio [3]. In 2016, Kir M et al. gradually degraded perchlorate to chlorate, chlorite and chloride through an electron donor. However, in the normal groundwater environment, perchlorate does not exist alone. Due to the large use of nitrogen fertilizer and the discharge of a large amount of industrial and agricultural wastewater, nitrate pollution in groundwater is increasing [4]. Zhang WJ et al studied the thyroid toxicity and reproductive toxicity of a mixture of DE-71, DE-79 and BDE-209 (52.1% DE-71, 0.4% DE-79, and 44.2% BDE-209). The results showed that 20 mg/ In the kg/day exposed group, the weight of kidney and liver increased and the expression of CYP1A and CYP2B genes were up-regulated [5].

Perchlorate pollution has the characteristics of wide range, long duration and fast flow. Toxicity research and detection technology development of perchlorate is an important part of reducing pollution [6]. Currently, studies on perchlorate toxicity mainly focus on animal models and cell models. Flow cytometry, due to the application of monoclonal antibody technology, quantitative cytochemistry and quantitative fluorescence cytochemistry, has become more and more widely used and important in many fields such as biology, clinical medicine and so on [7]. Modern flow

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cytometry, which combines monoclonal antibody technology, quantitative cytochemistry technology and quantitative fluorescence cytochemistry, makes its application in many fields such as biology, clinical medicine, pharmacology, materials and so on more rapid development [8]. The main technical indicators of the performance of flow cytometry are fluorescence resolution, fluorescence sensitivity, analysis parameters, sorting speed and purity. Fluorescence resolution refers to the minimum distance between two adjacent peaks, which is usually expressed by coefficient of variation (CV) [9]. The liquid column intersects the laser beam perpendicularly, and the intersection point becomes the measurement area. Cells that pass through the laser region are excited to produce fluorescence. An optical system is placed in a direction perpendicular to the incident beam and the liquid column to collect the fluorescent signal and the scattered light signal. At present, studies on the effects of perchlorate on human health have focused on the effects of thyroid function. However, there are no reports on the acute toxicity and genotoxicity of perchlorate. This study based on the acute toxicity and genotoxicity of perchlorate in flow cytometry [10].

2. Materials and Methods

2.1. Physicochemical Properties of Perchlorate

Perchlorate can affect the level of thyroid hormone, and thyroid hormone plays an important role in maintaining the normal growth and development of the body. Insufficient or excessive secretion of thyroid hormone will affect the growth and development of the body. Flow cytometry consists of four parts: liquid flow system, optical system, electronic system and analysis system. It can only detect signals from suspended single cells or particles. Usually, the cells or particles to be tested are stained by fluorescence and then made into suspension samples. Flow cytometry is a rapid measurement of cells or organelles in suspension. Total nucleic acids and proteins can only be measured in a cell, but not in a specific part of the cell. Flow cytometry is used for cell proliferation and apoptosis analysis in cell cycle; flow cytometry can easily analyze the cell cycle, promoting the development of botany based on this, supplemented by imaging instruments, flow cytometry It can carry out the growth and reproduction of microorganisms. It is highly inaccurate to use the toxic effects of a toxic substance to evaluate its toxic effects in the real environment. The interaction of two or more chemicals in an organism may result in the interaction of poisons.

2.2. Animal experimental study

Perchlorate can change the structure of cells, even act on chromosomes and interfere with gene expression. Animal experiments have shown that perchlorate has toxic effects on the body, not only on thyroid function, but also on nervous, reproductive, immune and other system functions. It was found that perchlorate was toxic to animal cell genetic material, but less toxic to bacterial cell genetic material. Sixty healthy rats, half male and half female, were randomly divided into three dosage groups according to body weight by flow cytometry. The dosage of exposure was set to 4.50, 22.00 and 560.00 mg/kg. The small round and epithelioid cells formed in the Petri dish were labeled under an inverted microscope. The colonies were picked up by mechanical scraping and transferred to 34 plates for purification culture. After passage, the cells were counted. Cyclophosphamide 40.00mg/kg, a known immunosuppressant, was injected intraperitoneally once a day for 5 days with 0.9% sodium chloride injection. Add an equal volume of 0.25% trypsin and 1 ml of DNase, digest for 15 min in a 37 ° C water bath (shake once every 5 minutes), then remove the tissue block with a 1 ml gun head, continue to place in a 37 ° C water bath for 10 min and then remove, with a 200 μl gun head After being blown off, the filter was passed through a 100 μm sieve and centrifuged at 800 × g for 10 min. On the 5th day, relevant indicators were tested. Since the charge and ionic radius of perchlorate are very close to that of iodide, it can competitively inhibit the transport of thyroid by transport, hinder the absorption of iodine by the organism, interfere with the synthesis of T3 and T4 by the thyroid, and cause the disorder of hormone regulation in the organism Normal physiological activity.

After centrifugation, precipitated cells were re-suspended in 50 ml centrifugal tube with 35%

Percoll separation solution of 10 ml. 33% and 70% Percoll separation solution of 10 ml were slowly added to the top and bottom of the centrifugal tube with micro-syringe respectively. After centrifugation for 30 minutes, stratification could be observed. Gradient dilution was performed and 10/mL and 5/mL were inoculated into 96-well plates respectively. Under the inverted microscope, the culture holes of a single colony were selected and labeled. When the cells converged to 70%, the labeled holes of a single colony were separated and subcultured to form an epithelioid monoclonal cell line. Using 2ml syringe to absorb a small amount of calf serum, rinse the marrow cavity, push tablets, and dry naturally. Fixed with methanol for 5 minutes, then dyed with Giemsa for 25 minutes and dried.Under the oil microscope, the polychromatic nucleus of the polychromatic erythrocytes was observed, and at least 1000 polychromatic erythrocytes were counted in each animal, the number of micronuclei was recorded, and the micronucleus rate was calculated. With the development of the cell sorting system, the flow cytometry analysis and sorting speed are significantly accelerated, such as the acquisition speed of 7000 cells / s, sorting speed of 50,000 cells / s, four-way sorting; In the study of acid neurotoxicity, it was found that lower doses of perchlorate can cause irreversible damage to synaptic transmitters during development. This experiment can infer that perchlorate affects the normal effects of neurotransmitters. The proportion of lymphocytes decreased with the increase of the concentration of the drug, the difference was statistically significant $P<0.01$; while the proportion of eosinophils, basophils and monocytes did not change significantly. The organ coefficients of the rats in each group after 5 days of perchlorate exposure are shown in Table 1.

Table 1 Visceral coefficients of rats exposed to perchlorate for 5 days (x±s).

	Thymus Index	Spleen coefficient	Kidney coefficient	Liver coefficient
4.50 mg/kg	0.20±0.21	0.23±0.05	0.76±0.06	2.17±0.38
22.00 mg/kg	0.14±0.03	0.21± 0.02	0.73±0.03	3.23± 0.27
560.00 mg/kg	0.13±0.05	0.23± 0.06	0.69±0.31	3.74±0.39

3. Acute Toxicity and Genotoxicity Analysis

3.1. Flow cytometry

In a short time, the digestive process can be completed quickly and the cell viability can be guaranteed. The monocyte layer containing microglia was successfully isolated by comparing the highest proportion of myelin sheaths in the central nervous system by flow cytometry. The visceral coefficient is a non-functional index. Its change can explain the damage of immune system of animals exposed to toxic chemicals or toxic chemicals. The most commonly used indexes are spleen index and thymus index. LD50 is one of the most commonly used parameters in acute toxicity test, and it is also the basis of micronucleus test and other genotoxicity test grouping. According to the results of this test, sodium perchlorate belongs to low toxic substances. Direct inhibition of thyroglobulin and thyroid peroxidase gene expression affects iodinated thyroxine deiodinase, resulting in decreased thyroid hormone secretion, AQP1, NIS and SLC22A3 protein transport genes.

3.2 Acute Toxicity and Genotoxicity Analysis

The ions to be measured are selected according to the target ionic mass-to-nucleus ratio. Under the impact voltage of the second quadrupole, the ions are fragmented into several fragment ions. The fragment ions impact the mass spectrometry detection plate, causing the change of the electrical signal of the detection plate, thus accurately determining and quantifying the perchlorate radical. Changes in antioxidant enzyme levels and changes in gene expression related to oxidative stress were used as indicators for oxidative stress detection. Apoptosis was detected by acridine orange (AO) staining and flow cytometry, and DNA damage was detected by single cell gel electrophoresis. The results of white blood cell count showed that the number of white blood cells decreased significantly with the increase of the concentration of poisoning, showing a trend of

immunosuppression. The leucocyte count in 4.50 mg/kg group was close to that in 22.00 mg/kg group, which was significantly higher than that in other groups, but with the increase of perchlorate concentration, the leucocyte count showed a downward trend. Hybrids obtained by allogeneic insemination of sperm produced by these females will die in the blastocyst stage or the organogenesis stage, and the sperm produced by the congenital male body can develop into a living combination with the eggs produced by the females in each treatment group. To prove that perchlorate can produce androgen effects. It has a certain damage effect on the process of chromosome and germ cell formation, and may have certain toxic effects, which is consistent with the toxic excitatory effect. Although autologous mature salivary gland cells maintain good functional characteristics in vitro, their proliferation is limited and aging is rapidly occurring. The discovery, isolation and in vitro expansion of salivary gland stem cells may make it an ideal source of seed cells.

The statistics and observation of toxicological endpoint index during tissue dense period can be used to observe the morphological changes without obstruction, so that the toxicological effects of compounds can be judged quickly. At the same time, the oocyte membrane transparency can be easily labeled and traced by cell lineage, and the normal and disturbed cell behavior can be effectively observed by combining with the genetically modified pathway. That is to say, the stimulation (excitation) effect is appropriate in low dose condition, but it is inhibited in high dose condition. Therefore, it can be inferred that the toxicity of perchlorate may have toxic excitation effect. The change of leukocyte classification in perchlorate poisoning group showed that neutrophils were higher than those in negative control group, while lymphocytes were more negative. Ion exchange column mainly uses detection ion and column ion to exchange, and then uses dilute alkali to elute the separation method. Liquid chromatography mainly uses column to selectively adsorb dissolved ions in mobile phase, and then changes the principle of mobile phase elution. The separation and purification methods are basically the same, but the principles are different. The epithelial-like monoclonal cells isolated and cultured in the submandibular gland of the rat have salivary gland stem/progenitor cell characteristics. The number of chromosomes deviates from normal and irregular in shape, and the degree of change increases with the degree of malignancy. The molecular basis of these changes, that is, the change in the level of expression, is positively correlated with the ability of in vitro clone formation. Affecting mitochondrial function can also alter lipoprotein metabolism, increase cholesterol, phospholipids, and triacylglycerol levels in experimental animal lipoproteins, resulting in an increase in the proportion of total cholesterol high-density lipoprotein cholesterol.

4. Conclusion

The acute toxicity and genotoxicity of perchlorate based on flow cytometry were studied. Toxic interactions refer to the toxic effects produced by various chemicals, which are different in quantity and quality from those produced by these chemicals alone. They often show very complex correlations in organisms, affecting the absorption, metabolism, transformation and distribution of organisms to each other. Molecular concentration decreased with the increase of concentration. It can be inferred that perchlorate can produce immunosuppressive effect on rats, and with the increase of the concentration of perchlorate, the inhibitory effect is strengthened. To formulate regulations and standards for the control of perchlorate and strengthen prevention and control research so as to completely eliminate and solve the problems caused by perchlorate and its residues. The specialization of flow cytometry is more obvious. The large-scale scientific research instruments and popularized application instruments have become the two mainstreams. With the development of photoelectric technology, after long-term experiments, the detection process is stable, that is, the high requirements of detection are achieved, and at the same time reduced. The financial burden of re-purchasing the instrument. Chromatographic detection technology has basically met the perchlorate detection requirements. Perchlorate does not have a genetic level of mutagenicity within this test dose range. Therefore, in this study, perchlorate is of low toxicity and has certain genetic damage effects. The specific mechanism is not clear and needs further

investigation.

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