Original Article

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Hematotoxicity of intratracheally instilled arsenic trioxide in rats

DOI: 10.1515/ii-2017-0163

Received November 28, 2017; accepted December 01, 2017; published online April 10, 2018

Abstract: The study aimed to investigate the correlation between concentration of inhaled arsenic trioxide and dynamic changes in hematotoxicity in rats. Wistar rats were randomly divided into four study groups that were treated with saline (control) or arsenic trioxide at a low (0.1 mg/mL), medium (1 mg/mL), or high (10 mg/mL) dose by intratracheal instillation. Blood samples were collected for analysis at 6, 12, 24, 48, and 72 h after exposure. Compared with the control group, intratracheal instillation of arsenic trioxide affected hematopoietic differentiation in rats, leading to blood cell changes that were related to observation time and concentration.

Keywords: arsenic, blood toxicity, dynamic changes, correlation

1 Introduction

Arsenic has been classified as a class 1 carcinogen by the International Agency for Research on Cancer (IARC) [1]. Several studies have investigated the effects of oral arsenic exposure on health [2,3]. In recent years, many cities in China have been exposed to a continuous haze that directly affects human health and travel safety [4,5]. The main pollutant present in this haze is fine particulate matter (PM_{2.5}), and analysis of the composition of PM_{2.5} identified that arsenic is one of the major pollutants [6-8]. Epidemiological studies have also found that various acute and chronic diseases are closely related to long-term exposure to environmental pollutants [1,9-11]. The impact of acute exposure to high air concentrations of arsenic on the human blood system has not been determined. Therefore, establishing an animal model of respiratory exposure to arsenic can provide an experimental tool to explore the influence of this pollutant on the human blood system.

2 Materials and methods

2.1 Reagents and equipments

Arsenic trioxide (ATO; 100 mg/mL; batch number: 14041) was purchased from China Institute of Metrology (Beijing, China). Chloral hydrate (batch number: 20150303) was purchased from Sinopharm Chemical Reagent Co. (Beijing, China), and the MEK7222K automatic blood analyzer (Nihon Kohden, Japan) was provided by the Institute of Disease Control and Prevention of the Chinese People's Liberation Army.

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2.2 Animal study groups

In total, 160 specific pathogen-free (SPF) Wistar rats weighting 100-150 g were purchased from the Experimental Animal Center of the Military Medical Science Academy of the Chinese People's Liberation Army, and the qualification number was 0023373. Using a random number table, the rats were randomly divided into four groups of 40 rats: the normal control group (CON), the low-dose group (LD), the medium-dose group (MD), and the high-dose group (HD). Each group was randomly divided into five subgroups of eight rats each, which were sampled at 6, 12, 24, 48, and 72 h after exposure. The rats were maintained in an SPF animal house at $22 \pm 2^\circ\text{C}$ for 1 week prior to the experiment, with a relative humidity of 50%-60%, a 12 h light/dark cycle, and *ad libitum* access to food and water. The study was approved by the Animal Ethics Committee of the Institute of Disease Control and Prevention of the Chinese People's Liberation Army.

2.3 Exposure and sampling methods

ATO was diluted to 0.1, 1.0, and 10.0 mg/mL with ultrapure water, and the pH was adjusted to 7.4 before use. The rats were anesthetized using 5% chloral hydrate, administered intraperitoneally at a dose of 0.75 mL/kg. Using a daily intratracheal instillation of 0.06 mL for 3 days, the CON group was treated with saline, whereas the LD, MD, and HD groups were treated with 0.1, 1.0, and 10.0 mg/mL ATO solution, respectively. Taking the last exposure time as the experimental starting point, blood (5 mL) was collected via the abdominal aorta into EDTA-K vacuum blood collection tubes at 6, 12, 24, 48, and 72 h and analyzed using a fully automated blood analyzer.

2.4 Statistical analyses

Analysis was conducted using SPSS 18.0. The results were expressed as the mean $\pm$ standard deviation. Groups were compared by one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls (SNK) post-hoc test. Pearson correlation analysis was used to analyze the relationship between blood cell parameters and concentration or time. Differences were considered to be statistically significant when $P < 0.05$.

3 Results

3.1 Dynamic effects of ATO exposure on erythroid parameters in rats

As shown in Figure 1, the only significant change in the red blood cell (RBC) counts was observed in the HD group after 48 h, and no significant group differences in hemoglobin (HGB) levels were observed. However, significant differences were observed in the rat RBC volume distribution width (RDW). This value was significantly reduced ($P < 0.01$, versus CON) in the LD group at 24 h; this effect was also present at 48 h ($P < 0.05$) and had disappeared by 72 h. RDW was also significantly lower in the MD group than in the CON group at 6 h ($P < 0.05$), 24 h ($P < 0.01$), and 48 h ($P < 0.05$), with no significant difference relative to CON at 12 h and 72 h. In the HD group, RDW was significantly decreased at 12 h ($P < 0.01$), and this effect was observed for up to 48 h.

3.2 Dynamic effects of ATO exposure on rat platelet parameters

Figure 2 shows that there were significant reductions in platelet (PLT) counts in the HD group 6 h after exposure, as compared with the CON group ($P < 0.01$); this difference had disappeared by 24 h. In contrast, sustained and significant decreases in PLT counts ($P < 0.01$) were observed in the LD and MD groups from 6 h to 12 h after exposure, and these had returned to the CON level by 24 h.

Fig. 1: Dynamic effects of ATO exposure on rat erythrocyte parameters. **A:** Effect of different concentrations of ATO on RBC count. **B:** Effect of different concentrations of ATO on HGB content. **C:** Effect of different concentrations of ATO on RDW. ATO, arsenic trioxide; RBC, red blood cell; HGB, hemoglobin; RDW, red blood cell distribution width; CON, control group; LD, low-dose group; MD, medium-dose group; HD, high-dose group. * $P < 0.05$, ** $P < 0.01$, as compared with CON.

Fig. 2: Dynamic effects of ATO exposure on rat PLT parameters. **A:** Effect of different concentrations of ATO on PLT count. **B:** Effect of different concentrations of ATO on MPV. ATO, arsenic trioxide; PLT, platelet count; MPV, mean platelet volume; CON, control group; LD, low-dose group; MD, medium-dose group; HD, high-dose group. * $P < 0.05$, ** $P < 0.01$, as compared with CON.

The mean PLT volume (MPV) was significantly smaller both in the HD and MD groups than in the CON group at 24 h, ($P < 0.01$), and the same change appeared at 72 h. In the LD group, a reduced MPV was observed at 12 h ($P < 0.05$) and at 24 h ($P < 0.01$).

3.3 Dynamic effects of ATO exposure on leukocyte parameters in rats

Figure 3 shows that the white blood cell (WBC) counts were significantly reduced in the LD, MD, and HD groups at 6 h after exposure, as compared to the CON group ($P < 0.05$). This difference had disappeared by 24 h in the LD group, by 72 h in the MD group, and by 48 h in the HD group.

Compared with the CON group, the lymphocyte (LYM) counts were significantly lower in the LD and MD groups at 6 h ($P < 0.01$); this difference had disappeared by 24 h in the LD group and by 48 h in the MD group. The LYM count also decreased significantly ($P < 0.05$) in the HD group from 6 h to 48 h, with the most significant change at 24 h ($P < 0.01$).

Fig. 3: Dynamic effects of ATO exposure on rat leukocyte parameters. **A:** Effect of different concentrations of ATO on WBC count. **B:** Effect of different concentrations of ATO on LYM count. **C:** Effect of different concentrations of ATO on MON count. **D:** Effect of different concentrations of ATO on NEUT count. **E:** Effect of different concentrations of ATO on BAS count. **F:** Effect of different concentrations of ATO on EOS count. **G:** Effect of different concentrations of ATO on leukocyte constituent ratio. ATO, arsenic trioxide; WBC, white blood cell; LYM, lymphocyte; MON, monocyte; NEUT, neutrophil; BAS, basophil; EOS, eosinophil; CON, control group; LD, low-dose group; MD, medium-dose group; HD, high-dose group. $*P < 0.05$, $**P < 0.01$, as compared with CON.

The neutrophil (NEUT) counts in the LD, MD, and HD groups had decreased significantly by 6 h after exposure. This change was the most significant in the HD group ($P < 0.01$) and was still apparent at 72 h, whereas the LD and MD groups had returned to the CON level at 72 h.

Monocyte (MON) counts were not altered over time in the LD group, whereas they significantly decreased in the MD group at 48 h ($P < 0.05$) and in the HD group at 6 h ($P < 0.01$), 12 h, and 48 h ($P < 0.05$).

At each time point, low basophil (BAS) and eosinophil (EOS) levels were observed in the study groups. LYMs and MONs, therefore, accounted for a large proportion of the WBCs, with relatively fewer NEUTs, BASS, and EOSs.

3.4 Correlation between hematotoxicity and ATO exposure level

Table 1 shows that the RDW at 12 h and 72 h and the proportion of NEUTs at 72 h were highly correlated with the exposure concentration (Pearson $|r| > 0.7$, $P < 0.05$). The proportion of LYM, PLT count, and MPV showed a moderate correlation with the exposure concentration at 6 h ($0.4 \leq |r| \leq 0.7$, $P < 0.05$). The data presented in Table 2 show that the proportion of NEUTs in the LD group and the PLT count and MPV in the MD group were highly correlated with the observation time after exposure (Pearson $|r| > 0.7$, $P < 0.05$). The RBC count in

Tab. 1: Correlation between blood parameters and exposure concentration

Item	6 h		12 h		24 h		48 h		72 h	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
RBC	−0.048	0.820	−0.340	0.104	−0.108	0.599	0.547	0.006	−0.245	0.227
Hemoglobin	−0.287	0.164	−0.274	0.195	−0.016	0.937	−0.044	0.127	−0.043	0.832
RDW	0.147	0.485	−0.496	0.014	0.092	0.654	−0.320	0.127	0.204	0.318
WBC	−0.220	0.291	−0.308	0.143	−0.315	0.117	0.353	0.090	−0.203	0.320
Lymphocyte%	0.440	0.028	0.387	0.062	0.616	0.001	0.400	0.053	0.185	0.365
Neutrophil%	−0.262	0.206	0.284	0.178	−0.311	0.122	−0.412	0.046	−0.753	0.000
Platelet	0.497	0.012	0.125	0.560	−0.168	0.413	−0.044	0.840	−0.382	0.054
MPV	−0.455	0.022	0.716	0.000	0.303	0.133	0.399	0.054	0.806	0.000

RBC, red blood cell; RDW, RBC distribution width; WBC, white blood cell; MPV, mean platelet volume.

Tab. 2: Correlation between blood parameters and observation time after exposure

Item	CON		LD		MD		HD	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
RBC	−0.103	0.580	0.528	0.020	0.280	0.195	0.330	0.040
Hemoglobin	0.069	0.714	0.185	0.448	0.334	0.119	0.344	0.032
RDW	−0.509	0.003	−0.564	0.012	−0.363	0.089	−0.359	0.025
WBC	−0.176	0.345	0.531	0.019	0.565	0.005	0.427	0.007
Lymphocyte%	0.052	0.781	0.419	0.074	0.470	0.024	−0.299	0.065
Neutrophil%	0.081	0.666	0.823	0.000	0.655	0.001	0.168	0.308
Platelet	−0.300	0.101	0.787	0.000	0.833	0.000	0.265	0.103
MPV	−0.627	0.000	−0.562	0.012	−0.726	0.000	−0.183	0.266

RBC, red blood cell; RDW, RBC distribution width; WBC, white blood cell; MPV, mean platelet volume; CON, control group; LD, low-dose group; MD, medium-dose group; HD, high-dose group.

the LD group and the LYM count and LYM% in the MD group showed a moderate correlation with exposure concentration ($0.4 \leq |r| \leq 0.7$, $P < 0.05$). In the CON group, the RDW and MPV showed a moderate correlation with observation time ($0.4 \leq |r| \leq 0.7$, $P < 0.05$).

4 Discussion

Previous studies have shown that ATO is absorbed into the bloodstream, where it binds with HGB and is rapidly distributed to tissues and organs [12,13]. Routine clinical erythrocyte analyses include seven parameters [14]: RBC count, HGB, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular HGB (MCH), mean corpuscular HGB concentration (MCHC), and RDW. The RBC count and HGB are absolute values that serve as important indicators of anemia, whereas the HCT expresses RBC as a percentage of the whole blood volume, reflecting the ratio of RBC to plasma. The MCV, MCH, and MCHC are calculated from the RBC count, HGB, and HCT. The RDW is the coefficient of variation in RBC volume, reflecting the heterogeneity of this parameter. The present study found no significant changes in the RBC count or HGB following ATO exposure; however, the absolute value decreased. The RDW showed little change among the different groups. The first among the possible reasons for these findings is that the total amount of arsenic entering the body by respiration was limited [15]; second, the differentiation of RBCs in the bone marrow hematopoietic system fully compensated for the effects of the arsenic; third, arsenic induced eryptosis was characterized by cell shrinkage [16]; and lastly, the effects on differentiation of bone marrow erythrocytes had not yet appeared [17]. In contrast to the results reported by Luo *et al.* [18], RDW was significantly decreased in the HD group at 12 h. It is likely that because arsenic and phosphorus are of the same group and have the same valence state, they have a competitive relationship in metabolism and that arsenic thus directly inhibits ATPase activity on the erythrocyte membrane, affecting glucose metabolism, and then affecting the morphology of RBCs [19,20]. Although the decrease in RDW was not considered to be clinically significant, this change still warrants further investigation.

PLTs are small cell fragments that are cleaved from the cytoplasm of mature megakaryocytes. PLTs play important roles in physiological and pathological processes, such as hemostasis, wound healing, inflammatory reactions, thrombosis, and organ transplantation rejection [21]. The present study found a very significant decline in the 6 h PLT counts in all groups exposed to ATO, which gradually recovered over time. The MPV is used to assess bleeding tendency and changes in bone marrow hematopoietic function. We found a significantly lower MPV in the ATO-exposed groups at 24 h after exposure, with subsequent gradual recovery. Combined with the change in PLT count, this indicated that ATO inhibited PLT differentiation within the bone marrow hematopoietic system, resulting in reduced production of PLT, morphological changes, and apoptosis [22]. Although the MPV decreased significantly in the MD and HD groups at 72 h, it was presumed that this was due to an incidental increase in the MPV in the CON group at this time point.

In general, WBCs can be divided into five groups according to their morphology, function, and origin: LYMs, MONs, NEUTs, EOSs, and BASs [23]. The main function of WBCs is the phagocytosis of bacteria and the prevention of disease. When bacteria invade the human body, WBCs can cross capillary walls by deformation, thus surrounding and engulfing pathogens. The present study found significantly reduced numbers of WBCs in rats exposed to ATO after 6 h. We also found that the changes in LYM counts showed basically the same trends as the changes in WBCs. LYMs are produced by lymphoid organs and represent an important component of the immune system, playing important roles in cellular and humoral immunity. We found that LYM and NEUT counts showed different degrees of decline after ATO exposure, resulting in an increased percentage of LYMs and a decreased percentage of NEUTs. The reason for this is unclear but we speculate that human exposure to arsenic induces oxidative damage in LYMs [24]. Additionally, ATO exposure may cause immune inhibition in rats, leading to changes in the LYM and NEUT populations [25]. MONs are an important component of the immune system because they can also destroy invading pathogens by phagocytosis and antibody production. After ATO exposure, MON levels were also reduced, presumably due to association with inflammation [26].

Some of the ATO exposure-associated changes in blood cell parameters were correlated with the concentration of ATO and observation time. For example, the MPV at 12 h was highly correlated with the exposure concentration, and the percentage of NEUTs in the LD group was highly correlated with the time after exposure. This indicated that higher concentrations of ATO had a greater effect on blood cell differentiation, and that these effects declined over time. It is worth noting that the RDW and MPV values in the CON group showed a moderate correlation with the observation time. This may reflect an effect of the exposure method used in this experiment, which was through a soft-sleeve protection needle inserted directly through the mouth into the trachea. The CON group received normal saline, which could also have had a slight impact on the lungs, initiating a stress response and leading to blood parameter changes [27].

This study indicated that even limited respiratory tract ATO exposure changed rat blood parameters within a short period, and that some of these changes lasted for at least 72 h. These hematological changes were related to the concentration of ATO and the observation time. These findings indicate that further study of the ATO toxicity threshold, duration, and mechanism following inhalation exposure is warranted in experimental animals.

Acknowledgments: Funding: This work was supported by grants from the Army Logistics Research Plan of China (AEP14C001), National Natural Science Foundation of China (81472478), and Public Science and Technology Research Funds Projects of Quality Inspection (201510024).

The authors thank all the technicians at the laboratory of the Institute of Disease Control and Prevention of the Chinese People's Liberation Army for their contribution during sample test.

Conflict of interest: The authors state no conflicts of interest.

Authors' contributions: Chao Liu and Qiang Wang designed the study. Mingxing Su, Congsong Sun, Haiying Wang, Chunyu Yuan, Ruixia Guo, and Yajie Liang performed the experiments. Mingxing Su discussed, drafted, and wrote the paper.

Abbreviations

ANOVA	One-way analysis of variance
As	Arsenic
ATO	Arsenic trioxide
BAS	Basophil
CON	Control group
EOS	Eosinophil
HCT	Hematocrit
HD	High-dose group
HGB	Hemoglobin
IARC	International Agency for Research on Cancer
LD	Low-dose group
LYM	Lymphocyte
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MD	Medium-dose group
MON	Monocyte
MPV	Mean platelet volume
NEUT	Neutrophil
PLT	Platelet count

PM _{2.5}	Fine particulate matter
RBC	Red blood cell
RDW	Red blood cell distribution width
SNK	Student–Newman–Keuls
SPF	Specific pathogen-free
WBC	White blood cell

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