Original Article

Effect of n-Butanol on Chromosomal Damage in Mice Bone Marrow Cells

Nahid Mansouri[1], Farhang Haddad[1], Masoud Fereidoni[1], Bita Pourkaveh[2*]

[1] Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
[2] Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received: 06 December, 2015; Accepted: 15 April, 2016

Abstract

Background: n-Butanol is a four-carbon alcohol used widely in foods, cosmetics industries, biology and chemistry research laboratories, and other fields. Long time-effects of inhalation or consumption of small amounts of Butanol on human health are still unknown. On the other hand, numerous reports about the development of n-Butanol toxicity are available. The main objective of the study was to investigate the effects of inhaled and oral administration of n-Butanol as a long-term *in vivo* investigation.

Materials and Methods: Small white laboratory, male mice (20-30 g) were used in 11 groups (n=4) including experimental 1 to 6, 1 to 4 control "A" and positive control groups. Experimental groups 1-3, for 10, 20, and 40 days; 5 hours a day were inside a box with ventilation facilities exposed to air saturated with n-Butanol vapor. Experimental groups 4 to 6, received water containing n-Butanol 0.2%, 1% and 5% for 10 days. Control groups B, 1 to 3 was placed for 10, 20, and 40 days inside a similar box exposed to normal air, respectively. Control group B 4 received water without any particular substance for 10 days. The positive control group received 30µl subcutaneous vinblastine. Bone marrow cells were extracted 24 hours after treatments and stained by May-Grünwald-Giemsa staining and the number of micronucleus was counted. Vinblastine, as a positive control, increased an average of micronucleus numbers significantly compared to control group ($P<0.001$).

Results: n-Butanol inhalation caused no significant difference in 1-3 experimental groups in the average numbers of micronucleus compared to control group, even in the 40 days treatment group, average numbers of micronucleus was decreased comparing to control group ($P<0.05$). Also, oral administration of 0.2% and 1% n-Butanol had no effect on the average micronucleus numbers compared to the control group, while oral administration of 5% n-Butanol caused even decrease in average numbers of micronucleus compared to control group ($P<0.05$).

Conclusion: n-Butanol inhalation may not cause chromosome damages in rat bone marrow cells that probably is due to its very fast metabolism and decomposition in the body. Therefore, the amount of n-Butanol in the systemic circulation and tissues is very low and, probably, the damaging potential is decreased.

Keywords: n-Butanol, Chromosomal damage, Bone marrow cells, Micronucleus

***Corresponding Author:** Bita Pourkaveh, Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: bitapourkaveh@yahoo.com

Please cite this article as: Mansouri N, Haddad F, Fereidoni M, Pourkaveh B . Effect of n-Butanol on Chromosomal Damage in Mice Bone Marrow Cells. Novel Biomed. 2016;4(3):110-5.

Introduction

Alcohols, especially famous linear alcohols such as Propanol, n-Butanol and Pentanol, have a vital role in macroeconomic competitions among the governments. n-Butanol can be used in cosmetic and non-cosmetics industries[1].In fact, n-Butanol consumption is not limited and covers a wide range of food industries, cosmetics and even pharmaceutical industry[2].The wide range of a chemical consumption, brings in the necessity for further research[3].

A few studies have been conducted in the field of the damaging effects of n-Butanol on human body or other livings' organs and also evolutional changes resulted from gene defects due to exposure to n-Butanol[4]. As the origin of many abnormalities and cancers is chromosomal damages and especially aneugenic damages, investigation of genotoxic effects of the substance is prior to other side effects of it[5,6]. The importance of this issue in academic terms on one hand, and weak literature of it on the other hand,resulted in this study to investigate the effects of n-Butanol on emergence of chromosome damages in bone marrow cells of laboratory small rats[7]. Also, the MN (micronucleus) assay in mice is the *in vivo* test on genotoxic effects chromosome aberrations with the best documentation concerning the number of tested chemicals[7]. However, as it has been mentioned before, the substance has not been examined under any condition and it is still unclear that whether long time exposure to n-Butanol in form of inhalation, oral, and even skin contact has the ability to create chromosomal damages from aneugenic or clastogenic type or not[7]. The main objective of the present study is to investigate the effects of inhaled and oral administration of n-Butanol as a long-term on chromosomal damage *in vivo* condition.

Methods

Animals: Male Balb/C mice, aged 5 to 6 weeks and weighing 20 to 30 g, were bought from Mashhad University of Medical Sciences. The mice were kept in an animal house at Department of Biology, Ferdowsi University of Mashhad, where free access to solid food and water was provided.

Treatments: The mice were categorized in 14 groups (n=4) experimentally 1 to 6, 1 to 7 control groups and positive control. Experimental groups 1 to 3, respectively, for 10, 20, and 40 days, 5 hours a day were in a box with ventilation facilities exposed to air saturated by n-Butanol (product of Merck Company) vapor. Experimental groups 4 to 6, respectively, received water containing n-Butanol 0.2%, 1% and 5% for 10 days. Control groups 1 to 3 (A control group) were placed for 10, 20, and 40 days inside a similar box exposed to normal air, respectively (this control group was considered to prove that putting mouse in the box cannot cause stress application on them and result information of micronucleus). Control groups 4 to 6 (B control group) were placed for 10, 20, and 40 days under normal conditions of animal house, respectively. The control group 7 was given water without any particular substance for 10 days. The positive control group was injected 30 µl vinblastine (product of Biosera company), subcutaneously. The chemical was given as a single dose and the animals were dead 24 hours after injection.

Collection and purification of erythrocytes from bone marrow: Animals were anesthetized to death with chloroform (product of Merck company) 24 hours after the treatments.

Mice were dissected to extract femoral bone marrow cells. Using syringe, 1.5 ml FBS (Fetal bovine serum) filtered and poured into the porcelain crucible. Afterwards, two ends of femoral bone were cut by seizer, so that cavities of two ends of bone could be observed. FBS in the porcelain crucible was injected into femoral bone by syringe. Using this method and pass of FBS through the bone, bone marrow cells were drained and were poured into a porcelain crucible. Then, the solution was discharged in the centrifuge pipe and was centrifuged with a speed of 7000 RPM for 8 minutes, so that desired cells could be precipitated. The supernatant was carefully aspirated, leaving erythrocytes and some nucleated cells attached at the bottom of the pipe.

Erythrocyte fixation and staining: The cells in each tube pipetted repeatedly and were poured on three slides prepared previously. In fact, in this method 3 slides would be provided for each mouse from both femoral bones. Samples were coded and stored for 1 to 2 hours, allowing cells to settle. Then, they were fixed

with 95% alcohol and were stained by May-Grünwald-Giemsa staining at the last stage.

Micronucleus test and calculation of MPCE frequencies: In this study, the micronucleous test was used to evaluate genotoxic effects of n-Butanol on mice bone marrow cells. The Slides were viewed in an optic microscope with a magnification of 100. In each sample, fields of vision were selected randomly and nucleus-free cells were counted through the portion (erythrocyte precursors). Also, frequency of MPCE (micronucleated polychromatic erythrocytes) was determined in the same region. Hence, to 1200 nucleus-free cells were counted by the counter and number of MPCEs among them was reported. 3 slides were existed for each mouse with numbers of 1, 2, 3, and number of MPCEs detected in them was reported. The reason of selecting erythrocytes for micronucleus test is that these cells lose their nucleus and micronucleus can be observed easily. Due to elimination of MPCE in the circulating blood, hip bone marrow erythrocytes were extracted for this test.

Data analyzing: In total, 56 mice were included in this experiment. They were assigned to 14 different groups (n=4). As it is mentioned, 12 slides were prepared for each group and in each slide, 1200 polychromatic erythrocytes were counted. Frequency of MPCE was also estimated. Then, mean value of MPCE and standard error of mean (SEM) was estimated for each group via using GraphpadInStat software (Table 1 and 2). Comparison between different groups was held by one-way ANOVA test. The P value <0.0001, is considered extremely significant. The P value >0.05, is considered non-significant.

Results

After different treatments and staining, slides were evaluated using optic microscope. In each slide nucleus-free erythrocytes and MPCE (micronucleated polychromatic erythrocytes) were counted (Figure 1).

Mean value of MPCE and standard error of mean (SEM) was estimated for each group (Table 1 and 2). Finally, obtained results from estimating mean value and SEM in control groups, inhalation and oral experimental groups, and a positive control group were compared using one-way ANOVA test.

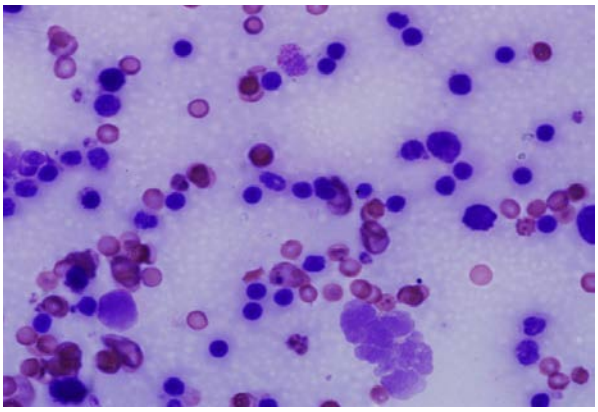

Figure 1. MPCE in inhalation experimental group (100×).

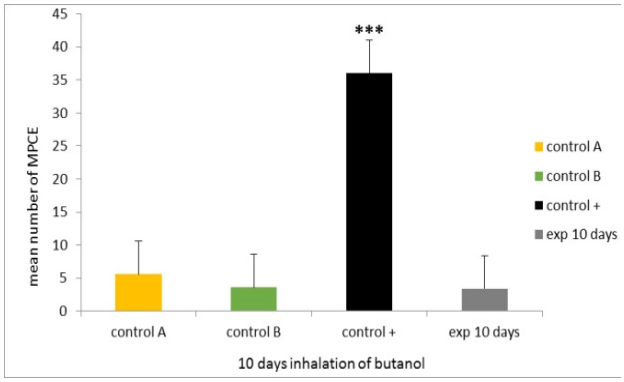

Figure 2. mean±SEM number of micronucleus in control groups A and B, positive control and experimental group 1.

In inhalation treatment, different control groups with positive control were compared, in addition to experimental groups with a positive control group.

As it was expected, p value of subcutaneous injection of vinblastine in the positive control group showed an extremely significant increase in the mean number of MPCE compared to control group (P<0.001) (Figure 2). Results assessment of the experimental group 1, which was under inhalation of n-Butanol for 10 days, indicated that n-Butanol has no significant effect on bone marrow cells in mice (P>0.05) (Figure 2). In the experimental group 2 same results achieved.

In the experimental group 3 with the longest time of n-Butanol inhalation, the previous results were confirmed which means 40 day inhalation of n-Butanol has not increased the number of micronucleus compared to control group, significantly (P>0.05). In addition, long-term and chronic inhalation of n-Butanol even caused a decrease in mean value of micronucleus compared to control group (P<0.05).

Table 1: The mean value and SEM of MPCE in mice bone marrow cells in different control groups.

Groups	Control						
	Inhalation control						Oral control
	Control A			Control B			
	10 days	20 days	40 days	10 days	20 days	40 days	
Mean ± SEM	5.583 ± 0.773	4.752 ± 0.652	5.083 ± 0.645	3.583 ± 0.558	3.583 ± 0.596	3.573 ± 0.538	5.254 ± 0.779

Table 2: The mean value and SEM of MPCE in mice bone marrow cells in different groups treated with butanol and vinblastine.

Groups	Vinblastine (positive control)	n-Butanol					
		Inhalation administration			Oral administration		
		10 days	20 days	40 days	0.2%	1%	5%
Mean ± SEM	36 ± 1.497	3.416 ± 0.570	2.916 ± 0.499	1.833 * ± 0.423	2.916 ± 0.398	4.253 ± 0.509	1.916 * ± 0.287

Regarding the oral administration group, obtained results indicated that oral administration of drinking water containing 0.2% and 1% n-Butanol caused no significant increase in the number of micronucleus compared to control group ($P>0.05$).

Experimental group, for which drinking water containing 5% n-Butanol was used, has not increased the number of micronucleus compared to control group significantly, even mean number of micronucleus was decreased compared to control group ($P<0.05$).

Discussion

Today, n-Butanol has extensive uses as an industrial solvent, chemical intermediate, spices and flavors in the food industry, cosmetics industry, and research laboratories[8,9]. To protect public health and people who are in contact with this material continuously, disadvantages of its usage like cytogenetic aberration induction must be identified, so that necessary precautions can be taken[10].

In order to measure liver toxicity secretion level of hormones such as glutamate dehydrogenase and glutamate pyruvate transaminase have been investigated[8]. It has been proven in many studies that increased levels of these enzymes may be seen in the case of exposure to Ethanol[8,11]. Given the evidence, it can be concluded that if Ethanol has traumatic effect on the chromosomes based on the mechanisms of membrane interference, n-Butanol may also be able to create such effects[8].

According to an interesting study regarding the effects of Ethanol and acetaldehyde on chromosomal maldisjunction, it should be concluded that lipophilic property of the alcohols is related to their polarity and this component can interference in the *Aspergillus nidulans* growth by disturbing the lipophilic structures of cell membrane[12]. In addition, there is a linear relationship between lipophilic characteristics and alcohols effect on disruption and turbulence in the separation of chromosomes[12]. McCann and his colleagues in 1975 and Kier and his colleaguesin 1986 demonstrated n-Butanol does not have mutagenic effects on *Salmonella typhimurium*[12].

Another group of scientists investigated mutagenic effects of n-Butanol to lung cells of Chinese hamster. They did not find any evidence of toxicity of Butanol to the 50 ml/µl concentration[13.]

In another research about genetic toxicity of n-Butanol *in vivo* condition, oral n-Butanol administration did not cause any clastogenic effects and symptoms of disorder in chromosomal distribution during mitosis.

Although, it was a short time survey and there wasn't justified reasons regarding genotoxic rejection of n-Butanol.

In animals, the major routes for exposure are oral and inhaled routes while dermal investigations are less important[14]. In the previous studies, effect of Butanol has been examined either *in vitro*, which lacks the similarity with that humans, or *in vivo* in a short period of treatment. Therefore, the purpose of the present study was to investigate cytogenetic effects of n-Butanol in the *in vivo* after long-term oral administration and inhalation treatment.

In previous studies *in vitro* or as short-term in terms of *in vivo*, because of lack of mammal bodies natural conditions or if there is a short treatment timely conclusion based on the results is not logical.

Our research showed that inhalation of n-Butanol compared with the control group do not cause a significant increase in the average number of micronucleus. The comparison done in all of the three groups of mice had inhalation treatment (10, 20, and 40 days) and in none of the groups chromosomal damages was observed. We expected to see an increase in damages with the elevation of inhalation time, but this did not occur. Our observations in the experimental group of inhaled treatment time of 40 days as well as 5% oral experimental group demonstrated a decline in the average number of micronucleus compared to the control groups ($P<0.05$). It can be said the possible antioxidant effects of n-Butanol or its metabolites in the body are responsible for the reduction of injuries.

Conclusion

To sum up, although n-Butanol has not the ability to create chromosomal damage in form of micronucleus according to this study, this cannot be denied that if the administrated n-Butanol or time of the experiments (in both oral and inhalation groups) for creating chromosomal damages in type of aneugenic and clastogenic has not been enough, increase in n-Butanol can result in damages. The results provide more evidence for other studies conducted *in vitro* or *in vivo*. However the results of the present study are derived from *in vivo* analysis with longer exposure to Butanol which makes the present study unique in comparison with previous studies.

However, due to the evidence, n-Butanol may have the ability to create chromosomal damages, so further preventive and protective actions should be taken while using the substance. Therefore, regarding carcinogenesis property of n-Butanol and according to the findings of studies in regard to non-mutagenic and non-clastogenic nature of n-Butanol, it can be claimed that the substance has a little potential for carcinogenesis.

Acknowledgment

We appreciate the colleagues who helped us in the genetics laboratory of Ferdowsi university of Mashhad.

Conflict of Interest

There is no conflict of interest for authors and there is no relationship with organizations that could influence the work.

References

1. Steen EJ, Chan R, Prasad N, Myers S, Petzold CJ, Redding A, et al. Metabolic engineering of Saccharomyces cerevisiae for the production of n-butanol. Microb Cell Fact. 2008;7(1):36.
2. Rappert S, Müller R. Odor compounds in waste gas emissions from agricultural operations and food industries. Waste Management. 2005;25(9):887-907.
3. Sarathy S, Thomson M, Togbé C, Dagaut P, Halter F, Mounaim-Rousselle C. An experimental and kinetic modeling study of n-butanol combustion. Combustion and Flame. 2009;156(4):852-64.
4. Gross J, Leblond C. Distribution of a large dose of thyroxine labeled with radioiodine in the organs and tissues of the rat. Journal of Biological Chemistry. 1947;171(1):309-20.
5. Ding W-X, Shen H-M, Zhu H-G, Lee B-L, Ong C-N. Genotoxicity of microcystic cyanobacteria extract of a water source in China. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 1999;442(2):69-77.
6. Hicks R, Wakefield JSJ. Rapid induction of bladder cancer in rats with N-methyl-N-nitrosourea I. Histology. Chemico-biological interactions. 1972;5(2):139-52.
7. Abramsson-Zetterberg L, Grawé J, Zetterberg G. The micronucleus test in rat erythrocytes from bone marrow, spleen and peripheral blood: the response to low doses of ionizing radiation, cyclophosphamide and vincristine determined by flow cytometry. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1999;423(1):113-24.
8. Bonte W. Congener content of German and foreign beers. Blutalkohol. 1979;16(2):108-24.
9. Barton H, Deisinger P, English J, Gearhart J, Faber W, Tyler T, et al. Family approach for estimating reference concentrations/doses for series of related organic chemicals. Toxicological Sciences. 2000;54(1):251-61.
10. Ema M, Hara H, Matsumoto M, Hirose A, Kamata E. Evaluation of developmental toxicity of 1-butanol given to rats in drinking water throughout pregnancy. Food and chemical toxicology. 2005;43(2):325-31.

11. Berezina OV, Zakharova NV, Brandt A, Yarotsky SV, Schwarz WH, Zverlov VV. Reconstructing the clostridial n-butanol metabolic pathway in Lactobacillus brevis. Applied microbiology and biotechnology. 2010;87(2):635-46.
12. Crebelli R, Conti G, Conti L, Carere A. A comparative study on ethanol and acetaldehyde as inducers of chromosome malsegregation in Aspergillus nidulans. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1989;215(2):187-95.
13. Obe G, Ristow H. Acetaldehyde, but not ethanol, induces sister chromatid exchanges in Chinese hamster cells in vitro. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1977;56(2):211-3.
14. McKee M, Sűzcs S, Sárváry A, Ádany R, Kiryanov N, Saburova L, et al. The composition of surrogate alcohols consumed in Russia. Alcoholism: Clinical and Experimental Research. 2005;29(10):1884-8.