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Araştırma Makalesi / Research Paper

The Acute Effect of Mercury Chloride on mtDNA

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ABSTRACT

Mercury is a highly toxic environmental pollutant. Molecular mechanisms of mercury toxicity are assorted. Basically, they block essential functional groups in biomolecules and also displace essential metal ions from them. Mercuric ion is known as one of the strongest thiol-binding agents. Although the oxidative properties of mercury have been studied and accepted the actual process of ROS generation is still unclear. The aim of the current study is to examine the acute effects of mercury chloride exposure on mtDNA damage and mtDNA copy number in *Drosophila*. Quantitative PCR method was used to measure mtDNA damage. In the mercury chloride application groups, mtDNA damage and mtDNA copy number were slightly greater than the control group but the difference was not statistically significant. We demonstrated that the mercury chloride application does not generate the damage on mtDNA of *Drosophila melanogaster* in 24 hours treatment.

Keywords: Mercury, mtDNA damage, mtDNA copy number, *Drosophila*

Civa Klorürün mtDNA'da Akut Etkisi

ÖZ

Civa çok zehirli bir çevresel kirleticidir. Civa toksisitesinin moleküler mekanizmaları çeşitlidir. Esasen, biyomoleküllerdeki temel fonksiyonel grupları bloke eder ve temel metal iyonlarını onlardan uzaklaştırır. Civa iyonu, en güçlü tiol bağlayıcı ajanlardan biri olarak bilinir. Civanın oksidatif özellikleri araştırılmış ve kabul edilmiş olsa da, ROS oluşumunun gerçek süreci hala belirsizdir. Bu çalışmanın amacı, civa klorür maruziyetinin, *Drosophila'*daki mtDNA hasarı ve mtDNA kopya sayısı üzerindeki akut etkilerini incelemektir. Çalışmamızda mtDNA hasarını ölçmek için kantitatif PCR yöntemi kullanıldı. Sonuçlara göre, civa klorür uygulama gruplarında mtDNA hasarı ve mtDNA kopya sayısı kontrol grubundan biraz daha fazlaydı, ancak bu fark istatistiksel olarak anlamlı değildi. Buna dayanarak, civa klorür uygulamasının *Drosophila melanogaster*'in mtDNA'sında, akut hasar oluşturmadığı sonucuna varılmıştır.

Anahtar Kelimeler: Civa, mtDNA hasarı, mtDNA kopya sayısı, *Drosophila*

INTRODUCTION

Mercury is a highly toxic environmental pollutant (Chen et al., 2005). Low dose mercury affects the nervous system, renal system, reproduction, immune system, cardiovascular system and motor activities (Zahir et al., 2005; Al-azzawie et al., 2013).

Molecular mechanisms of mercury toxicity are assorted. Basically, they block essential functional groups in biomolecules and also displace essential metal ions

from them. Mercuric ion is known as one of the strongest thiol-binding agents. The other functional groups besides SH for which mercury has high affinity include $CONH_2$, NH_2, COOH and PO_4. Moreover, low concentrations of mercury may trigger a cascade of events leading to impairment of mitochondrial energy metabolism and generation of reactive oxygen species (Zahir et al., 2005).

Although the oxidative properties of mercury have been studied and accepted the actual process of ROS generation is still unclear. In most studies, lipid peroxidation, DNA damage, and GSH imbalances caused by mercury have been assessed and have suggested an oxidative stress-like mechanism for mercury cytotoxicity (Chen et al., 2005).

$HgCl_2$-induced nuclear DNA damage has many similarities to those caused by X-rays; however, the single strand breaks induced by $HgCl_2$ are not readily repaired (Costa et al., 1991). There are conflicting research results on whether or not the mercury is carcinogenic. In some studies, it has been shown that mercury causes an increase in the incidence of cancer, while in some studies it has been suggested that it is not carcinogenic (Elemental mercury and inorganic mercury compounds: human health aspects, World Health Organisation, 2003). According to the carcinogenic classification prepared by International Agency for Research on Cancer (IARC), inorganic mercury compounds are classified as group 3; "not classifiable as to its carcinogenicity to humans" (Monographs, IARC, 2017).

Mitochondria are targeted by some environmental pollutants (Meyer et al., 2013). Mitochondrial DNA (mtDNA) damage is more extensive and persists longer than nuclear DNA (nDNA) damage in human cells following oxidative stress (Yakes and Van Houten, 1997). Some toxic materials generate mtDNA damage (Mutlu and Fiskin, 2009; Mutlu, 2012a; Mutlu, 2012b), which may trigger mitochondrial dysfunction (Lesnefsky et al., 2001). Damage to mtDNA could potentially be more important than deletions in nDNA because the entire mitochondrial genome codes for genes are expressed while nDNA contains a large amount of non-transcribed sequences (Liang and Godley, 2003). Nuclear DNA mutations generated by mercury have been investigated by some researchers but we need more information and direct evidence about the effects of mercury on mtDNA. The aim of the current study is to examine the effects of mercury chloride exposure on mtDNA damage and mtDNA copy number in *Drosophila*.

MATERIALS AND METHODS

Two-day-old, wild type *Drosophila melanogaster* were used. *Drosophila* (fruit flies) are useful model organisms because of their small size and short generation time, and are commonly used to facilitate experimental laboratory research (Hedges, 2002). Flies were fed corn meal, which contained water, corn flour, sugar, yeast and agar. They were housed in glass bottles and incubated at 24 ±1 °C (12-hour day-night cycles).

In the experimental groups treatments applied were: 0.1 mM, 1 mM and 10 mM mercury chloride in corn meal. Following the 24-hour treatment period, total DNA isolation was conducted. Twelve flies were analyzed from each group. SIGMA G1N350 Genomic DNA kits were used for total DNA isolation using the methods indicated in the technical bulletin. Invitrogen (Molecular Probes) Pico Green dsDNA quantitation dye and QUBIT 2.0 fluorometer were used for template DNA quantitation and for the fluorometric analysis of PCR products. A crucial step of the QPCR method is the concentration of the DNA sample. The accuracy of the assay relies on initial template quantity because all of the samples must have exactly the same amount of DNA. The Pico Green dye has not only proven to be an efficient method for template quantitation but also for PCR product analysis (Santos et al., 2002). DMSO (in a volume equivalen to 4% of total volume) was added to 5 ng of template total DNA in each PCR tube. Thermostabil polymerase used was Thermo Phire hot start II DNA polymerase.

Primers for *Drosophila* mtDNA small fragment (100 bp) were (Mutlu, 2012a; Mutlu, 2012b; Mutlu, 2013);

11426 5'- TAAGAAAATTCCGAGGGATTCA - 3'
11525 5'- GGTCGAGCTCCAATTCAAGTTA - 3'

Primers for large fragment (10629 bp) were (Mutlu, 2012a; Mutlu, 2012b; Mutlu, 2013);

1880 5'- ATGGTGGAGCTTCAGTTGATTT - 3'
12508 5'- CAACCTTTTTGTGATGCGATTA - 3'

For long fragment PCR amplification, DNA was denatured initially at 98°C for 1 minute; the material then underwent 21 PCR cycles of 98°C for 10 seconds, 52°C for 45 seconds, and 68°C for 5 minutes. Final extension was allowed to proceed at 68°C for 5 minutes.

For small fragment PCR amplification, DNA was denatured initially at 98°C for 1 minute; the material then underwent 21 PCR cycles of 98°C for 10 seconds, 55°C for 45 seconds, and 72°C for 10 seconds. Final extension was allowed to proceed at 72°C for 2 minutes.

The QPCR method was used to measure mtDNA damage. The lesion present in the DNA blocked the progression of any thermostable polymerase on the template, so a decrease in DNA amplification was observed in damaged templates. The QPCR method is highly sensitive to measurements of DNA damage and repair. mtDNA damage was quantified by comparing the relative efficiency of amplification of long fragments of DNA and normalizing this to gene copy numbers by the amplification of smaller fragments, which have a statistically negligible likelihood of containing damaged bases (Yakes and Van Houten, 1997; Santos et al., 2002; Venkatraman et al., 2004). To calculate normalized amplification, the long QPCR values were divided by the corresponding short QPCR results to account for potential copy number differences between samples (the mtDNA/total DNA value may be different in the 5-ng template of total DNA in each PCR tube). Decreased relative amplification is an indicator of the damaged DNA. The copy number results do not indicate damage.

Minitab Release 13.0 software was used for statistical analysis. The results were analyzed using the Mann–Whitney Test.

RESULTS AND DISCUSSION

The risk of chemical toxicity is recognized to be greatest in the rapidly industrializing and restructuring developing countries (Al-azzawie et al., 2013). Inorganic Hg compounds (mercury salts) are a significant source of Hg intoxication in some countries (Guzzi and La Porta, 2008).

Oxidative stress is being increasingly recognized as a possible mechanism in the toxicity of mercury (Stohs and Bagchi, 1995; Ercal et al., 2001; Durak et al., 2010; Al-azzawie et al., 2013). Metal-mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation and altered calcium and sulfhydryl homeostasis. The primary route for mercury toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins (Valko et al., 2005). However, some research indicated that the mercury chloride affects mitochondrial bioenergetics (Weinberg et al., 1982; Lund et al., 1993; Uyemura et al., 1997; Königsberg et al., 2001; Mieiro et al., 2015). Königsberg and colleagues observed disaggregation in mitochondrial inner membrane after mercury treatment (Königsberg et al., 2001).

The aim of the current study is to examine the acute effects of mercury chloride exposure on mtDNA damage and mtDNA copy number in *Drosophila*. mtDNA damage, mtDNA copy number and viability of fruit flies in response to the mercury chloride are shown in Table 1. In the mercury chloride application groups, mtDNA damage and mtDNA copy number were slightly greater than the control group but the difference was not statistically significant. We demonstrated that the mercury chloride does not generate the damage on mtDNA of *Drosophila melanogaster* in 24 hours treatment.

Table 1. Viability, mtDNA Damage and mtDNA Copy Number Results of the Experimental Groups. Decreased relative amplification is an indicator of the damaged DNA.

	Groups	% Viability	Relative amplification ± SE	mtDNA Copy Number (Small fragment amplification ± SE)
0,1 mM	Control	100	1,76±0,22	317,8±18,7
	$HgCl_2$	100	1,58±0,07	325,9±14,3
1 mM	Control	100	1,75±0,17	326,3±17,2
	$HgCl_2$	100	1,66±0,13	330,7±19,1
10 mM	Control	100	1,78±0,12	325,4±12,6
	$HgCl_2$	59	1,67±0,13	346,9±23,6

There is some evidence of mercury toxicity on DNA (Chen et al., 2005; Al-azzawie et al., 2013; Costa et al., 1991; Tran et al., 2007; Pereira et al., 2010; Barcelos et al., 2011) but there are few studies on the effect on mtDNA in literature. Wyatt and his team have come to the conclusion that, in their study on *C. elegans*, both inorganic and organic mercury have damaged the mtDNA (Wyatt et al., 2017). However, in our study, no statistically significant mtDNA damage was observed in Drosophila at the doses applied.

In Karouna-Renier's research, little brown bats that lived in mercury contaminated South river (Waynesboro, USA), had higher mtDNA damage than those from the reference location. But the levels of mtDNA damage exhibited weak correlations with fur and blood Hg levels and the mtDNA damage may not be connected

to mercury contamination in the river (Karouna-Renier et al., 2014). In another study, Sharpe and colleagues demonstrated that mtDNA damage was created by thimerosal. Thimerosal is generated by ethylmercury in aqueous solutions and is widely used as a preservative (Sharpe et al., 2012). But also, this research was not demonstrating the direct mercury effect.

In the current study, although 41% of the flies died in 24 hours on 10 mM $HgCl_2$ application there was no statistically significant oxidative mtDNA damage. According to these results, the effects of mercury on mitochondria may not be created primarily by oxidative stress. However, Inorganic Mercury is not carcinogenic (Boffetta et al., 1993; Lansdown, 2014) and it may be that the lack of carcinogenicity of mercury chloride is correlated with not forming the mtDNA damage.

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