Effect of Long Term Exposure to Sodium Nitrite on Gene Responsible for DNA Repair

تـأثير التعرض الطويل الأمد لنتريت الصوديوم في الجين المسؤول عن عملية إصلاح الحامض النووي الرايبوزي منقوص الاوكسجين

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المستخلص :
الهدف: تهدف هذه الدراسة الى معرفة تأثير التعرض المستمر لنتريت الصوديوم في أحدى الجينات المسؤولة عن عملية أصلاح الحمض النووي (الدنا). تلعب عملية أصلاح الحامض النووي دور مهم وكبير في المحافظة على أستقرار الدنا عند تعرضه للتلف, واستقرار الحامض النووي مهم جدا في الحفاظ على سلامة الخلية ومنع حدوث العديد من الاورام السرطانية. وهناك العديد من الجينات المسؤولة عن هذه العملية و يعتبر أوكسو كوانين كلايكوسليز (Ogg1) هو أحد هذه الجينات.
المنهجية: في هذه الدراسة عرضت مجموعتين من الفئران لنتريت الصوديوم أحداهما لمدة ستة أشهر والثانية لثمانية عشر شهرا وأستعملت مجموعة ثالثة سيطرة للمجموعتين, بعدها تم قياس حجم جين أوكسو كوانين كلايكوسليز.
النتائج: أظهرت النتائج في المجموعة غير المعرضة لنتريت الصوديوم (مجموعة السيطرة) وجود اليلين مختلفين لهذا الجين بينما أظهرت الفئران المعرضة الى نتريت الصوديوم تعدد في أشكاله بغض النظر عن فترة التعرض. نستحصل من هذه الدراسة الى ان نتريت الصوديوم له القابلية في العمل على هذا الجين مؤديا الى تعدد في أشكاله.
التوصيات: أجراء المزيد من الابحاث للتعرف على آلية تأثير النترات على هذا الجين.

Abstract:
Objective: The aim of this study is to detect the effect of continuous exposure to Sodium Nitrite on 8-oxoguanine DNA glycosylase (OGG1) gene which responsible on DNA repairs. DNA repair play a major role in maintaining genomic stability when DNA exposure to damage. Genomic stability is very important for keeping body cells healthy and to prevent many types of tumor development. Many genes are responsible for this job; one of them is OGG1 gene.
Methodology: In current study two groups of mice were chronically exposed to sodium nitrite for six months and eighteen months while third group was used as a control. Then sizes of OGG1 were estimated.
Results: The results exhibited in the unexposed (control) mice had two different alleles for this gene while the exposed animals showed more polymorphisms and this finding is independent to the period of exposure.
Conclusion obtained from this study indicated that sodium nitrite has ability to act on this gene that leads to develop more polymorphisms.
Recommendations: Other research should be done in order to detect how nitrite can act on this gene.
Keywords: Sodium nitrate, DNA repair genes, OGG1

Introduction:

The integrity of the genetic information in all living organisms is constantly threatened by a variety of endogenous and environmental insults [1]. Reactive Oxygen species (ROS) are produced both as a result of normal foreign chemicals and physical agents such as ionizing radiation [2].

Oxidative stress including DNA damage is implicated in the etiology of a broad spectrum of degenerative diseases, including, neurodegenerative like Alzheimer's and Parkinson's disease [2,3], both types of diabetes mellitus [4,5], Crohn's disease, ulcerative colitis [6], and cancer like colorectal [7] adenocarcinoma [8] Non-Hodgkin's lymphoma [9]. Among the various classes of DNA damage caused by Oxygen radicals, an oxidized form of guanine base (8-Oyoguanine) appears the most important, it can pair with cytocine and adenine and G: C to T: A transversion follows [10]. DNA lesions also accumulated spontaneously due to the chemical instability of DNA [11]. Multiple mechanisms have evolved to repair DNA damage and preserve genome integrity [7].there are several different DNA repair pathways that include enzyme specialized for repair of specific DNA lesion or type of DNA lesion. Base excision repair is the main pathway for repair of oxidative base lesions in DNA [11]. OGG1 encodes the 8-Oxoguanine glycosylase 1 enzyme which is the primary enzyme which is BER (base excision repair pathway) [7,12] responsible for the excision of 7,8 dihydro-8-oxoguanine (8- OxoG) a mutagenic base byproduct and other oxidatively damaged nucleobases from the DNA (7, 8) that occur as a result of exposure to reactive oxygen species [7]. OGG1 expression is increased upon inhalation or instillation of diesel exhausted particles indicating that base excision repair is activated [8]. Deficiencies in the DNA repair system are likely to cause chromosomal aberration which is in turn leads to cell malfunctioning, cell death and tumor genesis [13].So decreased repair of oxidative DNA damage is a risk factor for developing certain human malignancies [10].

Sodium nitrite a substance is used widely as food additive, mainly for curing meat [14, 15, 16] and also in medicine as therapeutic substance [17]. Sodium nitrite has ability to act as reactive oxygen species [15, 16] and also it is a genotoxic in human cell systems [18].

Methodology:

Fifty eight male and female mice were divided into three groups, the sodium nitrite was added to drinking water at 2000PPM for the first two groups while the third one used as a control group. The treatment persists for 6 months and 18 months for the first group and second one respectively.

Blood samples were collected by heart puncture and frozen in deep freeze until further use. Then DNA was extracted from each sample using Wizard Genomic DNA purification kit (Promega Corporation).

PCR was performed using Syber-Green PCR Master Mix (Kabba product).Samples were amplified with OGG1primers (5'-GAT TGG ACA GTG CCG TAA- 3' and 5'-GGA AGT GGG AGT CTA CAG-3') the cycling conditions comprised 2 minutes at 50 C, 10 minutes polymerase activation at 95 C and 40 cycles at 95 C for 15 seconds and 60 C for 1 minutes [19]. Then electrophoresis was done for each amplified DNA.

Results:

The data in the table (1) showed the size of bands of OGG1 gene in all groups including control group. There are no significant differences among groups in each band.

Table 1. Sizes of bands in the control and in the exposed mice

Bands Sizes	Exposed Groups					Control Group (18 mice)	
	6 Months (24 mice)		18 Months (16 mice)				
	No.	%	No.	%		No.	%
300	1	4.2	1	6.3		0	-

Table 1. Continues

400	**9**	**37.5**	**4**	**25**		**2**	**11.1**
700	**19**	**79.1**	**13**	**81.2**		**14**	**77.7**
1000	**18**	**75**	**9**	**56.2**		**11**	**61.1**

This table showed there are no significant differences among groups in each band in the control and exposed groups more one band were appeared in single animal (figure 1).

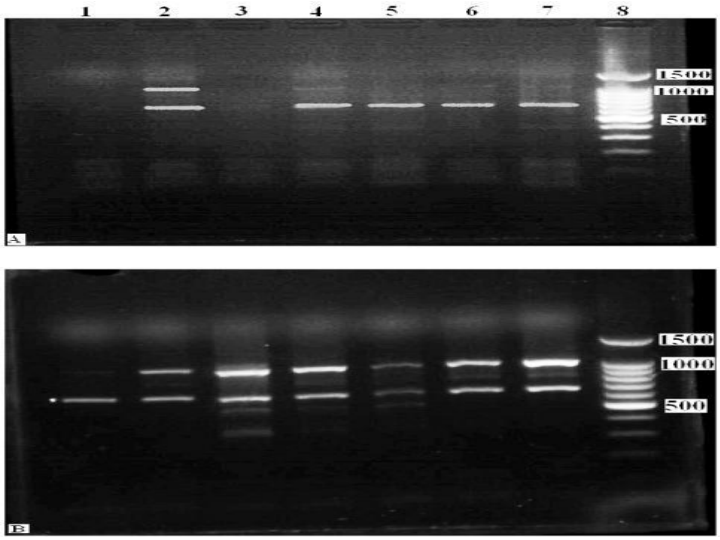
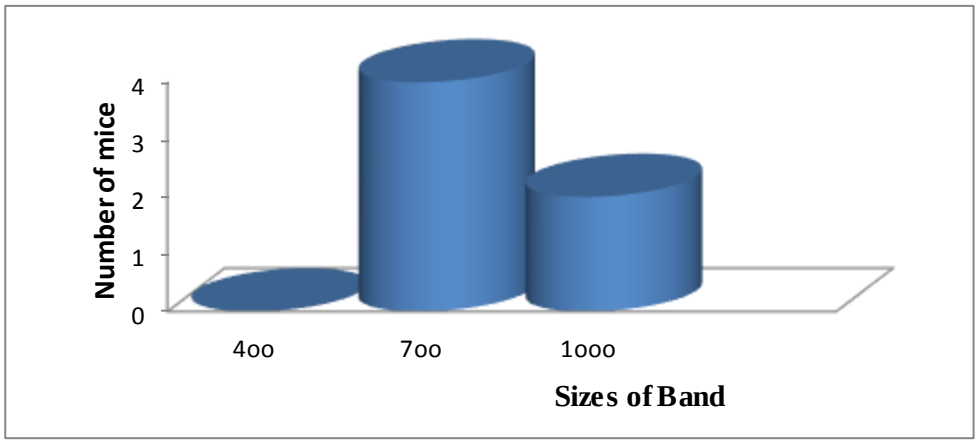


Figure 1. More one band appeared in the single animal. A) The lanes 2-7 represent the control animals while the lane 1 represents control for run (i.e. without DNA). B). the lanes 1-7 represent the exposed animals.

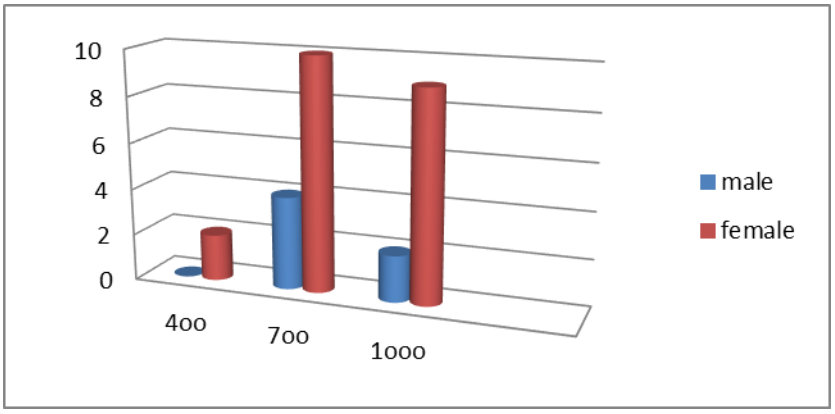
Figure 2. Sizes of bands in control group



Most mice appeared 700 bp (14 mice) and 1000 bp (11 mice) while 2 mice appeared 400 bp in size.

In the control group, the numbers of mice had 700 bp in size were four males and 10 females, while the size 1000 bp appeared in two males and 9 females, but the size of 400 bp appeared only in two females (figure 3).Statistic analysis showed there were significant differences between the size 400 bp and size 700 bp of bands among males and also among females at level 0.05 and also there was significant difference between band sizes 700 bp and 1000 bp only in females at the same level. However there were no significant differences between male and female at the same band.

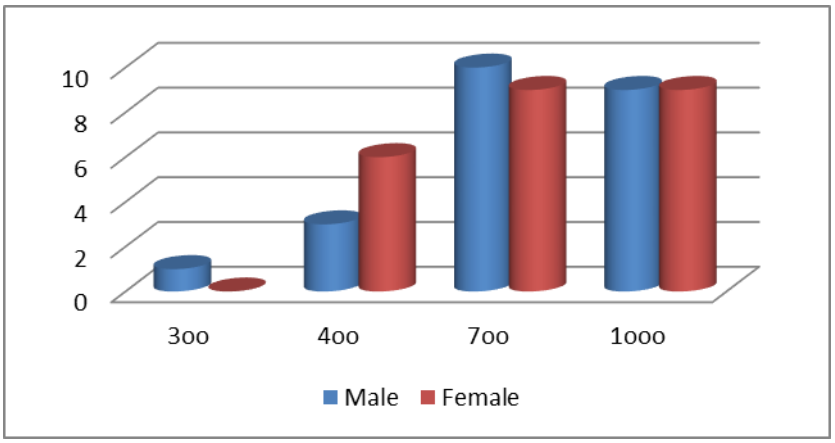
Figure 3. Showing the difference in sizes of band between male and female among control animals



There is significant difference between 400 and 700 bp

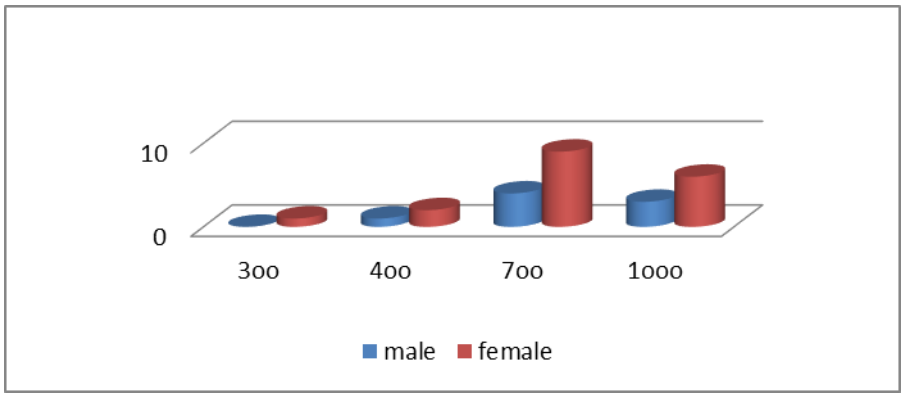
In exposed group for six months; the size 700 appeared in ten males and nine female while 1000 appeared in nine female and nine males, but the 400 pb appeared in three males and six females. There was other band (300 bp) appeared only in one male (figure 4).

Figure 4. Showing the difference in sizes of band between male and female among group 1



While in group exposed for eighteenth months, the band 700 appeared in four males and nine females, but band with size 1000 pb appeared in three males and six females while the size 400 pb appeared in 1 male and two females. However band 300 bp in size appeared only in one female (figure 5).

Figure 5. Showing the difference in sizes of band between male and female among group exposed for 18 months



Statistical analysis showed there were no significant differences at each band among the females in all groups while among the males significant difference appeared at level 0.05 in males exposed to sodium nitrite to 6 months at size of band 1000 when comparing with control group.

Discussion:

Our results in control group showed that the Ogg1 gene had different sizes of bands which lead to think that our laboratory mice normally had heterozygosity of OGG1 gene, that means the 700bp and 1000 bp bands act as two different alleles for this gene, and there was no difference between males and females in this concept (heterogenicity). Probably the size 700 bp band represents approximately the whole gene, while the 1000 bp band represents polymorphic gene [20]. This result is consistent with many researches' findings which found that Ogg1 had variant alleles and also is highly polymorphic among humans.

In spite 700 bp and 1000 bp bands there were other bands appear in the exposed groups, mainly 400 bp band and few 300bp. Although the appearance of these two bands is statistically not significant but it lead to believe that sodium nitrate acted by somehow on Ogg1 gene that promote to appear other places for primer attachment on this gene. The occurrence of 400bp band was independent on time of exposure. We suggest that the action of sodium nitrate probably acted by release reactive Oxygen species (ROS) our suggestion is supported by Bigot *et al*. findings [19] which they found that in the expression of 400bp of Ogg1 genes occurred normally in the tissues with high levels of stress and normally exposed to high level of reactive Oxygen species production like retina and CNS comparing with tissue normally exposure to low levels of ROS like liver [19].

In this study we have demonstrated that Ogg1 in our laboratory mice had variant alleles while the action of sodium nitrite appeared statically insignificants.

Recommendations:

1. Further studies should be done to detect the polymorphisms in this gene.
2. Sequencing of Iraqi laboratory animals of OGG1 gene is important to show if there is variety in this gene comparing with the global laboratory animals.

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