Age and exposure to arsenic alter base excision repair transcript levels in mice

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Arsenic (As) induces DNA-damaging reactive oxygen species. Most oxidative DNA damage is countered by base excision repair (BER), the capacity for which may be reduced in older animals. We examined whether age and consumption of As in lactational milk or drinking water influences BER gene transcript levels in mice. Lactating mothers and 24-week-old mice were exposed (24 h or 2 weeks) to As (2 or 50 p.p.m.) in drinking water. Lung tissue was harvested from adults, neonates (initially 1 week old) feeding from lactating mothers and untreated animals 1–26 weeks old. Transcripts encoding BER proteins were quantified. BER transcript levels decreased precipitously with age in untreated mice but increased in neonates whose mothers were exposed to 50 p.p.m. As for 24 h or 2 weeks. Treatment of 24-week-old mice with 2 or 50 p.p.m. As for 2 weeks decreased all transcript levels measured. Exposure to As attenuated the age-related transcript level decline for only one BER gene. We conclude that aging is associated with a rapid reduction of BER transcript levels in mice, which may contribute to decreased BER activity in older animals. Levels of As that can alter gene expression are transmitted to neonatal mice in lactational milk produced by mothers drinking water containing As, raising concerns about breastfeeding in countries having As-contaminated groundwater. Reduction of BER transcript levels in 24-week-old mice exposed to As for 2 weeks suggests As may potentiate sensitivity to itself in older animals.

Introduction

Weathering of the earth’s crust, mining and manufacturing release arsenic (As) into soil, water and air (1). Consequently, As is a common environmental contaminant prevalent in the groundwater of a number of countries including Bangladesh, Chile, Taiwan, The People’s Republic of China and the USA and can cause human health problems (2,3). Sublethal exposures to As have been associated with immune, vascular and liver disorders, a heightened risk of genomic instability, increased cancer incidence and a rapid burst of reactive oxygen species (ROS) in mammalian cells (4,5). Intracellular ROS cause oxidative DNA damage that, if left unrepairerd, can lead to mutations and cell death.

Base excision repair (BER) counters oxidative DNA base damage in mammalian cells (6). Most often, a glycosylase such as 8-oxoguanine DNA glycosylase 1 (OGG1) excises a damaged base. The resulting abasic site is incised 5’ by apurinic/apyrimidinic endonuclease 1 (APE1), leaving a 3’-hydroxyl and a 5’-deoxyribose 5-phosphate. DNA polymerase beta (Polß) (POLB) hydrolyses the 5’-sugar phosphate and adds at least one nucleotide to the 3’-hydroxyl end. The remaining strand nick is sealed by DNA ligase 1 (LIG1) or ligase 3 (LIG3). Poly (adenosine diphosphate-ribose) polymerase 1 (PARP1) may recruit the required proteins.

Exposure to very low As levels may enhance BER. Arsenic at 100 μmol/kg increases LIG1, LIG3, OGG1 and XRCC1 transcript levels when subcutaneously injected into mice (7). Furthermore, APE1, LIG1 and Polß expression and activity are stimulated in cultured cells by submicromolar As doses (8–10). Interestingly, OGG1 mRNA levels are elevated in individuals subjected to submicromolar As concentrations in contaminated drinking water (11), and lymphocytes from such individuals are more resistant to As challenge than those from unexposed individuals (12). It remains to be determined if this resistance is due to increased BER activity.

Transplacental As exposure can induce spontaneous abortion, developmental abnormalities, epigenetic alterations and tumours in mice (13,14). In addition to crossing the placental barrier, As has been shown to be transmitted via human breast milk or cow milk (15–17), although as of yet, no effects have been attributed to consumption of As in lactational milk. Although As levels transmitted in breast milk can be low compared to amounts in contaminated drinking water (18), they may nevertheless be significant if proliferating tissues in young animals are more vulnerable to carcinogens (19,20). This is an important consideration since children exhibit higher levels of oxidative DNA damage than adults, perhaps due to their higher metabolic rates (21). However, BER may protect them from this greater damage load and so fortuitously from the effects of As-induced ROS. Consistent with such possibilities, APE1, OGG1 and POLB transcript levels are higher in young mice or rats than adults, and BER appears to be more active in young than older animals (22–25). Whether expression of any other BER genes is influenced in vivo by age is unknown. Furthermore, the impact of As exposure via lactational milk on BER has not been investigated.

In this study, we examined the transcriptional profiles of several BER genes in untreated mice of different ages, as well as neonatal and young adult mice exposed to As via lactational milk or drinking water, respectively. Transcript levels for each gene declined rapidly with age. With one exception (POLB),
this age-dependent reduction was not affected by As. BER gene transcript levels tended to increase in neonatal mice feeding from mothers exposed to the highest As concentration used, whereas they decreased in the young adult mice subjected to the longest treatment period.

Materials and methods

As treatment

Animal experiments were conducted following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004), as approved by the Deakin University Animal Welfare Committee. Animals were always treated humanely and with regard for alleviation of suffering. Female BALB/c mice (1, 8 or 24 weeks old) were purchased from Monash University Animal Services (Victoria, Australia) and housed in an isolated temperature-controlled (±1°C) and moisture (50% relative humidity)-controlled room with a 12-h light–dark cycle. One-week-old mice or 8- and 24-week-old mice were housed in groups of two to five with one lactating mother or two to three, respectively, per polycarbonate cage. Mice had ad libitum access to water and standard mouse breeder chow (Ridley AgriProducts, Packenham, Australia) throughout the treatment periods. Mice were exposed to 2 p.p.m. (±15 μM) or 50 p.p.m. (~385 μM) NaAsO2 (Sigma–Aldrich™, Castle Hill, Australia) dissolved in tap water at the commencement of treatment. After treatment, mice were sacrificed by cervical dislocation. The lungs, liver, heart and kidneys were harvested and weighed. Lung tissue was then immediately placed in 5 volumes of Ambion RNA later (Applied Biosystems, Scoresby, Australia) incubated at 4°C overnight, removed from the RNA later® and stored at −80°C in 10 ml centrifuge tubes until use.

Quantitative transcript amplification

RNA was extracted from thawed lung tissue using TriReagent™ (Invitrogen™, Mt Waverley, Australia) and treated with Amplification Grade RNase-free DNase I (Invitrogen™) and its integrity was confirmed (RNA 6000 NanoChip®, 2100 Bioanalyzer; Agilent Technologies, Forest Hill, Australia). BER gene and 28S rRNA complementary DNAs (cDNAs) were quantitatively amplified (Rotor-Gene™ 3000, Rotor-Gene operating software version 6.0.24; Corbett Research, Sydney, Australia) following reverse transcription (Super-Script™ III Platinum® Two-Step qRT–PCR Kit with SYBR® Green; Invitrogen™) from RNA samples (1–2.4 μg) isolated from ≥3 mice. Primers (Table I) annealed at 58°C and yielded amplicons <200 bp in length whose identities were verified by size evaluation (DNA 7500 LabChip®, 2100 Bioanalyzer; Agilent Technologies), amplification and sequencing. Quantitative amplification reactions (20 μl) contained 10 μl SYBR® Green Master Mix reagent (Invitrogen™), 200 or 500 nM of BER gene- or 28S rRNA-specific primers, respectively, and 2 μl cDNA. Reaction mixes containing 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of the cDNA were used in linearity tests to ensure that critical threshold (Ct) value differences for the cDNAs remained constant despite variation in the amount of starting material. Primer concentrations were optimised to give the lowest Ct values and no amplification in no-template controls. The thermal profile for all quantitative amplification reactions was 95°C for 10 sec; 40 cycles of 95°C for 10 sec, 58°C for 15 sec and 72°C for 15 sec, followed by melt-curve analysis at 1.0°C increments using default parameters.

Data analysis

Ct values obtained for each cDNA were normalised to the Ct values for 28S rRNA obtained for each sample to give ΔCt values. Mean ΔCt values (derived from ≥3 independent samples) were transformed, using the comparative ΔΔCt method (26), into measures of absolute transcript levels expressed as fold change in relation to the untreated control (Table I).

Table I. Primers used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Amplicon length (bp)</th>
<th>Forward primer (5’ → 3’)</th>
<th>Reverse primer (5’ → 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APE1</td>
<td>149</td>
<td>GATGAATGCCCAGCTCTAAG</td>
<td>GATACACATGCTAGGTA</td>
</tr>
<tr>
<td>LIG1</td>
<td>150</td>
<td>CAACATGCAGAGAGTATT</td>
<td>CTCGGGACCCAGGTTATT</td>
</tr>
<tr>
<td>LIG3</td>
<td>157</td>
<td>ATGAAAACCCAGGAGAGACG</td>
<td>CTGTTGAAAGTCTGGTAG</td>
</tr>
<tr>
<td>OGG1</td>
<td>157</td>
<td>GTGAGACTGAGCAAGAAGG</td>
<td>GGAAAAGCCTATAGTGTG</td>
</tr>
<tr>
<td>PARP1</td>
<td>163</td>
<td>TGGAGGGTGAGACTGGTC</td>
<td>CCTCAGAATTCACACAG</td>
</tr>
<tr>
<td>POLB</td>
<td>150</td>
<td>CGCCATGAGGACACGCAA</td>
<td>TGGTACTTGGCTATCACAG</td>
</tr>
<tr>
<td>28S rRNA</td>
<td>179</td>
<td>TCGGCTCTCCTCATATG</td>
<td>CAAAATGTCTGAACCTGCG</td>
</tr>
</tbody>
</table>

Fig. 1. Age-dependence of BER gene transcript levels in untreated mice. Statistical signiﬁcance (P < 0.05) was assessed using a one-way analysis of variance with Dunnett’s multiple comparison post-test (all versus a single base state) to compare values for 1-week-old mice to values for other ages. In Figure 1 and Figure 2, an independent two-tailed t-test was used to conﬁrm signiﬁcance in cases where RNA samples were obtained from only three mice. Significant differences are denoted by asterisks above the columns.
percentages of control levels. Group mean values were compared by one-way analysis of variance. Dunnett’s post-test was used to compare all later values to initial transcript values in untreated mice, and Tukey’s post-test was used to perform all-with-all comparisons when assessing the effects of different As exposure durations and doses. Post-natal rate of decline in transcript levels under different As exposure doses was estimated and compared by fitting a one-phase exponential decay model using non-linear regression. Statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software, San Diego, CA, USA).

Results

Decline in BER gene transcript levels with age
To determine whether genes encoding BER proteins are differentially expressed in mice as a function of age alone, we compared transcript levels in lung tissue harvested from untreated animals at 1, 3, 8, 10, 24 or 26 weeks of age. Lung tissue was examined since lungs are considered target organs for As carcinogenicity in mice and humans (2,27). Transcript levels for APE1, LIG1, LIG3, OGG1, PARP1 and POLB declined as the mice aged, with the most substantial decrease occurring by 8 weeks of age (Figure 1). Analysis of the data involved normalization of the BER gene transcript levels to the 28S rRNA levels present in the same tissue. However, there was no downward trend in the amount of 28S rRNA that could have accounted for the age-related reductions in the BER gene transcript levels.

Effect of As on BER gene transcript levels
The ability of As to influence BER gene expression was investigated by providing As at 2 p.p.m. in drinking water for 24 h or 2 weeks. These time-points were chosen to represent a subacute versus chronic exposure period. Since treated mice only accumulate low As levels in lung tissue, likely due to rapid metabolism and excretion (28,29), but show increasing retention with increased dose (30), we also used 50 p.p.m. As. Both concentrations used are within ranges reported to have cocarcinogenic activity or induce gene expression and tumorigenesis in mice (13). No exposure regimen influenced lung, liver, heart or kidney weight in treated versus untreated mice of the same age (data not shown), suggesting that As did not affect organ development. The estimated water consumption per adult mouse varied only marginally between cages of treated or untreated animals (data not shown), indicating that As in the water did not deter drinking, and adult mice subjected to the same treatment conditions ingested similar amounts of As. In addition, results presented below are consistent with exposure of neonatal mice to As via milk produced by lactating mothers that drank As-containing water.

With respect to 24 h exposure, no significant differences in BER gene transcript levels were detected in 1-week-old mice whose mothers were treated with 2 p.p.m. As with the exception of an increase in the level of the LIG1 transcript (Figure 2). In contrast, significantly elevated levels of the APE1, LIG1, OGG1 and PARP1 transcripts were detected in mice whose mothers were exposed to 50 p.p.m. As. Furthermore, the transcript levels for genes other than LIG1 and POLB were significantly higher than observed for the neonates whose mothers were treated with 2 p.p.m. As. These observations suggest that transcript levels in neonates were affected by As ingested in lactational milk over a 24-h period. Exposure of 24-week-old mice to 2 or 50 p.p.m. As in drinking water for 24 h elevated the APE1 and LIG3 transcript levels (Figure 2). The levels of the POLB and PARP1 transcripts were increased by treatment with 2 or 50 p.p.m.,
respectively, whereas the level of the OGG1 transcript was decreased by treatment with 50 p.p.m. As. Treatment with 50 p.p.m. As for 24 h did not result in transcript levels significantly different from those observed for exposure to 2 p.p.m. As (Figure 2).

One-week-old mice exposed to As for 2 weeks via milk from mothers treated with 2 p.p.m. As in drinking water exhibited decreased levels of the LIG1, LIG3 and OGG1 transcripts and an increase in the level of the PARP1 and POLB transcripts (Figure 2). Maternal exposure to 50 p.p.m. As for 2 weeks was associated with increased levels of APE1, LIG3, PARP1 and POLB transcripts. In addition, the levels of all transcripts were significantly greater than observed for treatment with 2 p.p.m. As. All transcript levels were lower than controls in 24-week-old mice treated with 2 or 50 p.p.m. As for 2 weeks, although the reductions for LIG3 were not statistically significant (Figure 2). In no case was the transcript level significantly greater for treatment with 50 than 2 p.p.m. As.

Normalising transcript levels in treated mice to their untreated age-matched controls excluded effects of aging during the As exposure period so that the levels could be compared between mice treated with the same As concentration for 24 h or 2 weeks. Extending the exposure period seemed to increase, reduce or have no effect on transcript abundance depending on the gene and age group (Figure 2). However, for 24-week-old mice, lengthening the treatment with each As concentration to 2 weeks appeared to decrease transcript levels for all genes, although the decreases for LIG1 and OGG1 at 50 p.p.m. were not statistically significant.

Effect of As on age-related transcript level decline

We next examined whether exposure to As influences the decline of BER gene transcript levels in aging mice. The values for transcript levels in untreated or treated animals 1, 3, 8, 10, 24 or 26 weeks of age were fit to non-linear one-phase decay curves (Figure 3). More than one curve resulting per gene would indicate that As treatment can alter the transcript level decrease with age. For each gene except POLB, the three datasets gave one curve of best fit. With respect to the POLB transcripts, however, the curves deviated significantly from each other due to the effects of exposing 1-week-old mice to 2 or 50 p.p.m. As for 2 weeks (exclusion of the 3-week data points resulted in only a single curve). These observations suggest that As treatment may have induced an increase in POLB expression that attenuated the transcript level decrease with age in mice <8 weeks old.

Discussion

In the absence of As treatment, the levels of all BER transcripts investigated here were highest in 1-week-old mice, diminishing to much lower levels by 8 weeks of age. The initially higher transcript levels suggest that very young mice may rely heavily on BER, possibly because their greater metabolic rates might result in more oxidative DNA damage (21). In turn, the rapid decline in BER transcript levels suggests that the age-related decrease in transcript levels may begin in very young mice and is also consistent with an age-related decrease in BER activity reported elsewhere (31). If so, an age-dependent reduction in BER capacity may occur in a range of tissues and be a general feature of mammals. We detected a decrease in BER transcript levels with age in mouse lung tissue, and diminished BER activity has been observed in brain, liver, spleen and germ cells from older mice, brain, liver and neurons from aging rats and late passage human fibroblasts (23,24,32–36). Despite their consistency with other findings, our results must be interpreted cautiously. Mice 24 weeks old correspond in age to young human adults, and we do not have BER transcript level data for mice towards the end of their life expectancy. Furthermore, it might be that BER proteins play a role in embryonic development distinct from DNA repair, as it has been observed for APE1 (37,38). In this case, high BER transcript levels in neonatal mice, and their subsequent rapid decline with age, might reflect a decrease from elevated transcript levels required for embryonic functions to lower levels necessary for DNA repair in older mice.

![Fig. 3. Effect of As on the age-related decline of BER gene transcript levels. Mice at 1, 8 or 24 weeks of age were mock-treated (open circles) or treated with 2 (filled squares) or 50 p.p.m. (filled triangles) As for 2 weeks. Transcript levels were quantified at the beginning and end of each treatment period and expressed as percentages of those in 1-week-old mice. These values were fitted to a one-phase exponential decay model by non-linear regression using least squares (ordinary) fit, with statistical significance (P < 0.05) of differences in the curves assessed using a sum-of-squares F test to compare the rate constants (constant >0.0, with no weighting), the Y values from 10 to 26 weeks of age and the points where an association between the curves begins.](https://academic.oup.com/mutage/article-abstract/25/5/517/1047025)
On the basis of our data for 24-week-old mice treated with As for 2 weeks, it might be suggested that a reduction of BER activity in aging cells (23,25) could reflect a global decrease in transcription of BER genes. However, this would not be consistent with BER activity in vitro being rate-limited by specific proteins including LIG3 in germ line cell extracts prepared from young male mice. APE1 in extracts from older mice and Polβ in aging rat neurons (23,36). Furthermore, we did not measure mRNA stabilities and translation rates or the locations, stabilities and activities of the BER proteins encoded by the genes we studied. Consequently, while diminished transcript levels may contribute to a decline in BER activity as mice age, post-transcriptional changes in gene expression as well as protein redistribution (31) also may play an important role.

Exposure to As altered BER transcript levels in a gene, age-, dose- and duration-dependent manner. Overall, no consistent change was observed for 1-week-old mice whose mothers were treated with 2 p.p.m. As in drinking water for 24 h or 2 weeks. Although some small increases and decreases were observed, transcript levels still averaged 93% of those measured in untreated neonates. However, mice very efficiently metabolise and eliminate As (28,29). Thus, maternal ingestion of higher concentrations may have been necessary to transmit enough As via lactational milk to achieve doses sufficient to elicit a more general response in 1-week-old mice. Indeed, transcript levels tended to increase by as much as 3.4-fold in 1-week-old mice when mothers were treated with 50 p.p.m. As for 24 h or 2 weeks. These results indicate that exposure of neonatal mice to As via milk from mothers drinking water containing 50 p.p.m. As can induce changes in gene expression. Although As is excreted at very low levels in human breast milk (15–17), the treatments we used were of short duration compared to the chronic As exposures experienced by human populations. Clearly, the effects due to breastfeeding children in such populations cannot be directly extrapolated from our results. Nonetheless, the susceptibility of neonatal mice to low As concentrations via lactational milk from exposed mothers and the inverse correlation of As levels in drinking water with human skin cancer incidence (39) raise serious concerns.

Unlike the results for the 1-week-old mice, no overall increase was observed in 24-week-old mice subjected to any of the four exposure regimens. Instead, transcript levels declined by as much as 80% for all genes tested, though not significantly for LIG3, when 24-week-old mice were exposed to 2 or 50 p.p.m. As for 2 weeks. These reductions were in addition to those attributed to aging alone, and so might point to As exposure potentiating the reduction in BER activity that occurs in older animals, thereby increasing their sensitivity to As. Consistent with this possibility, female mice chronically exposed to 85 μM As in drinking water from post-natal day 21 through 1 year exhibited increased sensitivity to As-mediated carcinogenesis (40). Paradoxically, male mice showed the opposite response (40). Given that long-term in vitro As treatment provokes an increase in apoptosis of human small airway epithelial cells (41), it may be that chronically exposing mice to low As levels depresses BER activity but leads in male mice to an increase in apoptosis and a decrease in tumour development. Still, it is evident, as shown in Figure 3, that aging had a greater impact overall on BER transcript levels than did As treatment. Only in the case of POLB did exposure to As influence the decline in transcript levels with age. However, the transcript level decrease was offset rather than being further reduced and only in the youngest mice treated. Why this occurred for POLB is unclear. Although Polβ is a rate-limiting enzyme for BER in cultured human cells, APE1 and LIG3 are rate-limiting in rodent cell extracts (8,23,36). Yet the age-related declines we observed in the transcript levels for the latter two enzymes were unaffected by As treatment.

In conclusion, we found rapid decreases in the levels of transcripts encoding APE1, LIG1, LIG3, OGG1, PARP1 and Polβ in mice during the first 8 weeks of age that may contribute to the age-related reduction in BER capacity detected in rodent cells and tissues. Furthermore, transcript level alterations detected here in neonatal mouse suggest that the offspring of dams drinking As-contaminated water were exposed to As in lactation milk at concentrations sufficient to cause changes in gene expression. Finally, As-induced BER transcript level reductions in the young adult mice we tested point to the possibility that exposure to As may enhance As sensitivity in older animals.

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References


