Reproductive toxicity in boron exposed workers in Bandırma, Turkey

Nurşen Başaran a,∗, Yağıcın Duydu b, Hermann M. Bolt c

a Hacettepe University, Faculty of Pharmacy, Department of Toxicology, 06100 Sihhiye, Ankara, Turkey
b Ankara University, Faculty of Pharmacy, Department of Toxicology, 06100 Tandoğan, Ankara, Turkey
c Leibniz Research Centre for Working Environment and Human Factors (IfADo), Ardeystr. 67, 44139 Dortmund, Germany

A R T I C L E   I N F O

Article history:
Received 26 January 2012
Accepted 4 April 2012

Keywords:
Boric acid
Occupational exposure
Biological monitoring
Drinking water
Semen
Blood

A B S T R A C T

Boric acid and sodium borates have been considered as being “toxic to reproduction and development”, following results of animal studies with high doses. However unfavorable effects of boron exposure on reproduction and development have not been proved in epidemiological studies so far. The aim of the present study was to investigate the reproductive toxicity indicators in highly exposed workers employed in a boric acid production plant in Bandırma, Turkey. Two hundred and four workers participated in this study. The mean blood boron concentration of the high exposure group of workers was 223.89 ± 69.40 (152.82–454.02) mg/g. Unfavorable effects of boron exposure on the reproductive toxicity indicators were not observed.

© 2012 Elsevier GmbH. All rights reserved.

Introduction

Boric acid and sodium borates have been considered as being “toxic to reproduction and development”, following results of animal studies with high doses. Experimentally, a NOAEL (no observed adverse effect level) of 17.5 mg B/kg-bw/day has been identified for the (male) reproductive effects of boron in a multigeneration study of rats [1], and a NOAEL for the developmental effects in rats was identified at 9.6 mg B/kg-bw/day [2]. These values are being taken as the basis of current EU safety assessments [3]. In this context boric acid and sodium borates have been classified as toxic to reproduction in accordance with the Regulation (EC) No.: 1272/2008 (CLP). However, such effects have not been proven in humans so far.

The present study has been conducted to provide additional data concerning a possible relationship between chronic boron intake and reproductive toxicity outcomes in male workers employed at the Bandırma Boric Acid Production Plant. According to our results, boron has no apparent adverse effect on reproductive toxicity biomarkers in occupational boron-exposed workers. These results are compatible with the results of previously published studies on occupational boron exposed workers in China [4,5]. The results of this study are part of a project on reproductive effects of boron exposure in humans conducted in Turkey [6,7].

Materials and methods

The project plan was reviewed by both Ankara University and Hacettepe University, Ankara, Turkey, and was finally approved by the Ethics Committee of the School of Medicine, Hacettepe University (HEK 08/167, 22.10.2008). All study subjects gave their informed consent in writing prior to participation in the project.

Description of the cohort

The study was conducted at the Boric Acid Plant, Bandırma, Turkey. Bandırma is located at the south coast of the Marmara Sea, distant from the boron mining areas. The Bandırma district serves as a production and exportation zone for boric acid and sodium borates. Boron-exposed workers and most of the control workers were recruited from this zone.

The total number of workers (male) working in production of boron products was 428. One hundred and two of them participated in the study. The total number of workers (male) working in the same zone, but outside of the production area of boron products and not occupationally exposed to boron, was 432. One-hundred and two of them participated in the study. The other details of the cohort were published previously [6].

Biological sampling

Post-shift urine samples were collected in polypropylene containers and kept at −20 °C until urine and creatinine analysis. Blood was collected by venipuncture in suitable blood collection tubes for the determination of follicle-stimulating hormone (FSH),
luteinizing hormone (LH) and total testosterone levels by using an Immulite 2000 Immunoassay Analyzer.

Semen samples were collected in accordance with the recommendations of the World Health Organization [8]. Sperm concentration and motility parameters were determined by using SQA-V Gold Sperm Quality Analyzer in fresh semen samples. Additionally sperm morphology was also analyzed as per the Kruger’s strict criteria [9,10] by one experienced technician at the Research Center on Fertility, School of Medicine, University of Ankara.

**COMET (single cell gel electrophoresis) assay**

The DNA integrity of cryopreserved sperm cells was determined by using single cell gel electrophoresis assay. According to this assay protocol, the cells were embedded in agarose gel, lysed and fragmented DNA strands drawn out by electrophoresis (neutral conditions) to form a COMET. The details of the cryopreservation and COMET assay protocol were published previously [7].

**Boron analysis**

Blood, semen, and food samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) with a flow injection system. Urine, dust, and water samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). Standard reference materials were used for quality control. The details on the determination methods were described previously [6].

**Statistical analysis**

Boxplots, Pearson’s correlation coefficient and linear regressions were used as presented in the figures. For the data of the COMET assay, a Dunnett test was applied to compare with one-sided hypotheses the low, medium and high exposure group with the control group.

**Results**

According to the initial plan it was decided to sample 102 control and 102 occupationally boron exposed workers (n=204) from the boric acid production plant. However due to the high boron concentration in tap water of cafeteria and infirmary (located in boric acid production zone) the control and exposed workers were constituted according to their blood boron concentrations as shown in Table 1. The reproductive toxicity parameters were compared between these groups of workers. This situation is maybe the major limitation of this study. The analyzed endpoints concerning the reproductive toxicity parameters are shown in Fig. 1.

The mean levels of sperm concentration, motility and morphology parameters were statistically not significant between control and exposure groups (low, medium and high) of workers. Also, dose dependent increase in reproductive hormone levels were not observed with increasing blood boron concentrations of the workers as shown in Fig. 2 [6].

![Fig. 1. Reproductive toxicity parameters analyzed in blood and semen samples.](image1)

![Fig. 2. Hormone levels versus increasing blood boron levels. Box plot graphs: ○ outliers and ● extremes. The mean FSH level in high exposed group is not significantly higher than the control group (p > 0.05). The mean LH level in exposure groups is statistically not different from the control group (p > 0.05). The mean total testosterone levels in exposure groups are statistically not different from the control group (p > 0.05).](image2)
The DNA integrity of sperm cells was assessed by using the neutral version of the COMET assay in this study. In spite of a weak correlation coefficient, the correlation between blood boron concentrations and tail % intensity values was statistically significant (Fig. 3).

Discussion

Animal studies revealed that boric acid is toxic to reproduction and development in extreme doses. However these unfavorable effects have not been proven in humans so far. The main objective of this study is to provide additional data on this issue. In spite of the clear evidences of reprotoxic effects of boron in experimental animals, unfavorable effects on reproductive hormone (FSH, LH, and testosterone) levels, sperm concentration, motility and morphology parameters have not been observed in this study. These results are in agreement with the previously published studies on occupational boron exposure in China [4,5].

There are an increasing number of studies on the coherence of sperm DNA integrity and male fertility. High DNA damage in sperm cells appears as a predictor of infertility [11,12]. As expected, there were significant correlations between DNA strand breaks and several indicators of sperm status (motile sperm, number of motile sperm in ejaculate, the percentage of sperm cells with normal morphology, the percentage of neck-mid-piece defects, and the percentage of tail defects) (Table 2). However, our findings suggest that DNA strand breaks (tail % intensity) in the neutral COMET assay do not increase with increases in blood boron concentration, but rather, that a negative correlation apparently exists between this pair of variables (Fig. 3). Our findings suggest that exposure to environmental boron within expected ranges is not detrimental to sperm status and may have “beneficial” properties.

Our study did not demonstrate boron-mediated unfavorable effects on semen parameters, reproductive hormone levels, or DNA integrity in sperm cells. That is, we did not find unfavorable dose-dependent relationships between reproductive toxicity biomarkers and blood boron concentrations in a range of boron intakes common to boron production plant workers. In addition, the relatively extreme boron exposure conditions examined in this and other studies did not result in blood boron concentrations above those considered safe. In this context, the findings to date suggest that exposure to boric acid and sodium borates under normal handling and use conditions are not toxic for reproduction in men.

Acknowledgment

This project was funded by BOREN (2008-G0207) and Eti Mine Works General Management.

Table 2

<table>
<thead>
<tr>
<th>Correlations between DNA strand breaks with semen parameters and male reproductive hormone levels.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seminal parameters</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Motility (%)</td>
</tr>
<tr>
<td>Motile sperm (M/ejac.)</td>
</tr>
<tr>
<td>Sperm conc. (M/mL)</td>
</tr>
<tr>
<td>All sperm (M/ejac.)</td>
</tr>
<tr>
<td>Velocity (APV) (μm/s)</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
</tr>
<tr>
<td>Head defects (%)</td>
</tr>
<tr>
<td>Neck/mid-piece defects (%)</td>
</tr>
<tr>
<td>Tail defects (%)</td>
</tr>
<tr>
<td>Cytoplasmic droplets (%)</td>
</tr>
<tr>
<td>Male reproductive hormones</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
</tr>
</tbody>
</table>

Pearson’s corr. coeff.: -0.184, p<0.05

Fig. 3. Correlation between blood boron levels and tail % intensity values in neutral COMET assay. Linear regressions with 95% individual prediction intervals.

References