Biochemical Pharmacology 83 (2012) 687–693

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

The effect of sodium selenite on liver growth and thioredoxin reductase expression in regenerative and neoplastic liver cell proliferation

Suvd Erkhembayar [a,1], Annelie Mollbrink [a,b,1], Lennart C. Eriksson [a,*]

[a] Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, S-141 86 Stockholm, Sweden
[b] Division of Gastroenterology and Hepatology, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 7 October 2011
Accepted 2 December 2011
Available online 9 December 2011

Keywords:
Sodium selenite
Rat liver cancer
Liver regeneration
Tumour prevention
Thioredoxin reductase 1

ABSTRACT

Selenium in supra-nutritional doses is tumour-preventative in animal models and in humans. In this work, we have compared the effect of sodium selenite on tumour growth in a rat hepatocarcinogenesis model with the effect of sodium selenite on the regeneration of liver mass after partial hepatectomy. In the tumour model, 5 μg/mL sodium selenite in the drinking water reduced the rate of tumour growth for up to 12 months after initiation; the volume fraction of liver cancers was 14 ± 4% with a mean bromodeoxyuridine-index of 19 ± 11% in the treated rats compared to a volume fraction of 26 ± 7% with a mean bromodeoxyuridine-index of 42 ± 27% in the control rats. Despite its efficacy in reducing tumour growth, 5 μg/mL sodium selenite treatment did not affect the gain of liver mass or the rate of cell proliferation after partial hepatectomy. In the regenerating livers, the activity of the cytosolic selenoenzyme thioredoxin reductase (TrxR1) was briefly and transiently increased, an increase further potentiated by sodium selenite. TrxR1 was selectively over expressed in proliferating liver tumours in relation to the surrounding liver tissue in the tumour model, as shown using immunohistochemistry analyses. We suggest that sodium selenite is a suitable candidate for liver cancer prevention in patients with chronic liver diseases that are dependent on sustained liver regeneration due to its differential effects on neoplastic and regenerative cell proliferation. Furthermore, the over expression of TrxR1 in liver neoplasia can only partly be explained by increased growth.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

Selenium supplementation at supra-nutritional levels has been shown in multiple studies to have a tumour-preventive effect [1,2] (for reviews). Several possible mechanisms have been suggested to explain this effect, including the regulation of selenoproteins and direct effects on cellular growth and growth regulation [3–6]. Although a series of preclinical cell and rodent studies have shown that selenium reduces tumour cell growth [2], the tumour-preventive effects of selenium are still controversial and often vary depending on the selenium species, the type of selenium source used and the type of cancer studied [7]. Previous studies in our laboratory [8] as well as recent studies by others [9] have shown that selenite has a liver tumour preventive effect using sodium selenite in a rat liver model, namely by reducing tumour mass. We showed a dose-dependent effect of sodium selenite at doses of both 1 μg/mL and 5 μg/mL administered in the drinking water on both the volume fraction of liver nodules as well as on the rate of cell proliferation in the lesions [8].

The underlying mechanism of selenium tumour prevention and its effects on tumour cell growth are not completely understood [10]. Although the effects of selenite on cell proliferation have been addressed, most studies are performed on cell lines *in vitro*, and only a limited number of studies have been conducted on the effects of selenium on normal liver cells *in vivo* [6]. In this work, we investigated the effects of sodium selenite on liver regeneration after partial hepatectomy. Liver regeneration is regulated by growth factors that are also important during tumour development and progression [11,12], and recent publications have confirmed the relationship between inflammation, repair processes and tumour growth [13,14]. Patients with chronic liver disease (which is associated with increased cancer risk) are dependent on constant hepatocyte regeneration to maintain the functional liver mass and survive. The close relationship between neoplastic growth regulation and the regulation of regenerative growth as well as the opposing requirements for tumour prevention and liver regeneration motivated us to perform this study.

Cytosolic thioredoxin reductase (TrxR1) has been shown to be over expressed in many human cancers, including liver cancer [15–18]. TrxR1 expression was also shown to be markedly increased in

* Corresponding author. Tel.: +46 8 58 58 7858; fax: +46 8 58 58 7730.
E-mail addresses: suvd.erkhembayar@ki.se (S. Erkhembayar), annelie.mollbrink@ki.se (A. Mollbrink), lennart.eriksson@ki.se (L.C. Eriksson).
[1] These two authors share first authorship of this article.

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.12.004

the liver nodules from a rat liver cancer model in comparison both to the tissue surrounding the isolated liver nodules and to the normal liver tissue, though notably, TrxR1 had the same subcellular distribution pattern in the liver nodules and in the normal tissues [19]. TrxR1 is a selenoenzyme with a conserved penultimate C-terminal selenocysteine residue, which is essential for the enzyme's activity. In the liver, two main groups of thioredoxin reductases are expressed: one cytosolic form, TrxR1, and one mitochondrial form, TrxR2 [20,21]. The thioredoxin system is a general protein disulphide reductase system that plays a crucial role in cellular defence against oxidative stress [22]. Moreover, TrxR1 is involved in the regeneration of important antioxidants, such as ubiquinone, lipoic acid and ascorbic acid [23–25], some of which have been shown to be increased in liver neoplasia [26]. TrxR1 is also a key protein in cell proliferation and is necessary for DNA-synthesis [27]; accordingly, one of the suggested mechanisms for selenium tumour prevention is its perturbation of the cell cycle [28,29].

In this *in vivo* study, we have used a rat liver model of tumour development in combination with a model for rat liver regeneration after partial hepatectomy to investigate whether selenium affects the gain of liver mass during regeneration after partial hepatectomy in the same way that it slows down liver tumour growth during tumour promotion and progression. For this part of the study, we have evaluated the rate of volume or mass expansion and the rate of cell proliferation in the models with and without tumour-preventive doses of sodium selenite added to the drinking water. We have also used immunohistochemistry to study the expression of TrxR1 in the early and synchronous phases of tumour progression. The tumour data were compared with the effects of sodium selenite on TrxR1 mRNA expression and enzyme activity during rat liver regeneration.

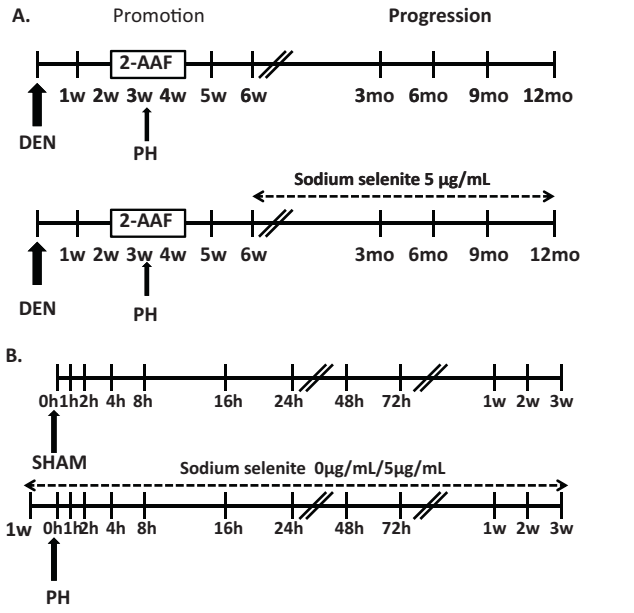

Fig. 1. Schematic presentation of the rat liver models employed. (A) The resistant hepatocyte model for the development of hepatocellular carcinoma in rat liver. Initiation was performed by intraperitoneal injection of a necrogenic dose of diethyl nitrosamine (DEN) at 200 mg/kg body weight. Promotion was performed by feeding a diet containing 0.02% 2-acetylaminofluorene (2-AAF) for 4 days followed by a 2/3 partial hepatectomy (PH). After PH, 2-AAF (20 mg/ml emulsified in agar) was given on day 2 and day 4 by gavage into the lumen of the stomach. Three days before harvest, bromodeoxyuridine (BrdU) Aztec osmotic mini-pumps were implanted subcutaneously. Three rats were harvested at 3, 6, 9 and 12 months after initiation. The relatively low number of rats in each group was compensated for by the study design with a time series. (B) A sham operation or 2/3 partial hepatectomy was performed under Isofluran Baxter® anaesthesia. Rats were harvested at the indicated time points. In the selenium-treated groups, sodium selenite was given in the drinking water starting 1 week before surgery.

2. Materials and methods

2.1. Animal models

Fischer-344 male rats aged 50–65 days and weighing 140–160 g upon arrival were purchased from Charles River Laboratories, Sulzfeld, Germany. The animals were maintained at 20 °C in a 12 h light and dark cycle and were acclimatised for 5 days before starting the experiments. Animal room conditions were controlled in accordance with international standards.

Two sets of animal experiments were performed (Fig. 1). For tumour progression studies, we used the resistant hepatocyte model described by Solt and Farber [30] with slight modifications, as described elsewhere [31] (Fig. 1A). Initiation was performed by a necrogenic dose of diethylnitrosamine (DEN). Two weeks after initiation, when the livers were completely compensated for the cell loss, promotion was performed with 2 weeks of exposure to 2-acetylaminofluorene (2-AAF) in combination with partial hepatectomy as a mitogen. After promotion, the liver weights were restored after 2 weeks as judged by relative liver weight determination. At that time, the toxic effect of the promoter was gone, and the substance was eliminated. Rat livers were harvested at 3, 6, 9 and 12 months after terminating treatment. An Aztec osmotic mini-pump with bromodeoxyuridine (BrdU) was implanted subcutaneously on the back of the rat 3 days before sacrifice. The livers were weighed at harvest and sliced into 4-mm-thick slices. One slice from each lobe was fixed in 4% buffered formalin and embedded in paraffin. The remaining slices were snap frozen in liquid nitrogen and stored at −70 °C until analysis.

For the studies of liver cell proliferation after partial hepatectomy, the animals were divided into 3 groups: two control groups drinking tap water and one selenium-treated group (Fig. 1B). One control group was exposed to a sham operation, while the two other groups were partially hepatectomised. The selenium-treated rats were treated with 5 μg/mL sodium selenite in the drinking water 1 week prior to surgery and were continually provided with selenite water during the entire experiment. The dose of selenite selected was the dose that was tumour preventive in earlier studies [8]. With an estimated water consumption of 10–13 mL/100 g/day, the daily intake of selenium corresponded to 25–30 μg/100 g/day from this source. The selenium content in the rat chow corresponded to 6 μg/100 g/day. The sham operation and partial hepatectomy were performed under inhalation anaesthesia using isoflurane (Isofluran Baxter®, Baxter Medical AB, Kista, Sweden). The sham operation was performed as a laparotomy where the liver was gently compressed *in situ* but not removed, while in the hepatectomised groups, 2/3 of the liver was removed, and the remaining third was allowed to regenerate. At the time points indicated in the figures, three rats were euthanised, and liver and blood samples were harvested. The rats were sacrificed by exsanguination through aortic puncture under Isofluran® narcosis, and the livers were harvested and stored as above.

2.2. Immunohistochemistry

Immunohistochemistry was performed and specificity verified as described before [8]. The following antibodies were used for immunohistochemical staining: for GST-π, anti-GST-Yp/Yf subunit 7 (Biotrin International, Dublin, Ireland); for TrxR1, anti-TrxR1 rabbit polyclonal IgG (Upstate, USA, Cat#07-078); for BrdU-labelled nuclei, anti-BrdU antibody M744 (Dakopatts, Denmark); for Ki-67, M 7248 MIB-5 antibodies (Dako Cytomatation, Glostrup, Denmark).

2.3. BrdU and MIB-5 indices

BrdU-, MIB-5 positive cells and mitotic figures were counted in random image fields until at least 1000 cells were counted. Relevant numbers of cells were determined by accumulated mean. Sections were evaluated under a light microscope (Nikon Eclipse E1000M) at 200× magnification with a Nikon Plan Apo 20×/0.75 DIC M ∞/0.17 WD 1.0 objective. A Nikon DXM 1200F camera was used to capture images.

2.4. Nodule and tumour density

The number and volume fraction of liver tumours were quantified using morphometric densitometry performed on immunohistochemically stained histological slides as previously described [32]. The tumours were identified by both their morphology and growth advantage in relation to the surrounding tissue.

2.5. RNA extraction

Frozen liver tissue (100 mg) was homogenised in 1% β-mercaptoethanol-containing lysis buffer, and total RNA was extracted using the RNeasy Midi Kit (Qiagen GmbH, Hilden Germany). Total RNA was quantified using the RiboGreen® RNA Quantitation Reagent and Kit (Molecular Probes, Leiden, Netherlands).

cDNA was synthesised from 2 μg of quality-checked DNase-treated total RNA with the OmniScript RT Kit (Qiagen GmbH, Hilden, Germany) and oligo(dT) 12-18-mer primers (Invitrogen, Carlsbad, CA, USA).

2.6. Quantitative PCR

Gene-specific primers were used for the amplification of TrxR1 and hypoxanthine guanine phosphoribosyl transferase (HPRT, housekeeping gene) transcripts using an iCycler iQ Detection System (BioRad, Hercules, CA, USA). Samples were analysed in triplicate under the following cycle conditions: 50 °C for 2 min, followed by 50 cycles of 94 °C for 15 s and 60 °C for 60 s. Confirmation of the PCR product was performed using Kiseq (KI, Stockholm, Sweden). Relative mRNA expression was determined according to the ΔΔCT method (Applied Biosystems; User Bulletin #2: Relative Quantitation of Gene expression).

2.7. TrxR1 enzyme activity

TrxR enzyme activity in the liver tissue homogenates was measured by a well-established enzyme assay according to Holmgren and Björnstedt [33] and published previously [8]. The enzyme activity was measured in 25,000 x g(av) supernatant from liver homogenate to adress the activity of the cytosolic TrxR1 family of enzymes and not the mitochondrial TrxR2.

Protein concentrations were determined according to the method described by Lowry et al. [34] using bovine serum albumin as a standard.

2.8. Ethical approval

The study was approved by the Stockholm South Local Community for ethical review of animal experiments.

2.9. Statistical analysis

The data were analysed using STATISTICA software. The Shapiro–Wilk's test was used for analysis of normal distribution. Statistical significance was calculated by either one-way analysis of variance (ANOVA), Student's *t*-test or the non-parametric Mann–Whitney *U* test. p-Values < 0.05 were considered statistically significant. Error bars represent ±SD. Figures were created using GraphPadPrism5 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. TrxR1 expression in growing liver neoplasia

In the promotion phase of the liver cancer model, during which the liver nodules were under the selection pressure of the promoter and expanding with a high growth rate, the nodules were all positive for both the GST-marker and TrxR1 [8]. In the progression phase, most nodules stopped growing in the absence of the promoter. The GST-marker positive areas were seen at all time points, while the

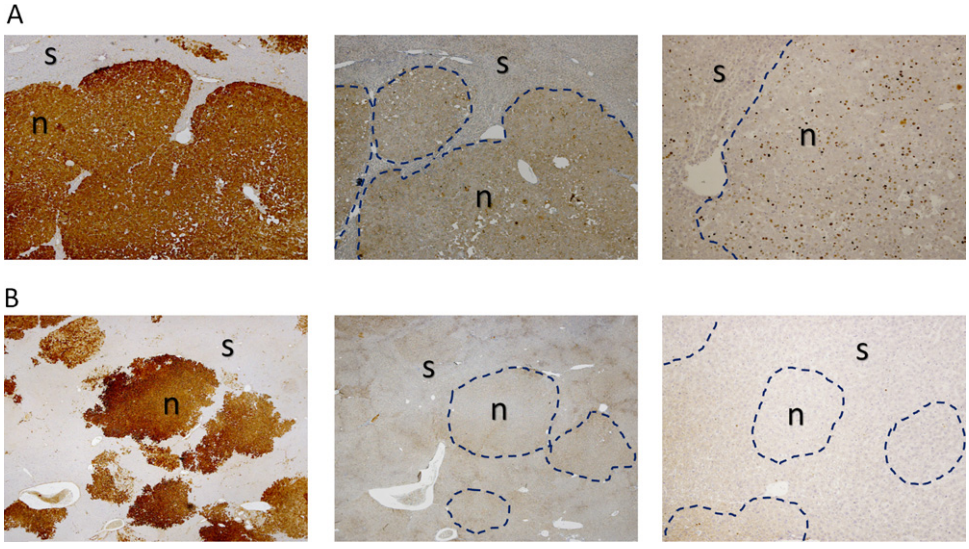

Fig. 2. Rat liver sections from the resistant hepatocyte model were harvested at 12 months. Three days before harvest, BrdU Aztec osmotic mini-pumps were applied subcutaneously. The sections were immunohistochemically stained with antibodies against glutathione S-transferase-π (GST-π), left column; cytosolic thioredoxin reductase 1 (TrxR1), middle column; BrdU, right column. Panel A depicts a GST- and TrxR1-positive neoplastic liver nodule that also showed signs of cell proliferation. In panel B, GST-positive, TrxR1-negative non-proliferating remodelling liver nodules from the same animal are shown (magnification 200×). Both liver nodule tissue (n) and surrounding tissue (s) appear in each slide.

TrxR1 signal faded and ultimately disappeared after 6 months. These nodule-like lesions are defined in the Solt and Farber model as remodelling nodules as they slowly lose their trabecular architecture, nodular structure and classical marker profile. During progression, a small subset of lesions appeared in the nodular liver and continued to grow in the absence of the promoter. These lesions increased in size, developed a marked trabecular architecture and became increasingly dysplastic, eventually acquiring the morphology and growth pattern of hepatocellular carcinoma. Lesions with a high growth rate expressing both the GST-marker and TrxR1 were regarded as neoplastic nodules.

In Fig. 2A, the immunohistochemical appearance of GST, TrxR1 and BrdU in a neoplastic nodule are shown from a rat liver 12 months after promotion. In panel B, a GST-positive, TrxR1-negative and non-proliferating remodelling nodule is shown from the same slide. When the TrxR1 immunohistochemical staining patterns were correlated to the BrdU index in either the same neoplastic lesion or between lesions, there was no correlation between the growth rates (LI-index varying between 10% and 80% (see Fig. 3A)) and TrxR1 expression of ⊕ to ⊕⊕ in the lesions on a relative scale between θ and ⊕⊕⊕ (not shown in figure). TrxR1 also did not reflect the heterogeneity of growth seen in the tumours.

3.2. The effect of sodium selenite on the growth of tumour mass and cell proliferation

In Fig. 3A, cell proliferation in the neoplastic nodules at 3, 6, 9 and 12 months after initiation is expressed as a BrdU index. In the TrxR1-positive lesions in the control rats, the growth rate was high with a mean above 20%. At 12 months, several nodules showed very high labelling indices as well as a marked increase in growth rate heterogeneity between lesions. The effect of selenium on reducing the neoplastic growth rate was significant at all time points ($p < 0.01$) with all lesions below the mean of the control except at 9 months. The heterogeneity in growth rate between lesions was less extensive in the selenium-treated animals, suggesting a regulatory effect by selenite on growth.

The volume fractions of the TrxR1-positive lesions increased with time, with lower values in the selenium-treated animals apparent at all time points, but only reaching statistical significance at 12 months ($p < 0.05$) (Fig. 3B). Variation was high within the cohort of animals drinking tap water. In the 9 months control group, drinking tap water, one rat was lacking TrxR1-positive lesions, which is expected in a cancer model with few neoplastic lesions. The number of GST- and TrxR1-positive proliferating nodules varied between 1 and 7 per liver, reflecting a transformation rate of 0.1–1% of the preneoplastic nodules at the end of promotion (not shown in figure).

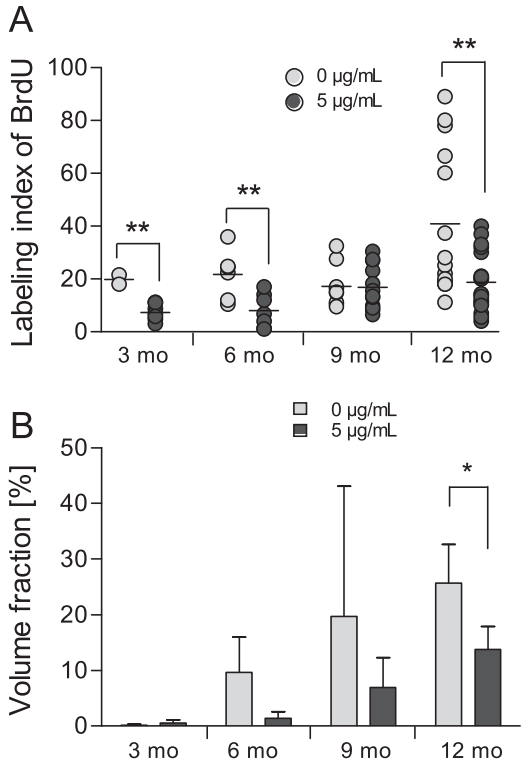

Fig. 3. (A) BrdU-labelling index in lesions positive for GST, TrxR1 and BrdU in rats drinking either tap water or tap water with 5 μg/mL sodium selenite at 3, 6, 9 and 12 months after initiation. Each dot represents one identified lesion with a growth rate above that of the surrounding tissue. All lesions that fulfilled the criteria were counted. Two slides from each rat were examined in a total of 3 rats from each group. (B) Volume fraction at 3, 6, 9 and 12 months after initiation of lesions positive for GST, TrxR1 and BrdU in rats drinking either tap water or tap water with 5 μg/mL sodium selenite. Each value represents the mean of three animals. The huge spread of tumour density seen at 9 months in the selenium-treated animals can be explained by one rat did not having any tumours, which is expected when studying infrequent processes with low probability.

3.3. The effect of selenium on liver regeneration after partial hepatectomy

Over the 3-week experimental time period, there were no significant differences in rat body weight in the experimental groups (Fig. 4A). The slight, consistent but insignificantly lower body weight in the group drinking water with 5 μg/mL sodium selenite did not increase with time. The gain of liver mass after partial hepatectomy was parallel in the two groups that were liver resected and reached the levels of the sham-operated animals after 2–3 weeks (Fig. 4B).

In Fig. 4C and D, the MIB-5 and mitosis indices are shown graphically. After partial hepatectomy (PH), the MIB-5 index was low, constant and not affected by selenium until the time point between 16 and 24 h. At 24 h post-PH, there was a sharp increase in MIB-5 and in the mitotic index. The MIB-5 response was lower in the selenium group than in the group drinking tap water at 24, 48 and 72 h post-PH but was significant only at 72 h ($p < 0.05$). The mitosis index in the tap water group peaked at 24 h, while in the 5 μg/mL selenium rats the index increased with time and showed its highest value at 72 h indication that the peak is later than 48 h ($p < 0.05$).

3.4. Regulation and expression of TrxR1 after partial hepatectomy

Fig. 5A–C graphically illustrates the TrxR1 mRNA variation over time post-surgery. In the animals exposed to sham surgery, there was a significant increase in TrxR1 mRNA at 4 h post-surgery and a decrease at 8 h. A small but insignificant increase in TrxR1 mRNA levels was observed at 24 and 48 h post-surgery, after which the mRNA levels were decreased to back-ground levels. The PH rats both with and without selenite, showed the same variation in mRNA levels over time as was seen in the sham group. The relatively high TrxR1 mRNA level at 0 h in the selenium group was explained by these animals having been drinking water with 5 μg/mL selenium since 7 days before surgery.

The TrxR enzyme activities in the three experimental groups are shown in Fig. 6A–C. The enzyme activity in the sham group was constant over the entire 3 week time period. In the PH group drinking tap water, the activity was low immediately after surgery and rose slowly over the next 8 h. The activity at 16 h was 4-fold that at 0 h and remained high over the next 56 h, ultimately decreasing back to background levels at 2 weeks post-PH. In the selenite-treated group, TrxR1 activity was high at 0 h, decreased markedly at 1 and 2 h post-PH, and increased again at 4 h, with

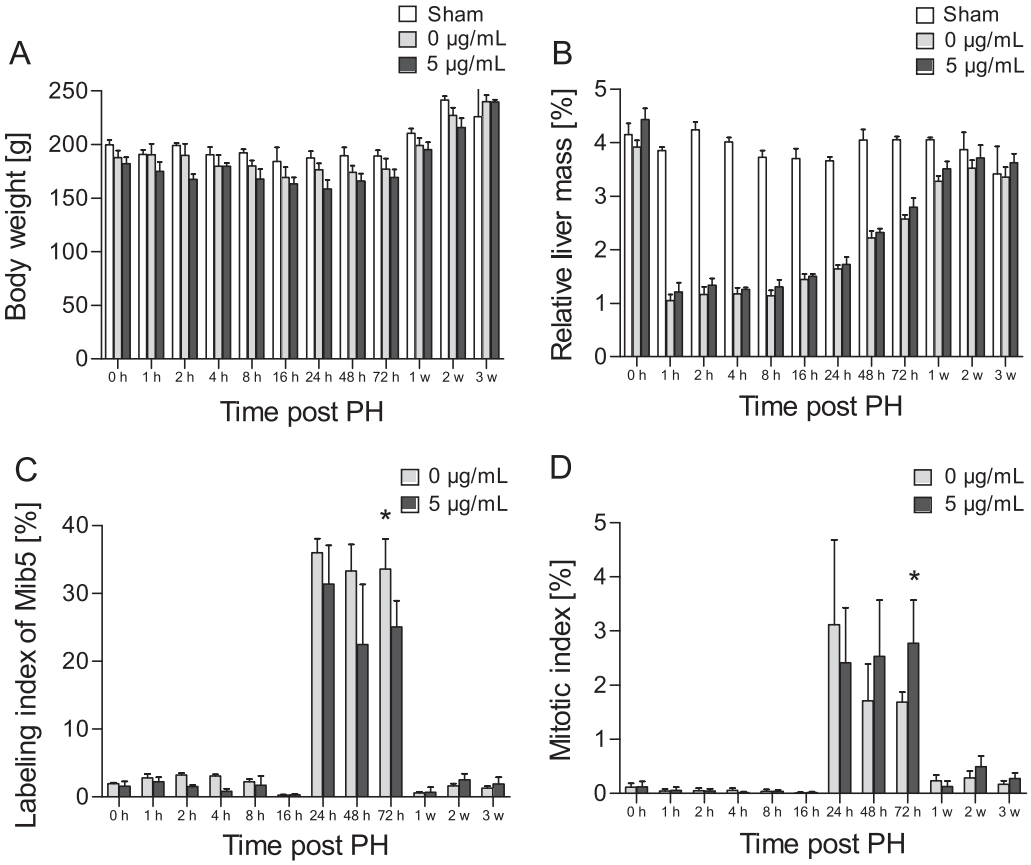

Fig. 4. (A) Rat body weights at different time points after either a sham operation or a 2/3 partial hepatectomy of rats drinking either tap water or tap water with 5 μg/mL sodium selenite as indicated in Fig. 1 (n = 4). (B) The relative liver weights (liver weight per 100 g of body weight) at different time points after either a sham operation or a 2/3 partial hepatectomy of rats drinking either tap water or tap water with 5 μg/mL sodium selenite as indicated in Fig. 1 (n = 4). (C and D) Ki67 (MIB-5) labelling (C) and mitotic index (D) are depicted at different time points after partial hepatectomy on rats either with or without selenium supplementation (n = 3 for 0 μg/mL and n = 5 for 5 μg/mL).

peak activity reached at 48 h. TrxR activity was 7-fold higher in treated animals than non-treated animals at 0 h. After 72 h post-PH, the activity decreased to the levels of the sham group for the rest of the experiment.

Immunohistochemical staining for TrxR1 in the livers of the rats in the group drinking tap water showed a uniform staining in the hepatocytes and bile duct epithelium. At 24 h post-PH, a weak periportal over expression of the enzyme was detected in the area of the liver lobule where MIB-5-positive cells and mitotic figures were observed (zone 1). This zone 1 to zone 3 gradient was observed in 2 out of 4 rats and only at 24 h post-PH. In the selenite-treated group, the immunohistochemical staining pattern was equal to that of the tap water group. We were unable to detect a selenite-induced increase in the active protein between the groups (not shown in figure). Zone 1 over expression of TrxR1 at 24 h post-PH was not obvious in the selenite-treated group.

4. Discussion

Our data and data from others indicate that selenium has a tumour-preventing effect and reduces neoplastic cell proliferation. Furthermore, the selenoenzyme TrxR1 is over expressed in different tumours, tumour cell lines and in our experimental liver tumours. Here, we asked whether TrxR1 is a marker for liver neoplasia and if the effects of sodium selenite on regenerative growth in liver healing processes resemble the effects of sodium selenite on tumours.

We concluded from our animal experiments that TrxR1 was over expressed in liver tumour development from the appearance of initiated liver cells and liver nodules through proliferating, neoplastic liver nodules and liver cancer in the progression phase, but not in the remodelling liver nodules remaining during progression. TrxR1 therefore seemed to be over expressed only in tumours with a neoplastic growth advantage, although there was no correlation between the intra- and intertumoural growth rate heterogeneity and the TrxR1 immunohistochemical signal. We could also observe that the enzyme activity was increased during regenerative cell proliferation; however, this increase was only transient over the time of cell proliferation from 16 h post-PH to 72 h post-PH. Our interpretation was that TrxR1 was indeed a marker for neoplasia, although TrxR1 expression could be partially explained by the fact that the cells were growing. TrxR1 could therefore be a component of resistant hepatocytes, induced in clonal adaptation and dysplasia as a response to the toxicity of the carcinogenic protocol, and could thus be a part of the liver cell defence against toxicity and free radicals. The selenoenzyme is a redox enzyme that can act either directly in the defence system or by regeneration of the lipid-soluble membrane-associated antioxidant ubiquinone (Q10), lipoic acid and ascorbic acid [23–25].

The relevance of TrxR1 in cancer was recently illustrated by TrxR1 removal using si-RNA-mediated knockdown in lung cancer cells resulting in reversal of the neoplastic phenotype and inhibition of malignant characteristics. Furthermore, a TrxR1-deficient cancer cell line was shown to lose growth self-sufficiency by inhibition of both S-phase progression and DNA polymerase expression essential for DNA replication [35]; however, Rollins et al. recently showed that neither normal liver cell proliferation nor regenerative cell growth was affected in transgenic mice with a

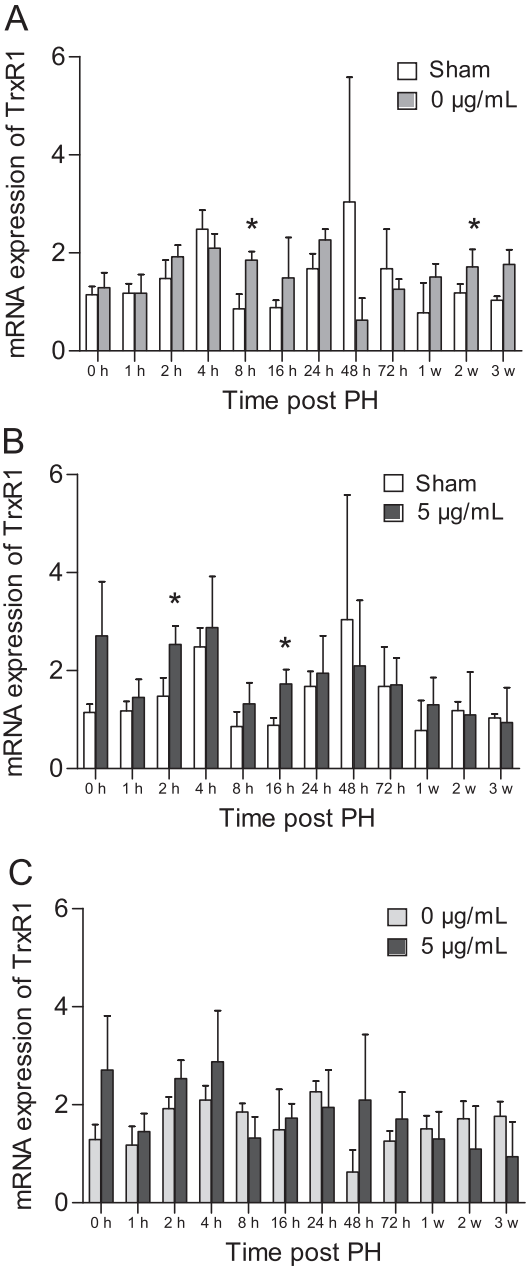

Fig. 5. The expression of TrxR1 mRNA at different time points after partial hepatectomy of Fisher 344 rats treated as in Fig. 4. We have chosen to group the data in this figure in three separate diagrams for easier comparisons of the groups (n = 3 for sham and 0 µg/mL and n = 5 for 5 µg/mL).

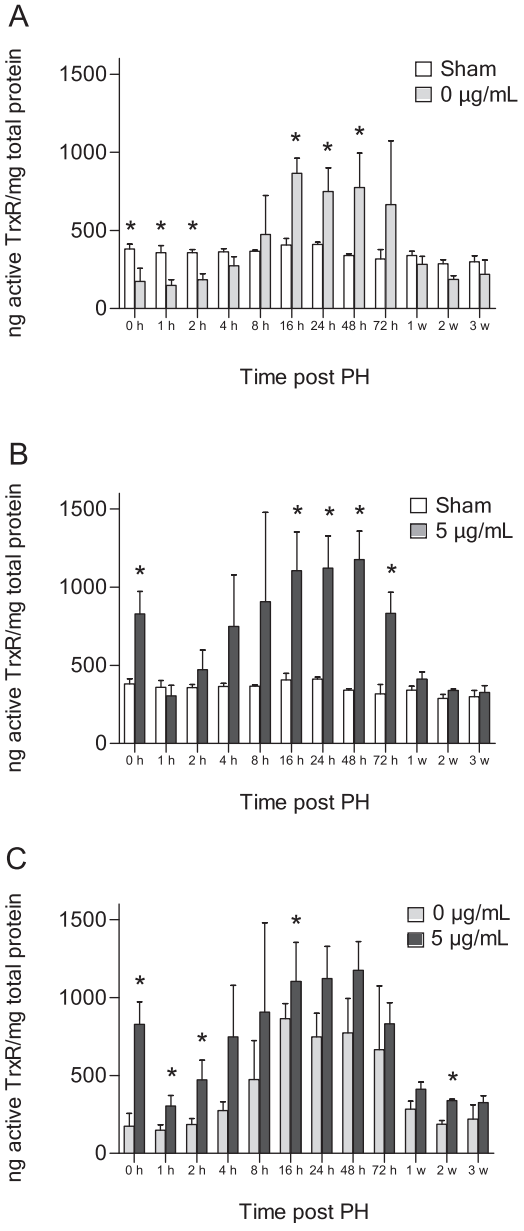

Fig. 6. The specific activity of TrxR at different time points after partial hepatectomy of Fisher 344 rats treated as in Fig. 4. We have chosen to group the data in this figure in three separate diagrams for easier comparisons of the groups. TrxR activity was measured in homogenate 25,200 × g_{av} supernatant. For the number of rats in each group, see Fig. 5.

TrxR1-deficient phenotype [36]. TrxR1-deficient cells have also been shown to effectively maintain a steady-state redox level by up regulating glutathione and inducing glutaredoxin activity [37]. These data indicate backup systems for TrxR1 in its role in activating ribonucleotide reductase in S-phase DNA-synthesis and also in maintaining the capacity for oxidative defence.

Although TrxR1 activity was increased in both proliferating normal hepatocytes and neoplastic cells in growing tumours, selenium did not affect the gain of liver mass or cell proliferation after partial hepatectomy while it significantly inhibited the mass expansion and cell growth in both preneoplastic and neoplastic liver lesions. TrxR1 cannot be excluded as a selenium target in neoplasia, but it is known that selenium can also act directly on growth regulation and growth-related signal transduction in the liver [6]. However, the role of TrxR1 in selenium induced tumour cell specific cytotoxicity might also be indirect, increasing the uptake of selenite and selenide in resistant tumour cells over-expressing NADPH-dependent redox proteins and multidrug resistant glycoprotein (MRP) [38]. The requirements for this explanation are fulfilled in this model over-expressing antioxidants GSH, TrxR1 and MRPs among other components in the resistant phenotype [19,26]. In general TrxR1 is expected to protect the liver nodules from further genetic changes by its antioxidative effect but also to participate in formation of the toxic selenide compound at doses of selenium in the pro-oxidative and oxidative range.

The lack of effect of selenium on the normal and regenerating liver makes it less probable that tumour prevention was mediated

through an effect on the non-neoplastic surrounding liver with secondary effects on the growth of the liver nodules. A probable explanation for the differentiated mitoinhibitory effect of selenium on regenerative and neoplastic growth was that the supra-nutritional and tumour-preventing doses of selenium created a situation of oxidative stress in the TrxR1-overexpressing neoplastic cells that could not be compensated for by further increases of the redox defence potential in the cell; instead, the selenium had a toxic effect that interfered with or compromised growth, possibly also causing cell death [39].

It was interesting to note that TrxR1 mRNA did display a consistent and transient peak at 4 h post-surgery (PH and sham) without a corresponding increase in enzyme activity. This peak correlated in time with the G0–G1 transition, which could be induced by the stress of anaesthesia and surgery. TrxR1 enzyme activity was only induced by the regenerative response after PH. Selenium only had a limited effect on TrxR1 mRNA levels at 4 h post-surgery.

Our experimental data on the differential effects of sodium selenite on neoplastic and regenerative cell proliferation and gain of liver mass support the use of sodium selenite in supra-nutritional doses for liver cancer prevention in patients with chronic liver disease. The effect of selenium on normal liver regeneration *in vivo* does not rule out using selenium in patients with chronic liver disease, including disease stages with an increased need of regenerative cell repair. To implement these finding in tumour-preventive protocols for patients with chronic liver disease, these encouraging experimental data need to be tested in humans. It is also critical to further explore TrxR1 based on existing data as a tool in the diagnostic pathology panel for patients with both increased liver cancer risk and with liver cancer.

Acknowledgements

The authors wish to thank Mrs. Ulla-Britta Torndal for excellent technical support and skilful animal handling and Petra Holmström for establishing the qPCR protocol for TrxR1 in the lab. The animal work was performed in the facilities of the Division of Comparative Medicine at Karolinska University Hospital Huddinge. This work was supported by grants from The Swedish Cancer Society and Karolinska Institutet.

References

[1] Combs Jr GF. Current evidence and research needs to support a health claim for selenium and cancer prevention. J Nutr 2005;135:343–7.
[2] Darvesh AS, Bishayee A. Selenium in the prevention and treatment of hepatocellular carcinoma. Anticancer Agents Med Chem 2010;10:338–45.
[3] Hatfield DL, Yoo MH, Carlson BA, Gladyshev VN. Selenoproteins that function in cancer prevention and promotion. Biochim Biophys Acta 2009;1790:1541–5.
[4] Zhao H, Whitfield ML, Xu T, Botstein D, Brooks JD. Diverse effects of methylseleninic acid on the transcriptional program of human prostate cancer cells. Mol Biol Cell 2004;15:506–19.
[5] Schrauzer GN. Selenium and selenium-antagonistic elements in nutritional cancer prevention. Crit Rev Biotechnol 2009;29:10–7.
[6] LeBoeuf RA, Laishes BA, Hoekstra WG. Effects of selenium on cell proliferation in rat liver and mammalian cells as indicated by cytokinetic and biochemical analysis. Cancer Res 1985;45:5496–504.
[7] Dennert G, Zwahlen M, Brinkman M, Vinceti M, Zeegers MP, Horneber M. Selenium for preventing cancer. Cochrane Database Syst Rev 2011;5:CD005195.
[8] Bjorkhem-Bergman L, Torndal UB, Eken S, Nystrom C, Capitanio A, Larsen EH, et al. Selenium prevents tumor development in a rat model for chemical carcinogenesis. Carcinogenesis 2005;26:125–31.
[9] Alwahaibi N, Mohamed J, Alhamadani A. Supplementation of selenium reduces chemical hepatocarcinogenesis in male Sprague–Dawley rats. J Trace Elem Med Biol 2010;24:119–23.
[10] Jackson MI, Combs Jr GF. Selenium and anticarcinogenesis: underlying mechanisms. Curr Opin Clin Nutr Metab Care 2008;11:718–26.
[11] Nilsson H, Eriksson LC. Growth factor induced mitogenic effects and inositol phosphate responses in primary hepatocyte cultures from normal rat liver and rat liver nodules. Carcinogenesis 1994;15:1821–6.
[12] Columbano A, Shinozuka H. Liver regeneration versus direct hyperplasia. FASEB J 1996;10:1118–28.
[13] Kasper M, Jaks V, Are A, Bergstrom A, Schwager A, Barker N, et al. Wounding enhances epidermal tumorigenesis by recruiting hair follicle keratinocytes. Proc Natl Acad Sci U S A 2011;108:4099–104.
[14] Schafer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol 2008;9:628–38.
[15] Berggren M, Gallegos A, Gasdaska JR, Gasdaska PY, Warneke J, Powis G. Thioredoxin and thioredoxin reductase gene expression in human tumors and cell lines, and the effects of serum stimulation and hypoxia. Anticancer Res 1996;16:3459–66.
[16] Lincoln DT, Ali Emadi EM, Tonissen KF, Clarke FM. The thioredoxin–thioredoxin reductase system: over-expression in human cancer. Anticancer Res 2003;23:2425–33.
[17] Gan L, Yang XL, Liu Q, Xu HB. Inhibitory effects of thioredoxin reductase antisense RNA on the growth of human hepatocellular carcinoma cells. J Cell Biochem 2005;96:653–64.
[18] Fernandes AP, Capitanio A, Selenius M, Brodin O, Rundlof AK, Bjornstedt M. Expression profiles of thioredoxin family proteins in human lung cancer tissue: correlation with proliferation and differentiation. Histopathology 2009;55:313–20.
[19] Bjorkhem L, Teclebrhan H, Kesen E, Olsson JM, Eriksson LC, Bjornstedt M. Increased levels of cytosolic thioredoxin reductase activity and mRNA in rat liver nodules. J Hepatol 2001;35:259–64.
[20] Miranda-Vizuete A, Damdimopoulos AE, Pedrajas JR, Gustafsson JA, Spyrou G. Human mitochondrial thioredoxin reductase cDNA cloning, expression and genomic organization. Eur J Biochem 1999;261:405–12.
[21] Miranda-Vizuete A, Damdimopoulos AE, Spyrou G. cDNA cloning, expression and chromosomal localization of the mouse mitochondrial thioredoxin reductase gene (1). Biochim Biophys Acta 1999;1447:113–8.
[22] Bjornstedt M, Hamberg M, Kumar S, Xue J, Holmgren A. Human thioredoxin reductase directly reduces lipid hydroperoxides by NADPH and selenocystine strongly stimulates the reaction via catalytically generated selenols. J Biol Chem 1995;270:11761–4.
[23] Xia L, Nordman T, Olsson JM, Damdimopoulos A, Bjorkhem-Bergman L, Nalvarte I, et al. The mammalian cytosolic selenoenzyme thioredoxin reductase reduces ubiquinone. A novel mechanism for defense against oxidative stress. J Biol Chem 2003;278:2141–6.
[24] Arner ES, Nordberg J, Holmgren A. Efficient reduction of lipoamide and lipoic acid by mammalian thioredoxin reductase. Biochem Biophys Res Commun 1996;225:268–74.
[25] May JM, Mendiratta S, Hill KE, Burk RF. Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. J Biol Chem 1997;272:22607–10.
[26] Eriksson LC, Andersson GN. Membrane biochemistry and chemical hepatocarcinogenesis. Crit Rev Biochem Mol Biol 1992;27:1–55.
[27] Thelander L, Reichard P. Reduction of ribonucleotides. Annu Rev Biochem 1979;48:133–58.
[28] Sinha R, Medina D. Inhibition of cdk2 kinase activity by methylselenocysteine in synchronized mouse mammary epithelial tumor cells. Carcinogenesis 1997;18:1541–7.
[29] Sinha R, Said TK, Medina D. Organic and inorganic selenium compounds inhibit mouse mammary cell growth in vitro by different cellular pathways. Cancer Lett 1996;107:277–84.
[30] Solt D, Farber E. New principle for the analysis of chemical carcinogenesis. Nature 1976;263:701–3.
[31] Eriksson LC, Torndal UB, Andersson GN. Isolation and characterization of endoplasmic reticulum and Golgi apparatus from hepatocyte nodules in male Wistar rats. Cancer Res 1983;43:3335–47.
[32] Stal P, Hultcrantz R, Moller L, Eriksson LC. The effects of dietary iron on initiation and promotion in chemical hepatocarcinogenesis. Hepatology 1995;21:521–8.
[33] Holmgren A, Bjornstedt M. Thioredoxin and thioredoxin reductase. Methods Enzymol 1995;252:199–208.
[34] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[35] Yoo MH, Xu XM, Carlson BA, Patterson AD, Gladyshev VN, Hatfield DL. Targeting thioredoxin reductase 1 reduction in cancer cells inhibits self-sufficient growth and DNA replication. PLoS One 2007;2:e1112.
[36] Rollins MF, van der Heide DM, Weisend CM, Kundert JA, Comstock KM, Suvorova ES, et al. Hepatocytes lacking thioredoxin reductase 1 have normal replicative potential during development and regeneration. J Cell Sci 2010;123:2402–12.
[37] Mandal PK, Schneider M, Kolle P, Kuhlencordt P, Forster H, Beck H, et al. Loss of thioredoxin reductase 1 renders tumors highly susceptible to pharmacologic glutathione deprivation. Cancer Res 2010;70:9505–14.
[38] Olm E, Fernandes AP, Hebert C, Rundlof AK, Larsen EH, Danielsson O, et al. Extracellular thiol-assisted selenium uptake dependent on the x(c)-cystine transporter explains the cancer-specific cytotoxicity of selenite. Proc Natl Acad Sci U S A 2009;106:11400–5.
[39] Selenius M, Fernandes AP, Brodin O, Bjornstedt M, Rundlof AK. Treatment of lung cancer cells with cytotoxic levels of sodium selenite: effects on the thioredoxin system. Biochem Pharmacol 2008;75:2092–9.