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

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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.

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

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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.

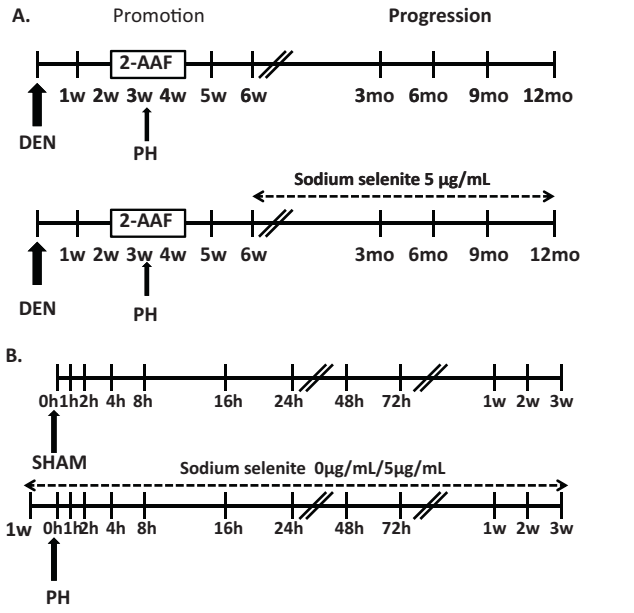

Fig. 1. Schematic presentation of the rat liver models employed. (A) The resistant hepatocyte model for the development of hepatocellular carcinoma in rat liver. Initiation was performed by intraperitoneal injection of a necrogenic dose of diethyl nitrosamine (DEN) at 200 mg/kg body weight. Promotion 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).