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Protective effects of meat from lambs on selenium nanoparticle supplemented diet in a mouse model of polycyclic aromatic hydrocarbon-induced immunotoxicity

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ABSTRACT

Increased environmental oxidative stress caused primarily by chemicals like polycyclic aromatic hydrocarbons, plays significant role in human diseases. A representative compound, 7,12-dimethylbenz(a)anthracene (DMBA), was used for modeling oxidative damages including the significant decrease of the antioxidant capacity of the blood. Selenium has antioxidant effects but with a narrow therapeutic window. In our current studies to avoid accidental overdose and toxicity selenium was given to meat-producing animals. The standard rodent diet of mice was replaced by meat from lambs either on standard or selenium-enriched diet. Selenium concentration of lamb meat was enhanced three times by nano-selenium administration and an increase in the antioxidant capacity of the blood of mice was measured after the indirect selenium supplementation. Protective effects were also observed against DMBA-induced immunotoxicity. Twice the amount of white blood cells and among them three times more phagocytes survived. Similarly, in their renewal system in bone marrow twice the amount of cells survived and regenerative capacity of granulopoiesis was four times higher than in control DMBA-damaged mice. Our findings suggest functional dietary benefits of lamb meat enriched with selenium by feeding lambs with nanoparticle selenium supplements.

1. Introduction

One of the most dangerous threats of our times is an increased level of oxidative stress caused by chemicals and environmental pollution (Braconi et al., 2011; Schoeters et al., 2011). Excessive amounts of reactive oxygen species (ROS) may damage DNA, proteins and membrane lipids; moreover they can weaken the immune system. As a consequence they correlate with development of many diseases (Saeidnia and Abdollahi, 2013). ROS play a critical role in diseases leading mortality lists worldwide – like cardiovascular and malignant diseases – as well as in early aging with increased risk for degenerative disorders (Roberts et al., 2009).

Free radicals are produced by both endogenous and exogenous mechanisms (Kryston et al., 2011). Many external sources of free radicals are known, namely ionizing and UV radiation (Ikehata and Ono, 2011), air pollutants (Rossner et al., 2011) and cigarette smoke (Valavanidis et al., 2009). Environmental pollutants lead to formation of large amounts of free radicals resulting increased oxidative stress (Aseervatham et al., 2013; Eom et al., 2013). Among these polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants (Cavalcante et al., 2012), which can be found primarily in fumes originating from incomplete combustion, such as fumes of vehicles, industry and household heating and in fumed or grilled foods (Lijinsky, 1991; Kitts et al., 2012). It is also known that environmental pollutants, like ubiquitous PAH-compounds, may spread through food web and may reach even humans e.g. by fish consumption (Vives et al., 2005), however their levels are below the margin of exposure recommended by the European Food Safety Authority (Ramalhosa et al., 2012; Benford et al., 2010) similarly to the PAH concentration of traditional meat products (Roseiro et al., 2011). In general, cooking processes are only of a limited value as a means of reducing PAH concentration in food (Perelló et al., 2009). The absorption of PAH compounds from the gastrointestinal tract or lung is good

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and metabolites can be found in almost any tissues and in urine (Feldt et al., 2013; Moon et al., 2012).

PAH compounds form harmful metabolites which have geno- and immunotoxicity with carcinogenic effect through free radical formation (Bjeldanes et al., 1982; Okona-Mensah et al., 2005; Song et al., 2012). In addition to these exogenous sources of free radicals certain endogenous biochemical processes (e.g. inflammation) generate a great number of ROS (Gupta et al., 2011). Endogenous antioxidant defense mechanisms limit and counterbalance the effects of oxidative stress by neutralizing ROS derived both from endogenous and exogenous sources (Birben et al., 2012). These endogenous mechanisms may be supported by exogenous sources of antioxidant materials (Pandey and Rizvi, 2009). Healthy diet contains proper amounts of such components but in recent decades the change in our diet resulted in suboptimal intake, leading to an imbalance between protective and damaging mechanisms.

The first line of endogenous defense against free radicals consists of some selenium containing enzymes. Selenium is an essential micronutrient mainly because of the antioxidant effects of these selenoproteins. Functionally there are at least two different enzyme families of selenoproteins: glutathione peroxidases (GSHPx) and thioredoxin reductases. Being a cofactor of these antioxidant enzymes selenium takes part in scavenging free radicals, thus protecting cells, membranes and cell organelles from lipid peroxidation, enzymes and nucleic acids from the harmful effects of ROS. Selenoproteins control the concentration of reactive oxygen species in various tissues and cells such as neutrophil granulocytes and monocytes (Sureda et al., 2004; Szuchman-Sapir et al., 2012), which have an important physiological role in immune responses by producing high amounts of ROS against microbes. The antioxidant defense capacity of these immune cells can be exhausted by either increased oxidative stress or decreased protection by selenoproteins (e.g. due to selenium deficiency) resulting in immunotoxicity (Song et al., 2012; Szuchman-Sapir et al., 2012; Gust et al., 2013).

It is well recognized that dietary selenium is important for a healthy immune response and regulate the inflammatory mediators in asthma. Selenium has a protective effect against some malignant diseases, it may enhance male fertility and decrease cardiovascular disease mortality (Brown and Arthur, 2001). The oxidative stress is also recognized as a constant feature in critical illness and a metaanalysis of randomized controlled trials comparing the exogenous supplementation of selenium versus standard therapy without any adjuvant showed a longer survival in critically ill adults (Landucci et al., 2013). In pregnant women selenium deficiency (plasma level <62 μg/L) was correlated with lower gestational age and Apgar scores of newborns (Ghaemi et al., 2013). Selenium deficiency is rather frequent in some populations. Moderate selenium deficiency (plasma level <70 μg/L) was measured in 62% of school children in Ethiopia (Amare et al., 2012). In a Spanish study the optimal plasma selenium concentration was established as 90 μg/L because it resulted in the maximal antioxidant activity of glutathion peroxidase in blood. According to this criterion, 50% of the men and 53% of the women were selenium deficient in Spain (Millán et al., 2012). Keshan and Kaschin-Beck diseases indicating serious selenium deficiency are endemic in some regions of China and Qing-Tibet plateau (Chen, 2012; Zhao et al., 2013).

These data support the importance of proper selenium intake but selenium has a narrow therapeutic window (Rayman, 2012; Gromer et al., 2005). Seventy-five to 120 μg/L is accepted as the normal range of selenium plasma concentration (Ghaemi et al., 2013; Zeng, Y.C., et al., 2012) and 1350 μg/L is associated with the risk of selenosis in humans, which is chronic selenium toxicity characterized by hair loss, fingernail changes and brittleness, gastrointestinal disturbances, skin rash, garlic breath and neurotoxicity. Disruption of endocrine function is another toxic effect with disorders in metabolism of thyroid hormones, growth hormones and insulin-like growth factor (Goldhaber, 2003). If it is used in uncontrolled high doses of sodium selenite as a dietary supplement chronic or even acute toxicity may develop with not too high plasma levels of selenium (134 μg/L; Kamble et al., 2009). A serious acute intoxication was published due to the accidental administration of selenium in a dose 10 times higher than the recommended. Serum levels were 350–380 μg/L which resulted in a gross hair loss, nausea, severe diarrhea and headache, complete onycholysis of all nails of the hands and feet. Almost 2 months afterwards the growth of nails and hair restarted (Schuh and Jappe, 2007).

The US National Academy of Sciences recommends that selenium intake should not exceed 400 μg per day and the recommended daily intake is 55 μg for adults. However recently some authors published that long-term selenium administration may worsen glucose homeostasis (Zhou et al., 2013; Zeng, M.S. et al., 2012). In diabetic patients 200 μg daily dose for 3 months resulted in hyperglycemia despite the normal selenium plasma concentrations (72 μg/L) (Faghihi et al., 2013). Genetic variants e.g. in selenoproteins may influence the individual requirements (Villette et al., 2002). Therefore finding other selenium sources with wider therapeutic window would be desirable.

We certified earlier that elemental selenium nanoparticles are less toxic than available inorganic selenium compounds (Benkő et al., 2012). In the current investigation the aim was to assess whether beneficial effects of selenium might be achieved through enhanced selenium amount in food chain to diminish the risk of accidental selenium overdose and toxicity. It is known that lamb meat contains high levels of micronutrients including selenium (Williams, 2007). We tried to further increase the selenium concentration of lamb meat by feeding the animals with the selenium nanoparticles.

Protective effects of selenium are known against ROS-mediated damages caused by arsenic or cadmium poisoning (Xu et al., 2013; Wang et al., 2013). The aim of our studies was to investigate in a mouse model whether selenium supplementation through the food chain has an effect on antioxidant defense mechanisms. Because mice kept on standard diet have no selenium deficiency we also tried to increase the sensitivity of our model by artificially creating an increased level of oxidative stress by using 7,12-dimethyl benz(a)anthracene (DMBA), a well-known PAH compound with geno- and immunotoxicity (Page et al., 2003). In this context DMBA-caused damage was used to model the effects of PAH environmental pollutants. The toxicity of DMBA on primary defense mechanisms was measured by its effects on the total antioxidant capacity of the blood and the immune system. Immunotoxicity was characterized by estimating the survival and renewal of phagocytes, the main actors of natural immunity. Immunotoxicity was measured by estimating survival and renewal of phagocytes, the main actors of natural immunity. This model was also applied to study the potential protective effects of selenium as a food component. For selenium supplementation the standard rodent diet was replaced by lamb meat originated either from lambs on standard diet or from lambs fed with nano-selenium enriched diet.

2. Materials and methods

2.1. Animals

(C57BL/6 xDBA/2) F1 mice (male, 20 weeks-old) were purchased from National Oncology Institute (Budapest, Hungary). They were housed in an animal room with 12-h light and dark periods a day, temperature of 23 ± 2 °C, relative humidity of 60 ± 10% with 5 animals per pen. Food and tap water were available *ad libitum*. As meat is not the main source of food for mice, the groups fed with lamb meat got straw as fiber source and vitamin supplementation into their