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Analysis of plasma indices of redox homeostasis in dairy cows reared in polluted areas of Piedmont (northern Italy)

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HIGHLIGHTS

► The effect of environmental pollutants on blood redox status of bovine was evaluated.
► Pollutant exposure negatively affects blood redox homeostasis of bovine cows.
► Blood redox status represents a biomarker of the extent of animal exposure to environmental pollutants.
► Lipophilic compartment is a selective target of oxidative damage in dairy cows.
► The extent of oxidative damage is correlated to the degree of milk dioxin contamination.

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ABSTRACT

Steel manufacturing is responsible for the emission of pollutants, including dioxins and transition metals, inducing reactive oxygen species generation and DNA damage. Dioxin pollution represents the major cause of milk and dairy product contamination, in Italy, and is associated with oxidative stress-related processes, that may impair health and performance of cows. We evaluated the effect of exposure to different concentrations of pollutants derived from steel manufacturing on blood redox homeostasis of bovine cows. We analyzed two groups of dairy cows (A, B), reared in two different polluted areas, and a control group of cows bred in an industry free area. The extent of exposure to contaminants was defined by measuring dioxin level in bulk milk samples collected from animals of each farm. This level was lower in milk of group A than in group B. Plasma concentrations of retinol, alpha-tocopherol and ascorbate, the total antioxidant capacity, and the activities of superoxide dismutase and glutathione peroxidase were higher in control group than in exposed groups. In particular, retinol and tocopherol levels were higher in the group with lower milk dioxin level. Plasma titers of protein-bound carbonyls (PC), nitro-tyrosine, and hydroperoxides were lower in control group than in A or B. Hydroperoxides and PC plasma concentrations were increased in the group with higher milk concentration of dioxin. Our results demonstrate that, irrespective of the nature of chemicals inducing oxidative modifications, the extent of damage to plasma protein and lipid, is correlated with the concentration of dioxin in milk. So, the characterization of blood redox status might be a useful tool for identifying animals exposed to environmental pollutants. Plasma concentrations of retinol, alpha-tocopherol, PC and hydroperoxides could therefore represent good indices of the extent of animal exposure, as they significantly change in groups with different milk concentrations of dioxin.

1. Introduction

Iron and steel manufacturing plants are responsible for the emission of noxious pollutants, such as dioxins, sulfur oxides, nitrogen oxides, hydrocarbons, carbon monoxide and transition metals, that can exert genotoxic and/or carcinogenic effects (Borská et al., 2003; Desoize, 2003; Mateuca et al., 2005; Cavallo et al., 2008). Waste incineration and illegal disposal of industrial waste represent major sources of environmental and food chain pollution by dioxins (Brambilla et al., 2004). In particular, dioxin pollution has occurred in Italy, especially

Abbreviations: Ret, retinol; Toc, alpha-tocopherol; Asc, ascorbic acid; N-Tyr, nitrotyrosine; PC, protein-bound carbonyls; LPO, lipid hydroperoxides; SOD, superoxide dismutase; GPx, glutathione peroxidase; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; PCBs, polychlorinated biphenyls; WHO, World Health Organization; TEQ, toxic equivalents.

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in Campania and in industrial areas of Piedmont, Lombardy, Tuscany and Puglia (Biasioli and Ajmone-Marsan, 2007; Ingelido et al., 2009; Turrio-Baldassarri et al., 2009), leading to contamination of dairy milk in sheep, cattle and river buffaloes (Iannuzzi et al., 2004; Perucatti et al., 2006; Cirillo et al., 2008; Esposito et al., 2009). Dioxins are polyhalogenated aromatic hydrocarbons, highly toxic and persistent, present at low level in air, soil, water, feed as well as in foods such as dairy products (Matsumura, 2003). Dioxins share a common toxicity mechanism, that is mediated via binding to a specific intracellular receptor, the aryl hydrocarbon receptor (AhR) (Alsharif et al., 1994; Mandal, 2005), whose activation is responsible for the enhanced expression of genes coding for cytochrome P450 1 family enzymes in the liver of several species, including cattle (Safe, 1986; Whitlock, 1990; Machala et al., 1998; Matsumura, 2003; Guruge et al., 2009). Dioxin exposure promotes highly reactive oxygen species (ROS) production (Slezak et al., 2000; Nebert et al., 2000; Dalton et al., 2002), and depression of several ROS quenching systems (Ishida et al., 2009), thus inducing increased DNA fragmentation, as well as production of superoxide anion, thiobarbituric acid reactive substances, and hydroperoxides (Shertzer et al., 1995; Zhao and Ramos, 1998; Slezak et al., 2000, 2002). Dioxin-dependent sustained oxidative conditions (Shertzer et al., 1998; Senft et al., 2002a, 2002b) overwhelm antioxidant defences, leading to oxidative stress-related processes (Cadenas and Davies, 2000; Halliwell and Gutteridge, 2000; Mandal, 2005; Pelclova et al., 2011). In physiological conditions, the antioxidant defence system scavenges oxygen and nitrogen-reactive species, thus limiting or preventing oxidative damage (Halliwell, 2012). The defence system includes enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants, such as ascorbic acid (Asc), retinol (Ret), and alpha-tocopherol (Toc) (Halliwell and Gutteridge, 2000; Galli and Azzi, 2010; Halliwell, 2012). Oxidative stress occurs as a consequence of an imbalance between ROS production and neutralizing capacity of antioxidant mechanisms, and is associated with modifications of physiological and metabolic functions (Halliwell and Gutteridge, 2000). In particular, it was reported that oxidative stress impairs health, fertility and zootechnical performance of dairy cows (Miller et al., 1993b), and is involved in the etiology of several diseases and metabolic disorders (Harrison et al., 1984; Smith et al., 1984; Gröhn et al., 1989; Lomba, 1996; Bernabucci et al., 2002, 2005). Feedstuffs are the major source of dioxin intake by dairy animals, as a consequence of the pollution of pastures and other feed ingredients (Brambilla et al., 2004). Dioxins accumulate in adipose tissue, liver, and muscles, and can be also transferred into milk and eggs, thus animal productions represent by far the major source of exposure for humans (Thomas et al., 1999).

The objective of this study was to analyze the effect of exposure to different concentrations of environmental pollutants derived from steel manufacturing on blood redox homeostasis of bovine cows, in order to define the potential targets of oxidative damage in blood, and to identify biomarkers useful for evaluating both the extent of animal exposure to environmental contaminants and the milk quality. Plasma concentrations of Ret, Toc and Asc, the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured, and used as indices of the antioxidant defence system. Also, plasma concentrations of nitro-tyrosine (N-Tyr) and protein-bound carbonyls (PC) were used as markers of oxidative damage to protein, and plasma level of lipid hydroperoxides (LPO) was used as index of the extent of lipid peroxidation induced by the interaction of free radicals with polyunsaturated fatty acids. It is worth mentioning that N-Tyr level represents the footprint of protein oxidative damage induced by peroxynitrite (Halliwell, 1997), while PC may be introduced into proteins by direct oxidative attack to proteins themselves (Kristal and Yu, 1992), or by reactions with aldehydes originated during lipid peroxidation processes (Uchida and Stadtman, 1993). These parameters were analyzed in plasma collected from bovine cows, reared in two different dairy farms localized in two different areas, near a high-temperature steel production plant, and in plasma obtained from dairy cows reared in the same valley, but in a farm located in an industry free area. Although steel manufacturing is associated with the emissions of numerous dangerous pollutants, the extent of exposure to environmental contaminants was quantified by measuring bulk milk dioxin and dioxin-like polychlorinated biphenyls (PCBs), expressed as toxic equivalents (TEQ). Indeed, due to the presence of dioxins in the food chain, TEQ measurement is widely used, in the context of animal production, to assess milk safety, and to evaluate the contamination of raw milk and dairy products (Cirillo et al., 2008; Esposito et al., 2009), according to European Union Regulation (Commission Regulation 1259/2011/EC).

2. Materials and methods

2.1. Materials

Bovine serum albumin fraction V (BSA), chemicals of the highest purity, Goat anti Rabbit IgG-horseradish peroxidase linked (GAR-HRP), Rabbit anti-dinitrophenylhydrazine (anti-DNP) IgGs, and standards for high performance liquid chromatography (HPLC) were purchased from Sigma-Aldrich (via Gallarate 154, 20151, Milan, Italy). The Nucleosil 100-NH_2 column (5 µm particle size, 250×4.6 mm I.D.) and the Nova-PAK C18 column (4 µm particle size, 125×2 mm I.D) were obtained from Macherey–Nagel (distributed by Delchimica Scientific, via Ruffini, 20123, Milan, Italy). Organic solvents were purchased from Romil (distributed by Delchimica Scientific, via fratelli Ruffini, 20123, Milan, Italy). Polystyrene 96-wells plates were purchased from Nunc (distributed by VWR International, via Stephenson, 94, I-20157, Milan). Nitrated BSA and the kit for titration of lipoperoxide of Cayman Chemical, as well as rabbit anti-nitrotyrosine IgG of Covalab were purchased by Vincibiochem (Via Ponte di Bagnolo, 10, 50059 Vinci, Italy).

2.2. Farm selection and animals

The study was carried out in the Susa Valley (Piedmont, northern Italy) on 36 dairy cows (mainly Piedmontese×Valdostana crossbreds) reared in two different dairy farms (A and B) localized in two different areas, both near a high-temperature steel production plant. These cows were fed on contaminated fodder, and were then regarded as groups exposed to dioxins and other environmental pollutants. Nineteen Valdostana dairy cows reared in a farm located in the same valley, but in an industry-free area, were also included in the study as control group (K). In particular, the extent of exposure to environmental pollutants was defined by measuring toxic equivalent (TEQ) values of polychlorodibenzodioxins (PCDDs), dioxin-like polychlorobiphenyls (DL-PCBs), and polychlorodibenzofurans (PCDFs) in bulk milk samples obtained from animals of group A (N=18), B (N=18), and K (N=19). These values amounted to 8.56 pg/g fat (farm A) and 18.56 pg/g of fat (farm B), thus exceeding those legally permitted (5.5 pg/g fat as the sum of PCDDs, PCDFs and DLPCBs), according to the European Union legislation in force (Commission Regulation 1259/2011/EC). Conversely, TEQ values measured in bulk milk from farm K (1.75 pg/g fat) were lower than those recommended by European Union (1.75 pg/g of fat as the sum of PCDDs and DL-PCDFs/PCBs) (Table 1). The milk sampling was performed by the Regional Veterinary Services, and the analyses of dioxin level in milk samples were carried out by Istituto Zooprofilattico Sperimentale of Piedmont, Liguria, and Valle d'Aosta using a validated High Resolution Gas Chromatography Mass Spectrometry method.

In each farm blood samples were collected, early in the morning, in the same day and under the same environmental conditions, into heparinized tubes. Plasma was obtained by centrifugation (500 g; 15 min; 4 °C), and processed, by the same operator, for titration of antioxidants, SOD and GPx activity, total antioxidant capacity (TAC), nitro-tyrosine (N-Tyr), protein-bound carbonyls (PC), and hydroperoxides (LPO).