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Changes in mammary histology and transcriptome profiles by low-dose exposure to environmental phenols at critical windows of development☆

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ABSTRACT

Exposure to environmental chemicals has been linked to altered mammary development and cancer risk at high doses using animal models. Effects at low doses comparable to human exposure remain poorly understood, especially during critical developmental windows. We investigated the effects of two environmental phenols commonly used in personal care products – methyl paraben (MPB) and triclosan (TCS) – on the histology and transcriptome of normal mammary glands at low doses mimicking human exposure during critical windows of development. Sprague-Dawley rats were exposed during perinatal, prepubertal and pubertal windows, as well as from birth to lactation. Low-dose exposure to MPB and TCS induced measurable changes in both mammary histology (by Masson's Trichrome Stain) and transcriptome (by microarrays) in a window-specific fashion. Puberty represented a window of heightened sensitivity to MPB, with increased glandular tissue and changes of expression in 295 genes with significant enrichment in functions such as DNA replication and cell cycle regulation. Long-term exposure to TCS from birth to lactation was associated with increased adipose and reduced glandular and secretory tissue, with expression alterations in 993 genes enriched in pathways such as cholesterol synthesis and adipogenesis. Finally, enrichment analyses revealed that genes modified by MPB and TCS were over-represented in human breast cancer gene signatures, suggesting possible links with breast carcinogenesis. These findings highlight the issues of critical windows of susceptibility that may confer heightened sensitivity to environmental insults and implicate the potential health effects of these ubiquitous environmental chemicals in breast cancer.

1. Introduction

Animal studies have unequivocally demonstrated that environmental chemicals can alter mammary gland development and subsequent cancer risk, depending on timing of exposure (Rudel et al., 2011). For example, prenatal exposure to bisphenol A (BPA) altered mammary gland morphology and gene expression signature indicative of transformation (Moral et al., 2008). We previously showed that lactating mammary glands were more sensitive to chemical exposures compared to glands at later stages of development (Manservisi et al., 2015). In the context of breast cancer, gestation, early childhood, puberty and pregnancy may represent 'windows of susceptibility' to environmental insults (Rudel et al., 2011), as all of these developmental stages are characterized by profound changes in mammary gland structure and function (Macias and Hinck, 2012) that may alter its susceptibility to cancer (Russo et al., 2001).

The translation of findings on environmental chemical effects from animal models into humans has been impeded by a number of limitations. First, doses tested in animals have usually been several orders of magnitude higher than human exposures (Rayner et al.,

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2005; White et al., 2007) when in fact environmental chemicals have been found to elicit adverse effects at low concentrations relevant to human exposures (Gioiosa et al., 2015; Jacobsen et al., 2012; Manservisi et al., 2015; Welshons et al., 2003). Indeed non-linear dose response relationships have been reported for several environmental chemicals (Welshons et al., 2003). Furthermore, understanding of the mechanisms of action of these chemicals during normal mammary gland development has been largely limited because most animal studies employ carcinogen-induced models (Jenkins et al., 2007; Lamartiniere et al., 2011).

Sources of environmental chemicals, including phenols, are diverse and ubiquitous. We focus on two environmental phenols, methyl paraben (MPB) and triclosan (TCS), both of which are commonly added in personal care and household products. Parabens are a group of lipophilic compounds easily absorbed through skin and are widely used as antimicrobial preservatives in cosmetics, toiletries and pharmaceuticals (CIR, 2008). Methyl paraben (MPB) is one of the most prevalent parabens detected in urine in the US population (Ye et al., 2006). Triclosan (TCS) is commonly used as an antimicrobial agent in cosmetics, pharmaceuticals, toys and medical devices, with major exposure occurring via dermal and oral routes (Yueh and Tukey, 2016). Both chemicals have been detected in breast milk (Ye et al., 2008) MPB was detected in human placenta (Towers et al., 2015) and TCS was found to accumulate in rodent placenta (Feng et al., 2016; Wang et al., 2015). Moreover, both chemicals were detected in cord blood (Pycke et al., 2014, 2015), indicating that MPB and TCS can enter the fetal environment through placental transfer, and also be transferred via breast milk. There are growing public health concerns with these chemicals because not only are their exposures prevalent in the general US population (CDC, 2015), they have been found to be associated with adverse outcomes, for example, TCS with body mass index (Lankester et al., 2013) and with earlier pubertal onset in girls (Wolff et al., 2015).

Using the Sprague-Dawley (SD) rat model, we examined effects of MPB and TCS on histology and transcriptome profiles of normal (non-cancerous) mammary glands at doses mimicking human exposure. Animals were exposed across several key developmental stages including perinatal, prepubertal and pubertal windows as well as long-term exposures from birth to lactation. Results from our study clearly demonstrate that even at levels comparable to human exposure, environmental phenols can induce changes in mammary histology and gene expression in a window-specific fashion and these changes may have potential implications for breast cancer development.

2. Materials and methods

2.1. Test chemicals

Methyl paraben (MPB; CAS # 99-76-3, lot # BCBG0852V, 99% purity) and triclosan (TCS; CAS # 3380-34-5, lot # 1412854 V, 97% purity) were supplied in plastic containers (Sigma Aldrich, Italy). Compounds were dissolved in the vehicle olive oil (lot #111275, Montalbano Agricola Alimentare Toscana, Italy). All compounds were stored in the dark at room temperature (20 °C). The solutions were prepared weekly on the basis of mean body weight of each group and were continuously stirred before and during the treatment. To minimize external contamination, the olive oil and chemicals were stored in glass containers and administered using 5 mL glass syringes. Biological samples were collected in polypropylene vials. Chemical analyses and stability testing have been described previously (Manservisi et al., 2015).

2.2. Experimental animals

Animal studies were carried out at Cesare Maltoni Cancer Research Centre/Ramazzini Institute (CMCRC/RI) (Bentivoglio, Italy) in accordance with the rules of Italian law for Animal Welfare (Decreto Legislativo 26, 2014), following the principles of Good Laboratory Practices and Standard Operating Procedures of the CMCRC/RI facility, which include authorization by the ethical committee. The experiment used female Sprague-Dawley (SD) rats which belong to the colony that has been used for over 40 years in the laboratory of the CMCRC/RI. There were no siblings in each treatment group and they were randomized so as to have minimal differences in body weight among them (standard deviation < 10% of the average). Animals were housed in makrolon cages (41×25×15 cm) at 2 or 3 per cage, with a stainless steel wire top and a shallow layer of white fir shavings as bedding (Giuseppe Bordignon, Reviso, Italy). All animals were kept in a single room at 23 ± 3°C and at 40–60% relative humidity. Lighting was artificial and the light/dark cycles were tended to be 12 h each. All animals were given the same standard “Corticella” pellet diet (Piccioni Laboratory, Milan, Italy). Feed and tap water were available ad libitum and were both periodically analyzed to exclude biological and chemical contamination (mycotoxins, pesticides, arsenic, lead, mercury, selenium). F0 generation corresponds to breeders of the experimental animals (F1). The breeder animals were weighed weekly to determine treatment dose, starting from gestation for the perinatal group and during lactation for prepubertal, pubertal, and long-term treatment groups and the dose to be administered was calculated on the basis of the weekly weight. For the perinatal group, all the F1 pups were housed with their dams until sacrifice at postnatal day (PND) 21. F1 pups from all the other groups were housed with their dams until weaning (PND 28), then separated from the dam, identified by ear punch, weighed individually every week and dosed by gavage based on the weekly mean body weight of each group. After weaning, each litter contributed 1 female pup to the study. For animals in the long-term treatment group, water and food consumption, and body weight were recorded weekly for the first 13 weeks of the experiment and every other week thereafter.

2.3. Chemical treatment

Female SD rats were treated with MPB or TCS at 0.105 and 0.05 (mg/kg/day), representing 1/10,000 and 1/1000 no observed adverse effect levels (NOAEL) of these chemicals, respectively (LA Goldsmith, 1983; Rodriguez and Sanchez, 2010; U.S. EPA, Offices of Prevention, Pesticides, and Toxic Substances.). These low doses were previously determined to produce urinary metabolite concentrations comparable to those reported for the US population (Teitelbaum et al., 2016). Control animals received olive oil. The number of rats used in experiments is shown in Table 1. There were three short-term windows (perinatal, prepubertal, pubertal) and one long-term window of

Table 1
Number of female Sprague-Dawley (SD) rats used in the study. Number of animals receiving treatment by oral gavage with MPB or TCS or vehicle control (olive oil) in each treatment window; (number of animals in histological analyses using Masson's Trichrome staining, number of animals in transcriptome analyses using microarrays). A grand total of 69 animals were used in this study. Age-matched female SD rats from the same RI breeding facility (‘untreated animals’) were also evaluated by histology; these animals are not shown in this table.

	Control	MPB	TCS	Total *n* in Windows
Perinatal	10 (9, 10)	10 (9, 10)	10 (10, 9)	**30 (28, 29)**
Prepubertal	5 (5, 5)	5 (5, 5)	5 (4,3)	**15 (14, 13)**
Pubertal	5 (5, 5)	5 (4, 5)	5 (5, 3)	**15 (14, 13)**
Lactation	3 (3, 3)	3 (3, 0)	3 (3, 3)	**9 (9, 6)**
Total *n* in control and treatment groups	**23 (22, 23)**	**23 (21, 20)**	**23 (22, 18)**	**69 (65, 61)**