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A mixture of Persistent Organic Pollutants (POPs) and Azoxymethane (AOM) show potential synergistic effects on intestinal tumorigenesis in the A/J Min/+ mouse model

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HIGHLIGHTS

- Can a mixture of POPs affect intestinal tumorigenesis in the A/J Min/+ mouse?
- Mice were exposed to POPs through the diet and received an injection of Azoxymethane.
- Results show an increased intestinal tumorigenesis in the A/J Min/+ mouse model.

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ABSTRACT

A multitude of cancer types, including breast, testicular, liver and colorectal cancer, have associations with exposure to Persistent Organic Pollutants (POPs). The present study aimed to investigate whether a mixture of POPs could affect intestinal tumorigenesis in the A/J Min/+ mouse, a model for human colorectal cancer (CRC). Pollutants were selected for their presence in Scandinavian food products and the mixture was designed based on defined human estimated daily intake levels. Mice were exposed through the diet, at control, low and high mixture concentrations, for 10 weeks. In a separate experiment, mice also received one subcutaneous injection of Azoxymethane (AOM) to explore whether this carcinogenic compound influenced the effect of the POPs. Intestinal tumorigenesis was examined by surface microscopy and histopathology. Moderate and dose-dependent increases in tumorigenesis were observed after dietary POP exposure. The AOM treatment alone stimulated the growth of colonic lesions, but did not increase the formation of new lesions. Combined AOM treatment and POP exposure demonstrated a synergistic effect on lesion formation in the colon, and to a lesser extent in the small intestine. This synergy was also evident by an increased number of malignant colonic tumors (carcinomas). In conclusion, the study shows that a mixture of POPs interacted synergistically with a known carcinogen (AOM), causing increased intestinal tumorigenesis in the A/J Min/+ mouse model.

1. Introduction

Persistent Organic Pollutants (POPs) are man-made chemicals that are toxic to humans and wildlife, resistant to degradation and have the potential to bioaccumulate and biomagnify in living organisms (UNEP, 2015). The compounds have adverse health effects and have been associated with an increased risk of breast cancer (Hoyer et al., 2000; Cameron and Foster, 2009), testicular cancer (McGlynn et al., 2008; Giannandrea et al., 2011), liver cancer (Filgo et al., 2015), and colorectal cancer (Howsam et al., 2004; Song et al.,

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2014). The main route of non-occupational exposure to POPs in humans is through ingestion (Darnerud et al., 2006; Vestergren et al., 2012), which makes the GI tract the first organ of exposure. Traditional animal experiments only assess the impact of POPs using single compounds (Sethi et al., 2017) or compounds belonging to the same chemical group (Colter et al., 2018). However, carcinogenesis is a multistep process, so focus on individual compounds may prevent the discovery of potential synergism between multiple chemicals.

Colorectal cancer (CRC) is the third most common cancer in humans worldwide and exposure to carcinogens through the diet is an essential risk factor (IARC, 2016). CRC develops as a result of several genetic and epigenetic changes that cause a transformation of intestinal epithelium from normal tissue, via benign neoplasms, into carcinomas (Kinzler and Vogelstein, 1996; Sancho et al., 2004). Up to 85% of CRC cases are considered sporadic and 1% are attributed to the hereditary CRC syndrome known as familial adenomatous polyposis (FAP) (Burt, 2000). Mutations in the tumor-suppressor gene adenomatous polyposis coli (*APC*) are responsible for FAP, and patients develop a vast number of adenomatous polyps in the intestine, which are likely to progress into malignant tumors (Kinzler and Vogelstein, 1996). In addition, dysfunctional *APC* alleles have been found in the majority of sporadic colorectal lesions (Fodde, 2002). Research on CRC caused by *APC* mutations is therefore highly relevant to human health.

The most widely used animal model for human CRC is the multiple intestinal neoplasia (Min/+) mouse. This mouse has a heterozygous mutation in the *Apc* gene, resulting in a truncated gene product at amino acid 850 (Su et al., 1992). Inactivation of the remaining functional allele in the intestinal epithelium appears to be the rate-limiting step in tumorigenesis (Luongo et al., 1994). Loss of *Apc* inhibits the formation of the β-catenin destruction complex, leading to accumulation of β-catenin in the cytoplasm and subsequent translocation to the nucleus. Here, it interacts with the transcription factor Tcf-4, creating an active complex that transcribes specific target genes (Fodde, 2002; Kretzschmar and Clevers, 2017). The conventional Min/+ mouse model, bred on a C57BL/6 genetic background (Moser et al., 1990), develops lesions primarily in the small intestine (Mollersen et al., 2004). The A/J Min/+ mouse, on the other hand, also develops a large number of lesions in the colon, many of which progress to carcinomas over time (Sødring et al., 2016b). Therefore, the A/J Min/+ mouse model more closely resembles CRC development in humans and was therefore chosen for the present study.

The A/J strain has been shown to be more susceptible to the induction of colorectal cancer by Azoxymethane (AOM) than its C57BL/6 counterpart (Nambiar et al., 2003; Meunier et al., 2011). AOM is a genotoxic chemical used to mimic sporadic CRC and to study the underlying mechanisms of sporadic colorectal carcinogenesis (Venning FA, 2013). Following metabolic activation by cytochrome P450 enzymes (mostly CYP2E1), AOM reacts with DNA and causes adduct formation, leading to DNA mutations initiating colorectal carcinogenesis (Takahashi and Wakabayashi, 2004).

The aim of this study was to investigate whether dietary POP exposure, alone or following AOM treatment, could affect intestinal tumorigenesis in the A/J Min/+ mouse model. The mixture was designed to simulate a real-life exposure scenario relevant to humans (Berntsen et al., 2017).

2. Animals, materials and methods

2.1. Ethics statement

The study was performed at the Section for Experimental Biomedicine at The Norwegian University of Life Sciences in Oslo, Norway. The animal facility is licensed by the Norwegian Food Safety Authority (https://www.mattilsynet.no/language/english/) and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (https://www.aaalac.org/). The animal experiment was approved by the unit's animal ethics committee (Institutional Animal Care and Use Committee/IACUC) and the Food Safety Authority (application ID: FOTS 8127) and executed in compliance with the local and national regulations associated with laboratory animal experiments. The rodent and rabbit section of the facility is a Specific Pathogen Free (SPF) unit and follows a health monitoring program recommended by Federation of European Laboratory Animal Science Associations/FELASA (http://www.felasa.eu/). The care of the animals was carried out by two veterinary nurses with FELASA B certification and the study was performed by a veterinarian with FELASA C certification.

2.2. Chemicals and experimental diet

A thorough description of the design and preparation of the POP mixture can be found in Berntsen et al. (2017). A list of the individual compounds can be found in Table 1. In brief, compounds occurring in Scandinavian food products reported in studies prior to 2012 were selected for the POP mixture. Human estimated daily intake (hEDI) levels were defined and adjusted to a 25 g mouse consuming 3 g feed/day. However, due to the possibility of background exposure and interspecies differences in compound metabolism, concentrations were adjusted up to 5000× (low dose) and 100 000× (high dose) hEDI. All polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and other organochlorines were purchased from Chiron AS (Trondheim, Norway). All perfluorinated compounds (PFCs) and hexabromocyclododecane (HBCD) were obtained from Sigma-Aldrich (St. Louis, MO, USA), with the exception of perfluorohexane sulfonic acid (PFHxS) potassium salt which was purchased from Santa Cruz (Dallas, US). All chemicals were dissolved in an appropriate solvent and added to corn oil (Jasmin, fully refined, Yonca Gida San A.S., Manisa, Turkey) intended for human consumption. Solvents were thoroughly evaporated under N_2-flow and the remaining oil was incorporated in AIN-93G mouse feed (TestDiets, St.Louis, MO) at the low and high mixture concentrations. The control diet contained only corn oil from which the solvent had been evaporated.

2.3. Study design

In Experiment 1, 66 mice were used and each litter was randomly divided into 3 exposure groups (control, low and high POP diet) at weaning and exposed for 10 weeks (Fig. 1). In Experiment 2, 21 mice were exposed to the mixture of POPs in the same way, but in addition, these mice were also given one subcutaneous injection of 8.5 mg/kg AOM (Sigma-Aldrich, St. Louis, MO, USA) during their second week after birth. After 10 weeks of POP exposure, all mice were sacrificed and sampled. Because of high offspring mortality after the AOM injection, the breeding of mice for Experiment 2 was terminated for animal welfare reasons prior to completion of breeding the individuals for the study. This resulted in a lower number of animals compared to Experiment 1.

2.4. Animal model

The A/J Min/+ mouse model was established by backcrossing the Min/+ trait onto the genetic background of the A/J strain for >12 generations (Sødring et al., 2016b). In the present study, a total of 87 A/J Min/+ mice were used. The animals were bred in-house. Female A/J +/+ mice were mated with male A/J Min/+ mice and their A/J Min/+ offspring were used in the present study. The pups