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Effect of polyhexamethylene biguanide on rat liver

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ABSTRACT

Polyhexamethylene biguanide (PHMB), an amphiphilic polymeric biocide, increased liver tumor incidence in male and female rats at 1000 and 1500 mg/L in drinking water, but not at 500 mg/L in previous studies. In another study, PHMB administered in diet at 4000 mg/kg was negative for hepatocellular tumors. The present studies evaluated bioavailability and distribution of PHMB administered in drinking water and diet and possible modes of action (MOA). PHMB in drinking water was unpalatable during the first 3 days, resulting in markedly decreased food consumption and decreased body weight. Ki-67 labeling index was increased in hepatocytes and endothelial cells dose responsively with PHMB administered in drinking water but not diet. Vitamin E had no effect on this. There was no cytotoxicity by histopathology or serum enzymes, and no increase in cytokines TNFα, IL-1α or NF-κB. Focal iron deposition in sinusoidal lining cells was detected. Microarray analyses were non-contributory. No effect on CAR or PPARα activation was detected. ^{14}C-PHMB administered at 500, 1000, or 1500 mg/L in the drinking water or 4000 mg/kg in the diet was nearly completely absorbed and excreted in urine, with some fecal excretion. The hypothesized MOA for liver tumors induced by PHMB in drinking water is: 1) severe dehydration and starvation because of unpalatability, followed by ingestion with rapid absorption and urinary excretion; 2) increased hepatocyte proliferation; and 3) induction of hepatocellular foci and tumors. The PHMB-induced rat hepatocellular tumors are unlikely to pose a human cancer risk. However, the actual MOA has not been determined.

1. Introduction

Polyhexamethylene biguanide (PHMB) is an amphiphilic polymeric biocide synthesized by polyaddition of sodium dicyanamide and hexamethylenediamine leading to the formation of poly (hexamethylene biguanide) hydrochloride (Paula et al., 2011). It is capable of acting as a strong chelator of divalent cations such as iron, copper, and other metal ions (Kaehn, 2010).

It is widely used as an EPA-approved (Environmental Protection Agency; case no 3122; docket id: EPA-HQ-OPP-2004-0305) sanitizer for swimming pools and spas, a preservative for cosmetics and leather (Paula et al., 2011), and a cleaning agent for contact lenses (Horner et al., 2015). PHMB is also used as an algaecide, fungicide and microbicide. It is a popular dressing agent for its wound healing properties (Roth and Brill, 2010). *In vitro* PHMB has been shown to have anti-HIV properties (Krebs et al., 2005). PHMB has several advantages over other cleaning agents. It is stable in sunlight, withstands a wide range of temperature (active decomposition at 392 °F) and pH (3.0–11.0), does not cause irritation to eye or skin at normal use concentrations, and is odorless (Unhoch et al., 1996).

PHMB is produced by different methods (Paula et al., 2011) starting with the same raw materials but with insertion of an intermediate at different stages of the synthesis (Patent GB1167429, August 1967, Polymeric Biguanide) which result in polymers with different chemical structures (chain length and in-chain distribution). Previous long term (two-year) bioassays utilizing a historical source of PHMB administered in the diet to mice and rats resulted in an increased incidence of vascular tumors in the livers of mice, but only at doses in excess of the maximum tolerated dose (MTD) (greater than 10% decreased body weight gain) (ECHA, 2011). No increase in hepatocellular tumors

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occurred in rats or mice. In a recent two-year bioassay, PHMB produced by Laboratoire Paréva (Saint-Martin de Crau, France) was administered in the drinking water to male and female rats (Rajsekhar, 2012) at doses of 500, 1000, and 1500 mg/L. The maximum tolerated dose of this PHMB in the drinking water was 1500 mg/L. In contrast, a dose of 4000 mg/kg in the diet was used in previous studies that utilized PHMB produced by a different manufacturer (ECHA, 2011). The liver was identified as the main target organ for PHMB in the two-year study involving administration in drinking water, with a greater tumor incidence in male rats than in female rats. Hepatocellular tumors were induced at administered water concentrations of 1000 mg/L and 1500 mg/L PHMB, but not at 500 mg/L (Chowdhury et al., 2016). The tumors had prominent vascular changes which resulted in an initial misclassification as vascular tumors. The vascular changes occurred in control rats and in rats treated with PHMB. The liver tissue slides for all rats in the study were reviewed by pathologists with expertise in rodent liver tumors. All tumors were reclassified as hepatocellular foci, adenomas, and carcinomas. The vascular changes in the tumors and in non-tumorous liver tissue were classified as ectasia. Since PHMB is non-genotoxic and non-mutagenic (EPA, September 2005), the hepatocellular tumors are induced by a mode of action (MOA) that functions ultimately by increasing hepatocellular proliferation (Cohen, 2010; Holsapple et al., 2006). Evaluation of the epigenetic effects of PHMB in *in vitro* studies suggested that it induced little increase in some cytokines and the transcription factor NF-κB, and there was little evidence of cytotoxicity or oxidative damage (Creppy et al., 2014). Increased cell proliferation could potentially be due to a direct effect of PHMB on hepatocytes, either by cytotoxicity with regeneration or by direct mitogenesis, or to an effect of PHMB on endothelial cells or Kupffer cells leading to a secondary mitogenic effect on hepatocytes (Cohen, 2010; Cohen and Arnold, 2011; Holsapple et al., 2006). The present study was designed to investigate the MOA for PHMB on rat liver, utilizing an administration protocol the same as that used for the two-year bioassay. The concentrations in the drinking water used in our studies were the same as in the two-year bioassay. We also included a group administered PHMB in the diet at the top concentration (4000 mg/kg in the diet) used in previous two-year bioassays, in rats in which hepatocellular tumors were not increased, to compare to the effects in rats administered PHMB in the drinking water. To evaluate a possible role of oxidative damage, vitamin E was co-administered to rats in the diet. In addition, ^{14}C-labeled PHMB was administered to evaluate the bioavailability and distribution of PHMB in the rat after repeated dose exposures to the substance.

2. Materials and methods

2.1. Chemicals

PHMB was provided by Laboratoire Paréva (Saint-Martin de Crau, France) and kept stored desiccated at room temperature until use in experiments. The purity of the test material as determined by Laboratoire Paréva was 98.92%. Phenobarbital (Sigma Aldrich, St. Louis, MO) with ≥99% purity and clofibrate (Cayman Chemicals, Ann Arbor, MI) with ≥98% purity (Experiment 2) were stored at room temperature until use. Adjustments were not made for percentage purities of less than 100%. ^{14}C PHMB (Experiment 3) was prepared by Quotient Bioresearch (Cardiff, UK) and stored at −20 °C until use.

2.2. Animals

Male Wistar Han [Crl:WI(Han)] rats were purchased from Charles River Laboratories, Inc. (Raleigh, NC, USA), the same strain and age of rats used in the two-year bioassay. Rats were housed in the animal facility at the University of Nebraska Medical Center (UNMC, Omaha, NE) which is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All animals were housed in polycarbonate cages with dry corn-cob bedding in a room with a targeted temperature of 22 °C, humidity of 50% and a light/dark cycle of 12 h (0600 lights on/1800 lights off). Animals in Experiments 1 and 2 were housed 2–3 per cage and quarantined for 7 days before the study. Animals in Experiment 3 were housed singly and quarantined for 59 days before the study due to a delay in obtaining the ^{14}C PHMB supplemented diet. After quarantine, all animals were randomized into treatment groups using a weight stratification method (Martin et al., 1986). All animals were fed pelleted Purina 5002 diet (Dyets Inc., Bethlehem, PA) or powdered Purina 5002 diet (LabDiet®, PMI, Richmond, IN) and Milli-Q water *ad libitum*. Nylabones® (Nylabone Products, Neptune, NJ, USA) were added to the cages for environmental enrichment. Experimental protocols were approved by the UNMC Institutional Animal Care and Use Committee (IACUC) and the level of care met or exceeded the basic requirements in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The dosing regimen in the drinking water was the same as that used for the two-year bioassay.

2.3. Study design

2.3.1. Experiment 1

Seventy male rats approximately 8 weeks old were randomized into 7 groups of 10 rats each and treated for 4 weeks following 7 days of acclimation (Table 1). Group 1 served as control for Groups 2–5. PHMB was administered to Groups 2–4 in drinking water at 500, 1000 or 1500 mg/L, respectively, (approximately 50, 100, and 150 mg/kg body weight, respectively) and to Group 5 in powdered diet at 4000 mg/kg (approximately 300 mg/kg body weight) for 4 weeks. Groups 6 and 7 were fed powdered diet supplemented with 1000 IU/kg vitamin E (di-α-tocopherol acetate, ≥99% purity, Glanbia Nutritional, Carlsbad, CA) to assess the effects of PHMB on oxidative stress. Diets supplemented with PHMB or vitamin E were prepared at Dyets Inc. (Bethlehem, PA). Group 7 also received PHMB at 1500 mg/L in drinking water. Body weights for all animals were measured on study days 0, 7, 10, 14, 28, and on the day of termination. Food and water consumption (weight of food or water offered minus the weight remaining at the end of the

Table 1
Study design.

Group No.	Treatment	Duration
Experiment 1		
1	Control-groups 2–5	4 weeks
2	500 mg/L PHMB in drinking water	4 weeks
3	1000 mg/L PHMB in drinking water	4 weeks
4	1500 mg/L PHMB in drinking water	4 weeks
5	4000 mg/kg PHMB in diet	4 weeks
6	Control-group 7, 1000 IU/kg vitamin E	4 weeks
7	1000 IU/kg vitamin E + 1500 mg/L PHMB in drinking water	4 weeks
Experiment 2		
1	Control	2 weeks
2	1500 mg/L PHMB in drinking water	2 weeks
3	5000 mg/kg clofibrate in diet-positive control for PPARα activation	2 weeks
4	500 mg/L phenobarbital in drinking water-positive control fo CAR activation	2 weeks
Experiment 3		
1	500 mg/L ^{14}C–PHMB in drinking water	7 days
2	1000 mg/L ^{14}C–PHMB in drinking water	7 days
3	1500 mg/L ^{14}C–PHMB in drinking water	7 days
4	4000 mg/kg ^{14}C–PHMB in diet	7 days
Recovery groups		
5	1500 mg/L ^{14}C–PHMB in drinking water	5 days
	Withdrawal of treatment	5 days
6	4000 mg/kg ^{14}C–PHMB in diet	5 days
	Withdrawal of treatment	5 days