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Evaluation of optimal water fluoridation on the incidence and skeletal distribution of naturally arising osteosarcoma in pet dogs

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Abstract

Experimental toxicological studies in laboratory animals and epidemiological human studies have reported a possible association between water fluoridation and osteosarcoma (OSA). To further explore this possibility, a case-control study of individual dogs evaluated by the UC Davis Veterinary Medical Teaching Hospital was conducted using ecologic data on water fluoridation based on the owner's residence. The case group included 161 dogs with OSA diagnosed between 2008–2012. Two cancer control groups included dogs diagnosed with lymphoma (LSA) or hemangiosarcoma (HSA) during the same period ($n = 134$ and $n = 145$, respectively). Dogs with OSA were not significantly more likely to live in an area with optimized fluoride in the water than dogs with LSA or HSA. Additional analyses within OSA patients also revealed no significant differences in age, or skeletal distribution of OSA cases relative to fluoride status. Taken together, these analyses do not support the hypothesis that optimal fluoridation of drinking water contributes to naturally occurring OSA in dogs.

Keywords

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Conflict of interest

The authors declare no conflicts of interest.

Introduction

A potential relationship between fluoridated drinking water and cancer has been investigated in a number of epidemiological studies. Investigation into water fluoridation initially stemmed from a study in 1977 that reported an increase in overall cancer mortality within subpopulations in 10 US cities following the implementation of drinking water fluoridation,[1] however, this study was later largely dismissed over methodological concerns. Follow-up studies found no consistent association between water fluoridation and cancer incidence with the possible exception of osteosarcoma (OSA).[2] Based on these concerns, extensive studies were undertaken to investigate the effects of sodium fluoride exposure in rats and mice. In 1990, the National Toxicology Program concluded that there was 'equivocal evidence' of carcinogenic activity of sodium fluoride in male F344/N rats, based on the occurrence of OSA in a small number of dosed animals.[3,4] 'Equivocal evidence' is a category for unclear findings defined as studies showing a marginal increase of neoplasms that may be related to chemical exposure. Identical studies showed no evidence of carcinogenic activity in male or female mice or female rats receiving sodium fluoride at concentrations up to 175 ppm (79 ppm fluoride) in drinking water despite the fact that many demonstrated lesions typical of dental fluorosis and increased osteoid formation or osteosclerosis in long bones. Interestingly, of the rats that developed OSA, three rats developed primary lesions in the vertebrae, one rat had an extraskeletal tumour, and one rat was found to harbour a microscopic OSA lesion of the humerus.

Since release of that report, several epidemiological studies have examined a possible association between fluoridated water and the incidence of OSA or bone cancers in human populations.[5–15] Three studies have implicated fluoride as a possible contributing factor,[9,12,14] but the vast majority found no association between fluoride and OSA. Studies have included ecological or case-control designs, each possessing identifiable strengths and weaknesses. Several studies included non-OSA bone tumours, while others used non-OSA bone tumours as controls. Many did not attempt to examine factors such as age or anatomical site, both of which may be important when considering the potential role of fluoride. Evidence regarding the true relationship between fluoride exposure and OSA in both laboratory animals and human studies therefore remains weak.[16,17] Detailed epidemiologic studies examining water fluoridation and OSA present significant challenges in that human OSA is a relatively uncommon disease. Furthermore, modern-day evaluation of true fluoride exposure in humans can be difficult to ascertain because of consumption of commercially bottled beverages/food or regular consumption outside of the home, also termed the 'diffusion effect'.[11,12] Exposure to fluoride supplementation and use of dental products can also contribute substantially to fluoride exposure, and can therefore further confound studies examining the possible contribution of fluoridated water among the human population.

Pet dogs have been proposed as a potential model, or sentinel population, to study the epidemiology, biology and treatment of human cancer because they share our environment and thus potential environmental risk factors.[18,19] Comparatively, OSA is much more common in pet dogs than in humans and is estimated to occur in more than 10,000 cases annually in the United States.[20] Large and giant breed dogs are predisposed to development

of appendicular OSA, with a biphasic peak incidence occurring around 7 years of age and a smaller peak between 18–24 months.[21] Canine appendicular OSA has been extensively investigated as a model for paediatric OSA,[22,23] and both clinical and molecular similarities have been well described.[24–26] Because OSA is much more prevalent in domestic dogs and because large-breed dogs represent a predisposed population, it seems plausible that the possible carcinogenic effects of water fluoridation may be more apparent in this species. In addition, while large-breed dogs are predisposed to appendicular OSA, older smaller-breed dogs comprise the majority of axillary OSA cases in this species. This may be particularly relevant because fluoride exposure in rats resulted in a potential increase in vertebral OSA. Previous carcinogenic studies using radioactive isotopes or chemical carcinogens have been found to induce appendicular tumours in rodents,[4] however, there is a shift towards axial lesions in both humans and dogs for plutonium-induced OSA.[27] While the 'diffusion effect' may still occur to some extent, pet dogs are generally less likely to be exposed to commercially available bottled water or beverages that could impact municipal water and overall fluoride consumption. Lastly, because dogs are not prone to dental caries, they are not exposed to fluoride-containing dental products that can significantly contribute to total fluoride exposure in young children.[28]

The purpose of this study was to explore if the occurrence of OSA in dogs may be associated with exposure to optimally fluoridated drinking water. We hypothesized that dogs residing in homes with optimally fluoridated community drinking water would have higher rates of OSA when compared with other cancers such as lymphoma (LSA) or hemangiosarcoma (HSA). We chose LSA and HSA specifically because: (1) they represent common canine cancers allowing optimally powered comparisons, (2) these tumours were not implicated in any fluoride toxicity studies and (3) these tumours are not commonly derived from tissues where high levels of fluoride accumulates. Based on fluoride toxicological studies in male rats, we also hypothesized that there would be a higher incidence of non-appendicular OSA in dogs exposed to optimally fluoridated drinking water when compared with dogs residing in households receiving non-optimally fluoridated water sources.

Materials and methods

Study subjects

A case-control study of individual dogs evaluated by the UC Davis Veterinary Medical Teaching Hospital was conducted using ecologic data on water fluoridation based on the owner's residence. Search of the UC Davis Veterinary Medical Teaching Hospital (VMTH) electronic medical database identified dogs with histologically confirmed OSA diagnosed between 2008 and 2012. Two separate cancer control groups (LSA and HSA) were used under the assumption that exposure to fluoridated water was not an etiologic determinant of either cancer type (based on previous toxicological animal studies and human epidemiologic literature) and included dogs with histologically confirmed HSA or LSA that were admitted to the VMTH during the same time period. The sex, breed, neutering status, age, weight and address were recorded for each dog. In addition, the bone location was also identified for the OSA group in order to allow classification into appendicular or non-appendicular locations.

Dogs were further classified as being predisposed to OSA based on whether they weighed 40 kg.[29–32]

Water fluoridation data

The geocoding function of ArcGIS was used to map client addresses. Addresses that were not matches to known addresses were excluded in subsequent steps. The water system node closest to each of the matched address locations was then identified, because system service area boundaries were not reliably available across the state at the time of mapping. System nodes may include water sources such as wells, treatment plants and storage tanks. Based on the water system to which the nearest node belonged, each matched address was assigned a nearest water system. The Water System IDs of these matched systems were related to the table provided by the California Department of Public Health, Division of Drinking Water and Environmental Management for fluoridation, and the data in that table was associated with the addresses that were previously matched. Using this data, each patient was assigned to one of three groups corresponding to whether there was no fluoridation of drinking water, mixed fluoridation or optimal fluoridation provided.

Statistical analysis

Descriptive statistics are presented as means, standard deviations and ranges for continuous variables and as total numbers and percentages for categorical data. When assessing if case and control groups were similar, differences between categorical variables (sex, fluoride status) were assessed by a chi-square test for homogeneity. Differences between cases and both control groups for continuous variables (body weight, age) were assessed by a one-way ANOVA with post-hoc contrasts using a Bonferroni adjustment for multiple comparisons. Within the OSA cases, differences between age and weight comparing axial or appendicular location were compared using a Student's two-group *t*-test with unequal variances.

Two separate analyses were carried out to test the hypothesis that dogs residing in homes with optimally fluoridated community drinking water would have higher rates of OSA when compared with either dogs with LSA or HSA. This analysis was also carried out after dividing the OSA dogs into groups based on either an axial or appendicular location. For the hospital-based case-control analyses, differences in exposure to optimally (versus non-optimally) fluoridated water in cases versus controls (LSA and HSA were treated as distinct control groups) were evaluated using logistic regression, and odds ratios (OR) with 95% confidence intervals (95% CI) and *P*-values were calculated. All models were adjusted for age and weight using linear and quadratic terms. We then also adjusted for dogs weighing over 40 kg to see if this group was more likely to have a history of fluoride exposure. The analysis was then repeated for those with appendicular OSA.

To test the hypothesis that there would be a higher incidence of non-appendicular OSA in dogs exposed to optimally fluoridated drinking water, a case–case analysis was carried out. Comparisons of the axial versus appendicular location of OSA between dogs exposed to optimally fluoridated water (versus OSA cases exposed to non-optimally fluoridated water) were assessed by a chi-square test. All data analyses were performed using a commercially

available software program (Stata 12.1; StataCorp, College Station, TX). *P*-values < 0.05 were considered statistically significant.

Results

For the hospital-based case-control study, the case group included 161 dogs with histologically confirmed OSA diagnosed between 2008–2012 that mapped using the geocoding function of ArcGIS. Two cancer control groups included 145 dogs with histologically confirmed HSA and 134 dogs with histologically confirmed LSA that mapped using the geocoding function of ArcGIS and were admitted to the VMTH during the same time period. Demographics of the OSA group along with the LSA and HSA groups are presented in Table 1. One dog each in the HSA and LSA group did not have a recorded weight or age (respectively) in the medical record and were not included when calculating the weight and age statistics, but were included in the fluoride exposure analysis. There were no differences between the three groups in relation to sex (*P* = 0.54) or fluoride exposure. No significant differences in age existed between the OSA group and the LSA group (*P* = 0.606), but dogs in the HSA group were older than those in the OSA group (*P* < 0.001). Dogs with OSA were also heavier than either the LSA group (*P* < 0.001) or HSA group (*P* < 0.001).

Forty-six of the 161 dogs (28.57%) diagnosed with OSA cases weighed 40 Kg, while 23 of the 134 dogs (17.16%) diagnosed with LSA and 23 of the 145 dogs (15.86%) diagnosed with HSA were 40 kg. Dogs who were 40 kg were more likely to have OSA than the control cases when LSA and HSA case controls were combined (OR 2.03, 95% CI = 1.27 – 3.23, *P* = 0.003) and in the LSA group (OR 1.93, 95% CI = 1.10 – 3.39, *P* = 0.022) or HSA groups (OR = 2.12, 95% CI = 1.21 – 3.72, *P* = 0.009) individually.

Dogs with OSA were not significantly more likely to live in an area with optimized fluoride (versus non-optimized fluoride) in the water than dogs with LSA (OR = 0.77, 95% CI = 0.44 – 1.37, *P* = 0.384) or HSA (OR = 1.36, 95% CI = 0.77 – 2.38, *P* = 0.286) after adjustment for age and weight. The lack of significant association remained when the analysis was restricted to predisposed dogs 40 kg and adjusted for age and weight (OR = 0.63, 95% CI = 0.18 – 2.24, *P* = 0.475 using LSA controls; OR = 0.70, 95% CI = 0.15 – 3.25, *P* = 0.652 using HSA controls). This also held when the analysis was restricted to non-predisposed dogs under 40 kg and adjusted for age and weight (OR = 0.87, 95% CI = 0.45 – 1.68, *P* = 0.685 using LSA controls; OR = 1.66, 95% CI = 0.88 – 3.12, *P* = 0.115 using HSA controls).

Dogs with appendicular OSA were not significantly more likely to live in an area with optimized (versus non-optimized) fluoride after adjustment for age and weight (OR = 0.74, 95% CI = 0.39 – 1.40, *P* = 0.351 for LSA controls, and OR = 1.35, 95% CI = 0.72 – 2.54, *P* = 0.350 for HSA controls). The findings were similar when the analysis was restricted to axial OSA (OR = 0.75, 95% CI = 0.34 – 1.63, *P* = 0.465 using LSA controls, and OR = 1.23, 95% CI = 0.56 – 2.71, *P* = 0.612 using HSA controls).

The locations of the OSA lesions are presented in Table 2. One hundred and eleven (68.9%) dogs with OSA had an appendicular lesion, and 50 (31.1%) had a non-appendicular lesion. In the OSA group, dogs with an appendicular lesion were heavier (*P* = 0.006) and younger (*P* = 0.021) than those with non-appendicular lesions (Table 3).

For the OSA case – case portion of the study, the same proportion of appendicular and non-appendicular lesions existed between groups of dogs with different fluoride exposures (*P* = 0.99, Table 4).

Discussion

Our study did not detect a significant difference in optimal water fluoride status between dogs with OSA and those with LSA or HSA. In addition, we found a similar distribution of tumour locations between OSA dogs when grouped according to fluoride status. Initial toxicity studies examining fluoridation in rats and mice revealed a potential increase in the number of OSA tumours in male rats.[4] Interestingly, only one out of five OSA tumours in rats were appendicular, which compares similarly to the 70% vertebral location of OSA reported in female mice treated with plutonium.[33] The altered tumour distribution in plutonium or radium induced OSAs, with a trend towards better vascularized, cancellous bone locations, might suggest similar findings would have been expected between spontaneously occurring canine OSA groups in our study if indeed fluoridated water did contribute to OSA development.[27] Others have postulated that fluoride may increase the incidence of OSA in metaphyseal long-bone regions in children or adolescents because it may act as a mitogen for osteoblasts and is preferentially taken up in bone during periods of rapid skeletal growth.[12]

Studies have shown that natural occurring canine OSA serves as an excellent model for OSA in people owing to the increased prevalence in dogs, the similarities between species in clinical behaviour and many concordant molecular and genetic aberrations found to be driving tumour development and progression.[24,26] One important difference is that the majority of human OSA occurs in adolescents whereas the disease tends to occur in skeletally mature dogs, indicating that the biological aetiology of these tumours may be different. However, an altered distribution of tumour locations was observed in both dogs and humans with plutonium-induced OSA, shifting to a predominantly axial location, compared with naturally occurring disease that primarily affects the limbs in both species.[27] It follows that epidemiological studies in canine OSA could legitimately be useful to infer possible associations to the equivalent human disease.

Case-control or ecological studies reported in human populations have been both consistent with and contradictory to our results.[5–12] Supporting our findings, however, is a study reporting no significant difference in bone fluoride levels between people with OSA and a control group.[34] Variation in the findings of epidemiological studies can likely be explained by differences in study design/analyses, including geographical differences, varying adjustment for confounding factors including duration of exposure, and differences in patient inclusion criteria including age and tumour location. We believe that the results reported in our canine study add to the previous human studies in that: (1) the incidence of

OSA is much higher in dogs, allowing for higher number of cases within a defined region over a short time-frame; (2) this is the first time that fluoride status has been evaluated on a population (large-breed dogs) with a known predisposition to OSA and (3) this is the first study to evaluate a potential shift in appendicular versus non-appendicular OSA relative to water fluoridation status.

Limitations of this study should be noted, some of which are not unique when compared with many prior human studies. Many previous ecological and case-control studies relied on historic residential data and were therefore subject to the 'ecological fallacy' in that they may not reflect the true exposure of fluoride at the individual level. Such inaccuracies could lead to both false positive and false negative results since true exposure is unknown. Observational epidemiologic studies to test the possible association between fluoride in drinking water presents significant challenges as it is difficult to evaluate overall exposure without obtaining bone measurements, however, one recent study indicates that typical levels used in community water fluoridation may not significantly affect bone mineral measures.[35] Regardless, it should be recognized that this study also relies on partially ecological data based on water fluoridation of the owner's address. We did not have information regarding an individual's duration of residence at that address and also had no information on previous places of residence. Additionally, it is possible that other variables such as diet could alter fluoride intake in a manner that is independent of fluoridation of drinking water. Analysis of the influence of such variables is of course impossible to achieve retrospectively.

Another limitation of this study relates to the limited number of reproductively intact cases. Previous rat and human data suggesting males may be more susceptible to the effects of fluoride intake on OSA development indicates that studying this disease in pet dogs within the United States may be problematic or even misleading because the common practice of neutering dogs could possibly have shielded male dogs from the potential full carcinogenic effect of fluoridated drinking water.[3,9] This scenario is unlikely if simply dependent on weight and water requirements because neutering often is associated with weight increases; however, this could be critical if risk associated with fluoride exposure were dependent on hormonal status. In contrast, others have described a possible protective role of hormonal exposure in dogs in regard to development of OSA, which may further confound studies examining the effects of fluoride on development of OSA in dogs.[36] Nevertheless, negligible numbers of hormonally intact male dogs were represented in our study, and thus no analysis or conclusion can be made in this subpopulation. To address this question, similar studies examining OSA in intact dogs would need to be performed in geographic locations outside of the United States where neutering is not a common practice. Lastly, while there is little evidence that fluoride exposure would be expected to alter the incidence of LSA or HSA in dogs, theoretically, if fluoride also played a role in the incidence of tumours in these controls, this could result in a Type II error regarding the contribution of fluoride in canine OSA patients. We chose to include both LSA and HSA control groups to minimize this possibility. Only one study that we are aware of found a possible association between fluoride and LSA[9] and this same study reported that over 60% of all cancers were positively associated with fluoride exposure. None of the other epidemiological studies that we are aware of have indicated an association between fluoride and haematopoietic tumours. We

are also not aware of any epidemiological studies evaluating a possible association between fluoride and HSA. Furthermore, extensive toxicity studies in rodent species demonstrated no association with either haematopoietic tumours or HSA. Lastly, it is possible that the limited number of cases presented was not powered correctly to detect a difference.

Taken together, results of this study indicate that exposure to optimally fluoridated water does not appear to alter the overall risk of developing OSA in pet dogs. These findings remained consistent regardless of weight, age or skeletal distribution (appendicular versus non-appendicular).

Acknowledgments

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Table 1

Demographics of the osteosarcoma, lymphoma and hemangiosarcoma groups

Characteristic		Osteosarcoma	Lymphoma	Hemangiosarcoma	All cases
Total		161	134	145	440
Age years	Mean	9.26	9.37	10.32	9.64
	SD (range)	2.72 (3.47–16.28)	3.29 (1.3–18.22)	2.62 (2.39–15.84)	2.91 (2.11–18.22)
Sex (%)	Female intact	4 (3)	5 (4)	3 (2)	12 (3)
	Female spayed	84 (52)	62 (46)	62 (43)	208 (47)
	Male intact	12 (7)	8 (6)	15 (10)	35 (8)
	Male neutered	61 (38)	59 (44)	65 (44)	185 (42)
Weight kg	Mean	35.67	25.84	31.05	31.16
	SD (range)	12.99 (9.7–88)	14.35 (2–80)	9.02 (7.8–50)	12.92 (3.1–88)
Fluoride status (%)	Not optimal	116 (72)	90 (67)	110 (76)	316 (72)
	Mixed	3 (2)	7 (5)	6 (4)	16 (4)
	Optimal	42 (26)	37 (28)	29 (20)	108 (24)

Table 2

Locations of osteosarcoma lesions

Anatomic location	Not optimal/ mixed fluoride (%)	Optimal fluoridation (%)	Total
Long bone	80 (67)	26 (62)	106
Vertebral	9 (8)	5 (12)	14
Head	11 (9)	5 (12)	16
Pelvis	4 (3)	0 (0)	4
Rib/sternum	5 (4)	1 (2)	6
Diffuse	4 (3)	1 (2)	5
Soft tissue	2 (2)	1 (2)	3
Scapula	2 (2)	3 (7)	5
Other (calcaneous, digit)	2 (2)	0 (0)	2
Total	119	42	161

Table 3

Characteristics of dogs with osteosarcoma of different locations

Characteristic		Appendicular	Non-appendicular	*P*-value
Total		111	50	
Age years	Mean	8.9	10.0	0.021
	SD (range)	2.69 (2.0–16.3)	2.65 (5.5–16.0)	
Sex (%)	Female intact	1.8	4.0	0.84
	Female spayed	53.2	50.0	
	Male intact	7.2	8.0	
	Male neutered	37.8	38.0	
Weight kg	Mean	37.5	31.5	0.006
	SD (range)	12.59 (18.6–88)	13.04 (9.2–69)	
Fluoride status (%)	Not optimal	71.2	74.0	0.70
	Mixed	2.7	0.0	
	Optimal	26.1	26.0	

Table 4

Characteristics of dogs with osteosarcoma with different fluoride exposure

Characteristic		Not optimal	Optimal	*P*-value
Total		119	42	
Age years	Mean	9.3	9.12	0.71
	SD (range)	2.77 (2.0–16.3)	2.59 (3.5–14.0)	
Sex (%)	Female intact	3.4	0.0	0.11
	Female spayed	48.7	61.9	
	Male intact	5.9	11.9	
	Male neutered	42.0	26.2	
Weight kg	Mean	35.7	35.5	0.94
	SD (range)	12.52 (9.2–86.0)	14.41 (9.7–88.0)	
OSA location	Appendicular	82 (68.9%)	29 (69%)	0.99
	Non-appendicular	37 (31.1%)	13 (31%)	