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Effects of Six Months of Daily Low-Dose Perchlorate Exposure on Thyroid Function in Healthy Volunteers

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Abstract

Context: Perchlorate has been detected in U.S. drinking water supplies at levels ranging from 4 – 200 μg/L, as well as in agricultural products. Perchlorate is known to be a competitive inhibitor of iodine uptake by the thyroid through the sodium-iodide symporter.

Objective: to determine whether prolonged exposure (six months) to low levels of perchlorate would perturb thyroid function.

Design: Prospective double-blinded randomized trial

Participants: 13 healthy volunteers

Intervention: placebo vs. 0.5 mg or 3.0 mg potassium perchlorate daily

Main Outcome Measures: serum thyroid function tests, 24-hour radioactive iodine uptake, serum thyroglobulin (Tg), urinary iodine and perchlorate, and serum perchlorate

Results: Mean urinary perchlorate value during ingestion of 0.5 mg perchlorate daily was 332.7 $\pm$ 66.1 μg/24h or 248.5 $\pm$ 64.5μg/g creatinine and mean values for the four subjects who received 3 mg perchlorate daily were 2079.5 $\pm$ 430.0 μg/24h or 1941.7 $\pm$ 138.5μg/g creatinine. There was no significant change in the thyroid RAIU during perchlorate administration. There were no significant changes in serum triiodothyronine, free thyroxine index, thyroid stimulating hormone (TSH), or Tg concentrations during the exposure period compared baseline or post-exposure values. Urine iodine values for the 3 mg perchlorate group were higher, but not significantly so, at baseline than during perchlorate exposure.

Conclusions: we observed that a 6-month exposure to perchlorate at doses up to 3 mg per day had no effect on thyroid function, including inhibition of thyroid iodide uptake, as well as serum levels of thyroid hormones, TSH, and Tg.

Introduction

Perchlorate salts have been used as pharmaceuticals, as oxidizers in solid propellants for rockets and missiles, in explosives, fireworks, and in road flares and air bag inflation systems (1). Since 1997, perchlorate has been found in drinking water throughout the U.S. at levels ranging from 4 - 200 μg/L, and in lettuce and milk (2). Environmental perchlorate contamination has been attributed to industries that manufacture and use perchlorate. However, detection of low perchlorate levels at locations far from such sources suggests that perchlorate may come from natural processes and from Chilean nitrate fertilizers (3).

Perchlorate is a competitive inhibitor of thyroid iodine uptake through the sodium-iodide symporter (NIS) (4). Prolonged major inhibition of iodide uptake can result in decreased synthesis of thyroid hormones. The ability of perchlorate to inhibit iodine uptake was the basis for its use in the 1950s and 1960s to treat hyperthyroidism (5), but after a few reported cases of fatal aplastic anemia in patients treated with high doses (600-1600 mg/day), use of perchlorate essentially ceased. However, more recent experience using perchlorate to treat Graves' disease and iodine induced hyperthyroidism demonstrated no serious side effects at doses less than 1000 mg/day for up to one year (6, 7).

In view of the reported environmental perchlorate contamination (2, 3), the potential health effects from this relatively low level perchlorate ingestion are a matter of public health interest. There have been several recent human studies regarding the health effects of perchlorate. U.S. studies have not shown any thyroid effects at drinking water perchlorate concentrations of 4 – 16 μg/L (8,9). Observational studies in the Atacama region of Chile, where drinking water perchlorate concentrations are up to 100-120 μg/L, have also not shown any effect of perchlorate on the thyroid in newborns, school-aged children, or pregnant women (10). In a recent study from an area in Israel where well water perchlorate contamination is > 300 μg/L, newborn serum thyroxine (T_4) values were normal (11).

Only one ecological drinking water study in the U.S. has reported thyroidal effects. Thyroid stimulating hormone (TSH) levels were elevated in newborns in Yuma City, Arizona, where the drinking water perchlorate concentration was 6 μg/L, as compared to newborns in Flagstaff, Arizona, where perchlorate is undetectable in the water supply (12). However, the demographics and altitudes of the two towns are different. When newborns from Yuma City

were compared to those from an adjacent town where perchlorate is not found in the drinking water, serum TSH values were normal and similar (13).

Studies in perchlorate production workers exposed to perchlorate via inhalation at doses up to 34 mg/day for a mean of three years have not indicated any adverse effects on thyroid structure or serum TSH, thyroid hormones, or thyroglobulin (Tg) concentrations (14,15) even though 14-hour thyroid ^{123}I uptakes (RAIU) were decreased by an average of 38% following three consecutive 12-hour shifts as compared with pre-shift RAIU.

Several controlled studies in normal volunteers have evaluated thyroid effects of 14-day exposures to perchlorate in drinking water (16,17,18). The thyroid RAIU's significantly decreased by 38% in healthy volunteers consuming 10 mg perchlorate daily. There were no significant changes in serum thyroid hormone or TSH concentrations during perchlorate administration. Administration of 3 mg perchlorate daily for 14 days resulted in an insignificant 10% decrease in the 24-hour RAIU (17). A perchlorate pharmacokinetic study was reported in which volunteers drank water for 14 days containing perchlorate (approximately 0.5, 1.4, 7, and 35 mg per day for a 70-kg individual) (18). On day 14, 24-hour RAIUs for the 0.5 mg/day exposure group were not significantly different from baseline, while 24-hour RAIUs at the other three doses had decreased significantly by 16%, 45%, and 67% from baseline, respectively.

Although the human drinking water and occupational studies provide some evidence that low-level perchlorate exposure does not adversely affect the thyroid, exposure was only for two weeks in the short-term studies and, in the worker studies, the exposures were not continuous. The aim of the present study was to determine whether more prolonged exposure (six months) to low perchlorate levels would perturb thyroid function.

Experimental Subjects

The protocol was approved by the Institutional Review Boards at Loma Linda University School of Medicine (LLUMC), the Jerry L. Pettis Memorial Veterans Administration Medical Center (JLPVAMC), and Boston University School of Medicine.

Subjects were recruited by direct mail advertising in the Loma Linda and San Bernardino, California area. Subjects with a known history of thyroid disease or taking thyroid hormone or anti-thyroid medications were excluded. During the first year, 94 subjects provided

informed consent, with 24 subjects eventually being randomized to study medication. Of the original 94 subjects, 15 declined further screening evaluation and 55 were excluded due to an abnormal baseline laboratory test or pre-existing illness. Of the original 24 randomized subjects, 14 completed the 7- month study. The 10 randomized subjects who did not complete the study withdrew due to personal scheduling conflicts. None of the 24 randomized study subjects developed an abnormal laboratory or clinical finding or a study-related adverse event. Of the 14 subjects (aged 25 – 65 years) completing the study, 9 were women, 12 were Caucasian, and two were African-American. Further volunteers could not be recruited due to adverse publicity (19).

Materials and Methods

Preparation of Capsules

Longwood Pharmaceutical Research, Inc. at the Massachusetts College of Pharmacy and Allied Sciences, prepared perchlorate (reagent grade potassium perchlorate) and placebo capsules using Good Manufacturing Practices as specified by the U.S. FDA. Capsules were checked every three months for stability.

Protocol

The 14 subjects were randomly and blindly assigned to four dose groups: placebo or 0.5, 1.0, or 3.0 mg perchlorate, taking the dose daily between 7 and 10AM for 6 months. Assuming an average daily water ingestion of 2 liters, the 3.0 mg dose corresponds to approximately 1500 μg perchlorate/L and the 0.5 mg dose to 250 μg/L. Physical examination by AF, including palpation of the thyroid, was done monthly during the 7 months of study. Blood (approximately two hours after capsule ingestion) and 24-hour urines were obtained at baseline and monthly during perchlorate or placebo administration and 1 month later. Serum thyroid function tests and perchlorate, and urine iodine, perchlorate, and creatinine were measured monthly. Capsule counts were monitored monthly. 24-hour thyroid RAIU and serum Tg and Tg antibodies (TgAb) were measured at baseline, 3 and 6 months during perchlorate or placebo ingestion, and 1 month after study medication had been discontinued. To be certain that perchlorate was not adversely affecting serum TSH and free T4 index (FTI) concentrations, somplete blood counts (CBC), or blood chemistries during the study, these parameters were assessed monthly.

Laboratory Tests

Serum perchlorate measurement was carried out using HPLC at Boston Medical Center as described earlier (14). The perchlorate content of the 24-hour urine samples was measured at the Centers for Disease Control and Prevention Laboratories in Atlanta, GA using ion chromatography-mass spectrometry (20). Serum TT3, T4, FTI and TSH concentrations were measured at the end of the study in the same assay at the Boston Medical Center Clinical Chemistry laboratories by chemiluminescence using the Bayer Advia Centaur automated system (Bayer Healthcare, Tarrytown, NY). Serum Tg and TgAb were measured using chemiluminescence on the Nichols Advantage (Nichols Institute Diagnostics, San Juan Capistrano, CA). Urinary iodine was measured using the Sandell-Kolthoff reaction. CBC and serum chemistries were carried out in the JLPVAMC assay laboratory. The 24-hour thyroid RAIU was measured at the LLUMC Department of Nuclear Medicine, in Loma Linda, CA. Subjects were given 100 μc ^{123}I orally approximately two hours after the morning ingestion of the perchlorate capsule. They returned to the laboratory 24 hours later for measurement of the thyroid ^{123}I uptake.

Statistical Analysis

Values are reported as the mean $\pm$ SD and significant differences as $p < 0.05$. Statistical analyses were carried out using SAS version 8 (SAS Institute, Cary, North Carolina). Baseline differences in mean values by group were assessed using ANOVA. Changes over time by treatment group were assessed using repeated measures ANOVA.

Results

Since only one subject received 1 mg perchlorate daily, this subject was omitted from the analysis. No subjects developed abnormal serum TSH or FTI values.

Urine perchlorate (Table 1)

Urine perchlorate was detected at low levels at baseline in all 13 subjects averaging 9.2 ± 5.7 µg/24h or 7.3 ± 5.5 µg/g creatinine. It remained low in the four subjects receiving placebo. In the five subjects given 0.5 mg perchlorate daily, perchlorate values over the six months rose appropriately (332.7 ± 66.1 µg/24h or 248.5 ± 64.5µg/g creatinine), as did mean values in the four subjects who received 3 mg perchlorate daily (2079.5 ± 430.0 µg/24h or 1941.7 ± 138.5µg/g creatinine). Thus, about 65-70% of the daily dose was excreted over a 24-hour period.

Serum perchlorate (Table 1)

Perchlorate was not detected in baseline samples or in those subjects receiving placebo. Serum perchlorate was detected in subjects receiving perchlorate, averaging over the six months 24.5 ± 16 µg/L in the 0.5 mg perchlorate group and 77.9 ± 18.2 µg/L in the 3 mg group. Perchlorate was not detected in any sera one month after perchlorate was discontinued.

Urine Iodine (Table 1)

Because subjects were not on a controlled diet, daily urine iodine values varied throughout the study, ranging from 54 - 840 µg/g creatinine (median 174 µg/g creatinine). Urine iodine values for the 3 mg perchlorate group were higher, but not significantly so, at baseline than during perchlorate exposure (322 ± 357 *vs*. 214.7±106 µg/g creatinine or 311.5 ± 263.2 *vs*. 238.2 ± 132.7 µg/24h).

Thyroid RAIU (Table 2)

The thyroid RAIU was measured at baseline, three and six months during perchlorate or placebo ingestion, and one month later. There was no significant change in the thyroid RAIU during perchlorate administration.

Thyroid Function Tests (Table 2)

There were no significant changes in serum TT3, FTI, TSH, or Tg concentrations during the exposure period as compared to values before and after perchlorate exposure. Tg antibodies were not detected in any subject.

Discussion

Although urinary iodine levels were variable both among subjects and across time, there was no relationship between urinary iodine levels and perchlorate levels in serum or urine. Iodine levels were comparable to those of the general population, with the median urinary iodine (174 μg/g creatinine) corresponding to approximately the 70th percentile value in the general population (21).

We observed that a 6-month exposure to perchlorate at 0.5 and 3 mg/day had no effect on thyroid function, including inhibition of thyroid ^{123}I uptake, and serum levels of thyroid hormones, TSH, and thyroglobulin. These results are similar to those of Lawrence *et al*. (17) in a 14-day study, but differ somewhat from those of Greer *et al*. (18) who observed a significant 16% decrease in RAIU following a 14-day exposure of 1.4 mg perchlorate daily for a 70 kg individual. The difference between our long-term results and those from the short-term studies may be due to the small number of subjects in our study, differences in the dosing regimen (once daily *vs*. semi-continuous), or due to upregulation of NIS as an adaptive response in the long-term study (22).

This is the first study to provide information regarding potential thyroid effects of continuous, long-term exposure to defined low levels of perchlorate. Although perchlorate plant workers were exposed to high amounts of perchlorate on a long-term basis, the exposure pattern in those studies involved three days of exposure followed by three days without exposure. The intermittent exposure pattern may allow the thyroid to recover, at least partially, from any perchlorate-induced inhibition of iodide uptake during the three non-exposed days, thus potentially dampening effects that might be observed with continuous exposure.

This study was limited by the small sample size, and is obviously underpowered. Unfortunately, further such studies can not be carried out at present in the U.S. In addition, the once per day dosing regime does not reflect how actual exposures *via* drinking water and food consumption would occur. Nonetheless, the results suggest that healthy, euthyroid individuals, with normal levels of iodine intake, can tolerate chronic exposure to perchlorate at doses up to 3 mg/day without any effects on thyroid function, including inhibition of iodide uptake.

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Table 1: The effect of perchlorate ingestion on serum and urine perchlorate and urine iodine values

	Timing	Placebo (n = 4)	0.5 mg Perchlorate (n = 5)	3.0 mg Perchlorate (n = 4)
		Mean ± SD	Mean ± SD	Mean ± SD
Urine Iodine (µg/Total Volume)	Before	300 ± 181.3	257.8 ± 119.4	311.5 ± 263.2
	During	264.2 ± 78.4	294.9 ± 78.1	238.2 ± 132.7
	After	238 ± 62.8	238.2 ± 132.7	188.4 ± 95
Urine Iodine (µg/g Creatinine)	Before	194.9 ± 87	184 ± 81.2	322 ± 357
	During	245.7 ± 106.4	206.2 ± 48.2	214.7 ± 106.6
	After	197 ± 59.2	256.9 ± 152.2	192.8± 110.1
Urine CLO4 (µg/Total Volume)*	Before	7.3 ± 1.0	11.9 ± 8.1	7.3 ± 3.1
	During	9.2 ± 5.0	332.7 ± 66.1	2079.5 ± 430
	After	7.1 ± 3.4	9.4 ± 4.3	10.1 ± 6.6
Urine CLO4 (µg/g Creatinine)	Before	5.4 ± 1.7	9.1 ± 7.7	7.4 ± 4.5
	During	8.2 ± 5.0	248.5 ± 64.5	1941.7 ± 138.5
	After	5.2 ± 1.8	8.2 ± 5.0	10.4 ± 7.2
Serum CLO4 (µg/L)**	Before	0	0	0
	During	0	24.5 ± 16	77.9 ± 18.2
	After	0	0	0

There were no significant differences in perchlorate and iodine values between groups at baseline.
*The minimum detection level for perchlorate was 0.025 µg/L in urine.
**The minimum detection level for perchlorate was 1.0 µg/L in serum.

Table 2: The effect of perchlorate ingestion for six months on thyroid function tests

	Timing	Placebo (n = 4)	0.5mg Perchlorate (n = 5)	3.0mg Perchlorate (n = 4)
		Mean ± SD	Mean ± SD	Mean ± SD
TSH (μIU/ml)	Before	1.1 ± 0.5	1.6 ± 0.7	2.2 ± 0.6
	During	1.1 ± 0.4	1.5 ± 0.6	2.2 ± 0.9
	After	1.2 ± 0.5	1.6 ± 0.5	2.6 ± 1.8
Free Thyroxine Index	Before	2.8 ± 0.2	2.5 ± 0.4	2.3 ± 0.1
	During	2.6 ± 0.2	2.4 ± 0.4	2.3 ± 0.3
	After	2.3 ± 0.2	2.4 ± 0.2	2.4 ± 0.3
Triiodothyronine (ng/dl)	Before	181.9 ± 39.1	160.7 ± 41.3	119.6 ± 24.0
	During	179.4 ± 51.1	160.0 ± 39.7	118.0 ± 26.4
	After	162.3 ± 34.8	163.5 ± 44.9	115.2 ± 16.5
Thyroglobulin (ng/ml)	Before	19.8 ± 14.0	23.7 ± 8.5	36.9 ± 32.9
	During	20.3 ± 13.7	24.5 ± 4.2	41.7 ± 35.6
	After	19.0 ± 12.8	25.0 ± 8.5	44.5 ± 42.7
24-Hour Thyroid ^{123}I Uptake	Before	17.7 ± 4.9	15.6 ± 1.6	19.6 ± 4.9
	During	15.8 ± 3.8	14.1 ± 4.4	19.8 ± 5.7
	After	17.6 ± 2.4	16.6 ± 3.3	22.5 ± 6.1

There were no significant differences in TSH, free thyroxine index, triiodothyronine, thyroglobulin, or 24-Hour Thyroid ^{123}I Uptake values between groups at baseline.

Normal ranges: TSH 0.35 – 5.5 μIU/ml; Free thyroxine index 1 – 4; Triiodothyronine 60 – 181 ng/dl; Thyroglobulin 4 – 40 ng/ml. Intraassay coefficients of variation are: thyroxine < 3.2%, T3 uptake < 3.2%, triiodothyronine < 3.2%, and TSH < 2.5%. For triiodothyronine, the conversion factor to go from conventional to SI units is 0.0154.