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In vivo Formation of Halogenated Reaction Products Following Peroral Sodium Hypochlorite

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To date the principal concern of the disinfection of potable water has centered on the formation of halogenated organic reaction products and the adverse health effects that these products may have. However, an additional area for concern relating to water disinfection is the potential for in vivo production of halogenated organic reaction products resulting from the direct action of residual chlorine with endogeneous organic material. It has been shown that chloroform is produced in vivo after ingestion of sodium hypochlorite (VOGT et al. 1979). The likelihood of the in vivo formation of halogenated organics is also suggested by findings (TREHY & BIEBER 1981) that dihalogenated acetonitriles are generated by the chlorination of various amino acids. Dichloroacetonitrile was previously shown to have mutagenic activity in the Ames bioassay (SIMMON et al. 1977). Similarly, the reaction of hypochlorous acid and hypochlorite with nucleic acids (OLIVIERI et al. 1980), uracil (DENNIS et al. 1978), and pyrimidine and purine bases (HOYANO et al. 1973) has also been reported. Consequently, samples of gut content and plasma were taken from rats dosed with sodium hypochlorite (NaOCl) and analyzed by GC/MS after diethyl ether extraction and methylation. The major objective was to identify any halogenated organic reaction products produced in vivo.

EXPERIMENTAL

Biological Sampling

Twelve male Sprague-Dawley rats weighing approximately 400 g were divided into two control groups (3 fasted/3 non-fasted) and two dosed groups (3 fasted/3 non-fasted). Food was removed from the fasted animals 16 h prior to dosing. The six dosed rats received 7 mL of an 8 mg/mL solution of NaOCl at pH 7.9 by gavage. The six control animals received distilled H_2O at pH 7.9 by gavage also. One hour post-dose all rats were sacrificed under light diethyl ether anesthesia, and as much blood as possible was withdrawn from the inferior vena cava into a heparinized syringe. Immediately following bleeding, the stomach was removed, and the contents were placed into clean glass vials and sealed. No weights were taken as quanitation was not the major objective at this time. The whole blood was then centrifuged, and the plasma was pipetted into clean glass vials having Teflon-lined screw caps.

All biological samples samples were then stored at 4°C and prepared for chemical analysis as soon as possible.

Sample Preparation

All biological samples were extracted with diethyl ether and then methylated for GC/MS analysis. A 1-5 mL aliquot of sample was pipetted into a screw-capped, glass centrifuge tube (approximately 15 mL). The sample was then acidified to approximately pH 1 using 6N HCl. An aliquot of commercial peroxide free diethyl ether equal to the sample volume was added to the tube. The tube was capped and shaken vigorously for one min and then centrifuged for 5 min at 2000 rpm. The ether fraction was then transferred through a jumbo Pasteur pipet (8 mm i.d.) filled with 5 cm of anhydrous sodium sulfate to remove any water and collected in a ground glass stoppered test tube. The sample was again extracted with ether and passed through the sodium sulfate column. The column was then rinsed with 2 mL of ether. All aliquots were combined into a clean glass test tube as they eluted. A boiling chip was added to the extract. A micro evaporation concentrator was placed on the test tube and the sample was concentrated to 1 mL using a water or oil bath set at 45°C. The 1-mL sample was then transferred to a small Teflon-lined, screw-cap glass vial (approximately 8 mL), methylated with 1-2 mg purified 3-methyl-1-p-tolyltriazine (HUMBERT & FERNANDEZ 1976), capped, and set aside at room temperature for at least 10 min to insure complete methylation. Just prior to GC/MS analysis, three internal standards (1-chlorohexane, 1-chlorooctane, and p-bromofluorobenzene) were added to the ether extracts in concentrations of 26 ng/µL each. These internal standards were used to monitor GC/MS stability and calculate relative retention times of unknown peaks.

Chemical Analysis

GC/MS analyses were performed on a Finnigan Model 3300 quadrupole mass spectrometer equipped with an INCOS Model 2300 data system computer. A Carlo-Erba Fractovap 4160 gas chromatograph was equipped with a Grob-designed splitless injector and 60 m long x 0.25 mm i.d. wall-coated-open-tubular fused silica SE-30 column. One end of the column was inserted into the MS ion source chamber just to the edge of the ion beam, by way of a transfer oven with a fixed temperature of 275°C. Two microliters of each ether extract, with internal standards added, were injected with the column vent of the splitless injector closed. The column vent (20:1 ratio) was opened 30 s after injection. The helium carrier gas flow rate was approximately 3 mL/min at 25°C; the linear velocity through the GC column was 25 cm/sec with a helium head pressure of 1.2 kg/cm^2 (17 psi) without a flow controller. The injector temperature was maintained at 260°C. Samples were injected at 20°C, held for 6 min, then the oven temperature was programmed to

250°C at a rate of 4°C/min. Mass spectra were acquired at the rate of 1 spectra/sec (mass range: 14-450 amu at 70eV; source temp: 90°C). Source pressure was approximately 5×10^{-6} torr when the GC column temperature was 25°C. Data acquisition was terminated after 5000 mass spectra (approximately 80 min) were obtained. The survey nature of this study precluded the use of shorter analysis times.

RESULTS AND DISCUSSION

Sample results at this time can only be expressed as detectable or non-detectable as a direct result of the low levels of reaction products found. Typically, chlorinated reaction products detection limits, from subsequent inhouse studies, range from approximately 0.06 to 1.3 µg/mL of plasma in this type of broad spectrum capillary column GC/MS scanning. The critical point of these preliminary studies was to establish the presence (if above detection limits) of reaction products from water disinfectants *in vivo*. Several previously unreported reaction products were discovered.

The formation of trichloroacetic acid (and other chlorinated organic reaction products) in the gut content and plasma in fasted and nonfasted rats one hour after dosing was our primary finding (Table 1). The detection of trichloroacetic acid and dichloroacetic acid in the presence or absence of food in the upper gastrointestinal tract would tend to indicate that *in vivo* formation of these particular chlorinated acetic acids is not dependent on NaOCl's interactions with foreign organic material in the gut. The specific site of biotransformation and the mechnism of action are not known at this time. Acetic acid was found in relatively large quantities (chromatographed without significant methylation) in the stomachs of all 12 rats tested. In all dosed rats dichloroacetic acid and trichloroacetic acid were detected (Table 1). In only two of six plasma samples from dosed rats was trichloroacetic acid detected versus five out of six contained dichloroacetic acid. The minimum detection limits of 0.3 µg/mL (dichloroacetic acid) versus 1.3 µg/mL (trichloroacetic acid) could possibly account for this result. Chloroform was also found to be present in all but one of the samples where trichloroacetic acid was detected. Chloroform is known to be a break down product of trichloroacetic acid upon heating (GARRETT & LAMBERT 1966). Chloroform could be an *in vivo* decarboxylation product of trichloroacetic acid and/or could be produced directly from residual chlorine's interaction with endogenous organic material (VOGT et al. 1979).

Dichloroacetonitrile was found in the gut contents of 2 of the 3 non-fasted rats (Table 1). If the gut is totally void of food content dichloroacetonitrile is not detected (visual inspection of rat #2DF indicated that food content was present in the gut). This would support past findings (TREHY & BIEBER 1981) that specific

Table 1. *In Vivo* Halogenated Organic Reaction Products

Group	Sample Size	$CHCl_3$	Acetic Acid	DCAN	TCAN	DCA	TCA
Blood Plasma							
1 DF[a]	0.9 mL	-[b]	+[c]	-	-	+	-
2 DF	2.5 mL	-	+	-	-	+	+
3 DF	1.1 mL	-	+	-	-	-	-
1 CF[d]	3.2 mL	-	+	-	-	+	-
2 CF	NM[e]	-	+	-	-	-	-
3 CF	2.3 mL	-	+	-	-	-	-
1 DN[f]	3.0 mL	+	+	-	-	+	+
2 DN	3.0 mL	-	+	-	-	+	-
3 DN	0.8 mL	-	+	-	-	+	-
1 CN[g]	2.6 mL	-	+	-	-	+	-
2 CN	5.0 mL	-	+	-	-	-	-
3 CN	3.1 mL	-	+	-	-	-	-
Stomach Content							
1 DF	<1.0 g	+	+	-	-	+	+
2 DF	2.1 g	+	+	+	-	+	+
3 DF	3.3 g	+	+	-	-	+	+
1 CF	<1.0 g	-	+	-	-	-	-
2 CF	<1.0 g	-	+	-	-	-	-
3 CF	<1.0 g	-	+	-	-	-	-
1 DN	NM	+	+	+	-	+	+
2 DN	1.8 g	+	+	-	-	+	+
3 DN	2.8 g	+	+	+	-	+	+
1 CN	3.5 g	-	+	-	-	-	-
2 CN	2.0 g	-	+	-	-	-	-
3 CN	1.9 g	-	+	-	-	-	-

[a]Dosed, fasted; [b]Not detected; [c]Detected; [d]Control, fasted; [e]Not measured; [f]Dosed, non-fasted; [g]Control, non-fasted.

amino acids react *in situ* with OCl^- to form dichloroacetonitrile. A preliminary *in vitro* addition of the 8 mg/mL NaOCl dosing solution to non-fasted control rat gut contents produced dichloro-

acetic acid, trichloroacetic acid, dichloroacetonitrile, and trichloroacteonitrile. These could possibly be due to direct chlorination in the stomach. Acetic acid mixed with NaOCl *in vitro* did not produce any of the aforementioned compounds. Dichoracetonitrile was not detected in any plasma samples. Time course studies are presently being investigated to determine oral dose dichloroacetonitrile pharmacokinetics.

Other related preliminary studies have indicated that NaOCl/KBr solutions (approximately 6:4 w/w ratio) by oral gavage not only produced dichloroacetic acid, trichloroacetic acid, and dichloroacetonitrile in the non-fasted rat gut, but also bromodichloromethane, dibromomethane, dibromoacetic acid, and dibromoacetonitrile. (Table 2) Several trihalogenated organic compounds were also detected in the plasma (i.e., tribromomethane and chlorodibromomethane).

Table 2. Non-Fasted Rat Dosed With 48 mg Cl(NaOCl) and 32 mg Br (KBr) one h Post-Dosed

Compound	Plasma	Stomach Content
Chloroform	-	+
Ethanol	-	+
Acetic Acid	+	+
Dichloroacetonitrile	-	+
Trichloroacetonitrile	-	-
Dichloroacetic Acid	-	+
Trichloroacetic Acid	-	+
Bromoform	+	+
Bromodichloromethane	-	+
Chlorodibromomethane	+	+
Dibromoacetonitrile	-	+
Dibromoacetic Acid	-	+
Dibromomethane	-	+

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