Vol. 179, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1991 Pages 436-441

1-BROMOALKANES AS NEW POTENT NONTOXIC GLUTATHIONE DEPLETORS IN ISOLATED RAT HEPATOCYTES

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Received July 19, 1991

Summary: The effect of 1-bromoalkanes on intracellular glutathione (GSH) was studied in freshly isolated rat hepatocytes. Treatment of cells with bromoalkanes depleted cellular GSH levels without causing cytotoxicity. The extent of GSH depletion was directly proportional to the concentration and increasing chain length of 1-bromoalkanes (C_2-C_7). Bromoheptane (100μM) depleted GSH by 87% in 30 mins which remained depleted for the 4 hr study period without causing cytotoxicity. A 30 fold higher concentration of bromoheptane was required before cytotoxicity ensued. Bromoheptane would therefore be particularly useful for studying the role of GSH in modulating xenobiotic cytotoxicity.

Glutathione (GSH) plays an important role in detoxifying many electrophilic compounds by GSH: conjugate formation (1) or by reducing various oxidising agents (2). Several compounds are however activated following conjugation with GSH (3,4).

GSH depletion in vivo, in cells or homogenates by other glutathione S-transferase substrates such as diethyl maleate, phorone, dinitrofluorobenzene or acrolein can result at slightly higher concentrations in a lipid peroxidation associated cytotoxicity which can be prevented or delayed with antioxidants or ferric chelators (5-8). It has therefore been suggested that GSH depletion promotes endogenous oxidative stress and that GSH plays an antioxidant role in cells (5-7).

Oral administration of 1-halogenoalkanes to rats has been shown to cause hepatic GSH depletion (9) presumably as a result of their effectiveness as GSH-S-alkyltransferase substrates (10). Alkyl mercapturic acids are also excreted in the urine (11-14).

Results reported in this communication show that bromoheptane is much more effective at depleting GSH in isolated hepatocytes than diethylmaleate or phorone used by other investigators. Furthermore cytotoxicity did not ensue until a concentration thirty times higher than that required to deplete GSH. The other GSH-S-transferase substrates caused marked cytotoxicity at a concentration less than two fold that required to deplete GSH. Depletion of

0006-291X/91 $1.50

cellular GSH with bromoheptane would therefore be a particularly useful method for studying the role of GSH in the cytotoxic mechanism(s) of xenobiotics.

Methods and Materials

Fluoro-2,4-dinitrobenzene, iodoacetic acid, GSH, and Trypan blue were obtained from Sigma Chemical Co. (St. Louis, MO). 1-Bromoalkanes were purchased from Aldrich Chemical Co. (Milwaukee, WS). Collagenase (from Clostridium histoliticum), Hepes, and bovine serum albumin were obtained from Boeringer-Mannheim (Montreal, Canada). All other chemicals used were of the highest purity commercially available.

Hepatocytes were isolated from adult male Sprague-Dawley rats (220-250g) following a collagenase perfusion of the liver as described by Moldeus et al (15). Cell viability was measured by the Trypan blue exclusion method, and the hepatocytes used in this study were at least 85% viable. Cells were incubated at a concentration of 1 x 10^6 cells/ml in rotating round-bottom flasks at 37°C in Krebs-Henseleit buffer, pH 7.4, supplemented with 12.5 mM Hepes under an atmosphere of 95% O_2, 5% CO_2. The final incubation volume was 20 ml, and hepatocytes were preincubated for 30 min before addition of 1-bromoalkanes. Stock solutions of 1-bromoalkanes in dimethyl sulfoxide (DMSO) were prepared immediately prior to use. The vehicle (0.5%) alone neither depleted GSH nor caused cytotoxicity.

The total amount of GSH and GSSG in isolated hepatocytes was measured in deproteinized samples (using 5% metaphosphoric acid) after derivatization with iodoacetic acid and fluoro-2,4-dinitrobenzene, by high-pressure liquid chromatography, using a μBondapak NH_2 column (Water Associates, Milford, MA)(15). GSH and GSSG were used as external standards. A Waters 6000A solvent Delivery system, equipped with a Model 660 solvent programmer, a Wisp 710A automatic injuctor, and a Data Module were used for analysis.

Results and Discussion

1-Bromoalkanes at micromolar concentrations depleted intracellular GSH levels markedly after addition to isolated rat hepatocytes (Table 1). The degree of GSH depletion increased with increasing chain length of bromoalkanes (C_2-C_7). Thus, 1-bromohexane and 1-bromoheptane at 100μM were the most effective at depleting intracellular GSH levels (83% and 87% respectively) in isolated hepatocytes after 30 minutes. In the absence of cytosolic GSH-S-transferases, GSH reacted much more slowly with bromoalkanes (results not shown). This indicates that the substrate specificity for the GSH-S-transferases catalysing the GSH depletion is determined by the chain length and lipophilicity of the 1-bromoalkane. Fig. 1 shows that the rate and extent of GSH depletion is also dependent on the concentration of 1-bromoheptane. Although 100μM bromoheptane depleted GSH 87% in 30 mins and GSH levels remained depleted for 2-3 hours, no cytotoxicity ensued even at 4 hours. Furthermore, as shown in table 2, bromoheptane was not cytotoxic at 2mM and higher concentrations were required. Lipid peroxidation also did not occur with concentrations less than 2mM bromoheptane (results not shown).

However as shown in table 1 it can be seen that diethyl maleate or phorone, GSH-S-transferase substrates used by other investigators to deplete hepatocyte GSH, deplete GSH slower and less completely than 1-bromoheptane. Furthermore as shown in table 2, GSH depletion by

Table 1. Intracellular GSH Depletion by 1-Bromoalkanes in Isolated Rat Hepatocytes

1-Bromoalkanes (100 μM)	GSH content (nmol / 10^6 cells)			
	1 min.	10 min.	30 min.	60 min.
none	63.2 ± 4.6	62.5 ± 5.1	63.1 ± 4.8	60.0 ± 4.8
1-Bromoethane	59.2 ± 3.6	55.3 ± 3.7	48.4 ± 3.9	45.3 ± 3.2
1-Bromopropane	58.4 ± 4.1	52.3 ± 3.6	46.4 ± 3.2	40.8 ± 2.9
1-Bromobutane	51.6 ± 3.8	48.5 ± 4.0	40.0 ± 2.9	38.3 ± 2.9
1-Bromopentane	46.5 ± 4.0	43.2 ± 3.7	37.8 ± 2.6	31.6 ± 2.7
1-Bromohexane	45.5 ± 3.5	22.7 ± 2.1	10.9 ± 1.3	9.3 ± 0.5
1-Bromoheptane	42.3 ± 3.2	19.3 ± 1.8	8.4 ± 0.9	7.1 ± 0.5
1-Bromooctane	50.4 ± 4.9	49.7 ± 4.2	32.4 ± 3.1	17.6 ± 2.3
Diethyl maleate (350μM)	50.8 ± 4.6	36.2 ± 4.2	21.8 ± 2.5	15.6 ± 1.4
Phorone (300μM)	54.2 ± 4.5	43.2 ± 3.8	27.3 ± 2.7	20.1 ± 1.6
L-Buthionine (S,R)-Sulfoximine (4mM)	62.0 ± 5.2	51.3 ± 4.7	39.4 ± 3.5	31.2 ± 2.9

Results are expressed as an average of three experiments ± SD.

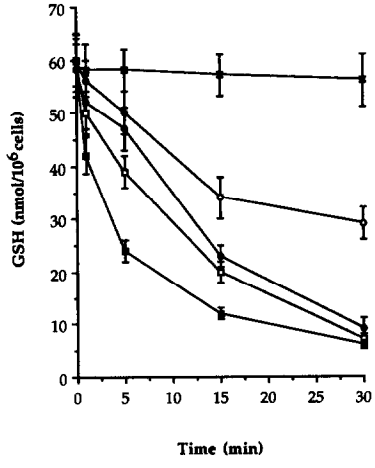

Figure 1. Intracellular glutathione depletion on treatment of hepatocytes with various concentrations of 1-bromoheptane. Control cells —✕— ; 1-bromoheptane- 50μm—○—; -100μm —●—; -200μm —□— and 300μm —■— .

Table 2. 1-Bromoalkanes Induced Cytotoxicity Torwards Isolated Rat Hepatocytes

Condition	Trypan blue uptake (%) at time			
	0.5 h	1.0 h	2.0 h	3.0 h
Control	17 ± 2	18 ± 1	20 ± 3	23 ± 2
1-Bromoethane				
(5mM)	24 ± 3	29 ± 2	30 ± 3	36 ± 5
1-Bromopropane				
(5mM)	25 ± 2	34 ± 4	38 ± 3	51 ± 5
1-Bromobutane				
(5mM)	29 ± 3	45 ± 4	54 ± 5	63 ± 8
1-Bromopentane				
(5mM)	33 ± 4	52 ± 8	63 ± 9	76 ± 8
1-Bromohexane				
(3mM)	27 ± 2	31 ± 4	39 ± 3	56 ± 5
(4mM)	29 ± 4	42 ± 6	63 ± 8	90 ± 7
(5mM)	50 ± 6	91 ± 6	100	100
1-Bromoheptane				
(3mM)	21 ± 3	22 ± 3	23 ± 3	25 ± 2
(4mM)	26 ± 3	29 ± 4	37 ± 3	43 ± 5
(5mM)	28 ± 4	39 ± 5	57 ± 7	75 ± 12
1-Bromooctane				
(5mM)	21 ± 3	27 ± 4	36 ± 6	44 ± 6
Diethyl maleate				
(0.7mM)	35 ± 3	40 ± 3	43 ± 4	48 ± 4
Phorone (0.4mM)	36 ± 4	42 ± 3	43 ± 4	48 ± 4
Ethacrynic acid (0.8mM)	39 ± 3	49 ± 3	62 ± 5	85 ± 8

Results are expressed as an average of three experiments ± SD.

diethyl maleate or phorone resulted in cytotoxicity at concentrations only slightly higher than the concentrations used to deplete GSH. Following GSH depletion with these substrates, lipid peroxidation ensues (results not shown) and cytotoxicity occurs. Furthermore cytotoxicity can be prevented or delayed with antioxidants (11-14, 17) which suggests that lipid peroxidation and GSH depletion contributes to the cytotoxicity. Diethyl maleate also inhibits rat liver microsomal drug metabolism by modifying cytochrome P-450 (18). However, Bromoheptane (2mM) had no effect on hepatic aniline hydroxylase activity (data not shown).

Recently another transferase substrate ethacrynic acid currently in use as a diuretic drug has also been used as a reversible transferase inhibitor (19) to overcome acquired resistance of various tumor cell lines to the anticancer alkylating drugs (20,21) chlorambucil and melphalan. However as shown in tables 1 and 2, GSH depletion and cytotoxicity also occur with the concentrations required and would be expected to contribute not only to the increased anticancer activity but also to the adverse effects associated with such therapy. Investigators are also using buthionine sulfoximine, an inhibitor of GSH synthesis to deplete GSH in tumor cells to increase efficacy of anticancer drugs (22). However as can be seen in Table 1 it depletes GSH less completely than 1-bromoheptane.

Other 1-bromoalkanes were also compared for their effectiveness at inducing cytotoxicity towards isolated hepatocytes (Table 2). Cytotoxicity increased with the chain length of 1-bromoalkanes with bromohexane being the most cytotoxic. Bromooctane and bromoheptane were much less cytotoxic. This presumably indicates that high concentrations of bromohexane can modify membrane macromolecules by direct alkylation or via peroxidation in the GSH depleted cell which results in cytotoxicity.

In conclusion, the present study demonstrates that 1-bromoheptane is the most effective GSH-S-transferase substrate tested, the least cytotoxic and therefore can be used as a tool to modulate cellular GSH levels in isolated hepatocytes. It is also clear that GSH depleted cells have adequate antioxidant and enzymic systems to counteract physiological oxidative stress. The cytotoxicity and lipid peroxidation following GSH depletion by the GSH-S-transferase substrates used by other investigators (11-14) may indicate that metabolism of these substrates results in radical formation and lipid peroxidation (23).

Acknowledgments

This research was supported by grants from the Medical Research Council of Canada and the Natural Sciences and Engineering Research Council, Canada.

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