AIM

Association for Information and Image Management

1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202

Centimeter

Inches

MANUFACTURED TO AIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

DISCLAIMER

Conf-9209280-9

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

BNL-48695

SYNTHESIS AND PRELIMINARY *IN VIVO* TOXICITY EVALUATION OF AN IODINATED SULFIDOBORATE.

M. Miura,¹ P.L. Micca,² J.C. Heinrichs² and D.N. Slatkin²

Brookhaven National Laboratory,

¹Department of Applied Science

²Medical Department

Upton, New York 11973

INTRODUCTION

An iodine-labeled ¹⁰B-carrier would enable the biodistribution of ¹⁰B to be imaged noninvasively in patients so as to optimize the timing of neutron exposure for BNCT. While Na₂B₁₂H₁₁SH [BSH] is in clinical use for BNCT in Japan, its disulfido dimer Na₄B₂₄H₂₂S₂ [BSSB]¹ is also under consideration for BNCT in Japan and elsewhere. Herein we describe the synthesis of an iodinated product of the dimer for possible use in BNCT and compare its toxicity in normal mice to that of BSSB.

METHODS

Chemistry

The ¹⁰B-enriched (95 atom%) sulfidohydroborate dimer K₄H₁₁B₁₂SSB₁₂H₁₁ [K₄BSSB], which was ion-exchanged from the cesium salt (Callery Chemical Co., Callery, Pennsylvania), was treated with iodine and iodine monochloride in tetrachloroethane and allowed to stir at reflux under nitrogen overnight (Figure 1) as described previously for periodination of B₁₂H₁₂-2.² A product was precipitated under these oxidizing conditions using tetramethylammonium chloride and was isolated by recrystallization in 24% molar yield from BSSB. The product was characterized by elemental analyses (Galbraith Laboratories Inc, Knoxville, Tennessee) and infrared [IR] spectroscopy and shown to be, most likely, the periododimer (Me₄N)₄I₁₁B₁₂SSB₁₂I₁₁ [(Me₄N)₄IBSSB]. There was no BSSB identified in the recrystallized product by thin layer chromatography. The biege-colored tetramethylammonium salt was ion-exchanged to the sodium salt for toxicity experiments.

MASTER

Figure 1. Synthesis of IBSSB from BSSB.

Toxicity

Three groups of female mice (BNL Hale-Stoner strain) were each given a single intraperitoneal [i.p.] injection of A) 17 μg B per gram of body weight [gbw] of Na_4BSSB , B) 50 μg B/gbw of Na_4IBSSB or C) given a single i.p. injection of 0.9% NaCl in water [saline]. Injection volumes were all 0.015 ml/gbw. Five days after the injection, mice were euthanized by deep halothane inhalation anesthesia, thoracotomy, and removal of $\approx 0.7\text{--}0.9$ ml blood through the right ventricle; hematological and chemical analyses were performed on the blood specimens from each mouse.

Table I. Hematologic analyses of the blood of mice given BSSB (Group A), IBSSB (Group B) and saline (Group C) in doses described. Mouse group and number [MS], leukocytes [WBC] ($10^3/\mu\text{l}$), erythrocytes [RBC] ($10^6/\mu\text{l}$), hemoglobin [HGB] (g/dl), hematocrit [HCT] (%), mean erythrocyte volume [MCV] ($[\mu\text{m}]^3$), mean erythrocyte hemoglobin [MCH] (pg), mean erythrocyte hemoglobin concentration [MCHC] (%), erythrocyte distribution width [RDW] ($[100\sigma/\text{MCV}]$ %), platelets [PLT] ($10^3/\mu\text{l}$), mean platelet volume [MPV] ($[\mu\text{m}]^3$), granulocytes [GRN] ($10^3/\mu\text{l}$), lymphocytes [LYM] ($10^3/\mu\text{l}$), midrange-sized leukocytes [MID] ($10^3/\mu\text{l}$).

MS	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV	GRN	LYM	MID
A1	3.4	7.76	14.8	42.2	54.4	19.1	35.0	24.8	1090	4.80	0.10	3.2	0.10
A2	5.1	8.81	17.1	49.2	55.8	19.4	34.8	24.9	1382	4.80	0.40	4.2	0.50
A3	4.0	7.74	15.5	44.1	57.0	20.0	35.1	23.9	1101	4.90	0.30	3.4	0.30
A4	4.9	8.81	17.0	47.9	54.4	19.3	35.5	23.7	1391	4.80	0.30	4.5	0.10
A5	5.6	8.46	16.7	47.7	56.4	19.7	35.0	24.2	1316	4.50	0.70	3.9	1.0
B1	2.6	8.05	15.6	44.8	55.6	19.4	34.8	24.2	1405	4.70	0.20	2.2	0.20
B2	4.8	8.28	15.2	45.7	55.2	18.4	33.3	23.6	1395	5.10	0.20	4.5	0.10
B3	4.4	7.92	15.5	43.4	54.8	19.6	35.7	23.9	1536	4.80	0.20	4.0	0.20
C1	4.7	7.88	15.6	44.5	56.5	19.8	35.1	23.9	1384	4.50	0.30	4.2	0.20
C2	4.4	8.72	17.5	48.7	55.9	20.1	35.9	23.8	1223	4.80	0.20	3.9	0.30
C3	3.7	8.66	16.9	48.2	55.7	19.5	35.1	24.3	1334	5.00	0.50	2.6	0.60
C4	3.3	8.64	16.7	48.3	55.9	19.3	34.6	24.5	1249	4.80	0.30	2.8	0.20
C5	4.4	8.85	17.4	50.1	56.6	19.7	34.7	23.9	1222	4.80	0.40	3.6	0.40

Table II. Chemical analyses of plasma of blood from mice given BSSB (Group A), IBSSB (Group B) and saline (Group C) in doses described. Mouse group and number [MS], glucose [GLU] (mg/dl), blood urea nitrogen [BUN] (mg/dl), blood creatinine [BCR] (mg/dl), aspartate transaminase [AST] (U/l), alanine transaminase [ALT] (U/l), alkaline phosphatase [ALP] (U/l), creatine phosphokinase [CPK] (U/l), total protein [TPR] (g/dl), albumin [ALB] (g/dl), calcium [CA] (mg/dl), chloride [CHL] (meq/l), inorganic phosphorus [P] (mg/dl), potassium [K] (meq/l), sodium [SOD] (meq/l).

MS	GLU	BUN	BCR	AST	ALT	ALP	CPK	TPR	ALB	CA	CHL	P	K	SOD
A1	311	16	0.4	46	28	128	50	5.7	3.4	11.7	119	11.7	10.0	156
A2	217	26	0.4	41	21	115	37	5.3	3.3	10.9	118	8.8	6.9	154
A3	315	23	0.4	56	36	130	64	6.0	3.6	11.1	117	9.6	9.2	154
A4	268	23	0.5	70	34	103	141	5.3	3.2	11.3	119	10.9	9.9	153
A5	249	23	0.6	80	28	132	215	6.0	3.6	11.6	121	9.0	6.5	161
A6	269	30	0.5	40	19	143	58	5.8	3.4	11.0	120	9.4	7.2	158
B1	332	15	0.4	80	33	108	89	5.5	3.0	11.2	119	10.1	7.9	155
B2	206	27	0.4	77	47	123	148	5.8	3.3	11.9	119	10.8	7.8	156
B3	252	26	0.4	89	66	123	75	5.8	3.2	11.3	117	9.8	6.0	155
B4*	278	28	0.5	44	22	110	35	5.9	3.3	11.5	116	9.9	7.1	154
C1	307	24	0.5	63	26	149	134	5.6	3.0	11.6	121	11.7	11.3	153
C2	298	17	0.4	58	26	156	113	5.3	3.2	11.1	121	11.0	11.0	154
C3	286	31	0.5	49	26	120	36	5.5	3.2	11.3	115	8.8	6.3	154
C4	295	28	0.5	41	28	112	35	5.5	3.3	11.1	119	9.0	6.6	155
C5**	234	32	0.5	112	54	126	245	5.9	3.5	11.4	123	9.7	9.5	158
C6	226	33	0.5	50	19	136	99	6.1	3.6	11.6	121	10.1	7.9	159
C7	410	29	0.6	30	10	164	58	5.8	3.3	12.0	121	11.2	10.5	157

*Mouse not given entire dose of IBSSB; excluded from analyses.

**Mouse appears to have had liver dysfunction by its abnormal liver enzyme values; excluded from analyses.

RESULTS AND DISCUSSION

The IR spectrum shows a marked reduction in intensity of the strong absorbance at $\approx 2500 \text{ cm}^{-1}$ corresponding to the B-H stretching frequency of the starting dimer. This could be due to a minor contamination by BSSB or to incomplete iodination. Two absorbances appeared at 968 and 944 cm^{-1} which correlate with the B-I stretching frequencies observed in $\text{B}_{12}\text{I}_{12}^{-2}$ (940 and 925 cm^{-1}).² The higher frequencies in IBSSB than in $\text{B}_{12}\text{I}_{12}^{-2}$ may be due to inductive effects, which would indicate that sulfur exerts a greater electron-withdrawing effect than iodine.

A single i.p. injection of IBSSB at a dose corresponding to $50 \mu\text{g B/gbw}$ does not appear to be more toxic than an injection of one-third the amount of boron as BSSB, as judged by hematological and chemical tests of the blood 5 days later. Fourteen chemical and thirteen hematological tests of blood from mice given either BSSB (Group A) or IBSSB (Group B) showed no significant differences between the two groups using the Wilcoxon two-sample test.³ Alanine transaminase [ALT] levels in the mice given IBSSB were significantly different from those in mice given physiological saline (Group C).

Wilcoxon analyses³ showed that the only significant difference ($P = 0.05$) between any of the groups in Tables I and II occurred for ALT between groups B and C (mice given IBSSB and mice given saline, respectively).

Table III. Ranks and rank sums of the ALT values for groups B (IBSSB) and C (saline).

Rank	1	2	3	4	5	6	7	8	9	Rank sum
IBSSB							33	47	66	24
Saline	10	19	26	26	26	28				21

CONCLUSIONS

Periodination of BSSB can be carried out under reaction conditions similar to those used for $B_{12}H_{12}^{2-}$. We surmise from this preliminary *in vivo* experiment that IBSSB may be slightly hepatotoxic to mice. Since the putative weight ratio of iodine to boron-10 in IBSSB is 11.5, the presence of a therapeutically adequate concentration of ^{10}B in tumor, for example $30 \mu g$ ^{10}B per gram tumor, would be associated with $345 \mu g$ I per gram tumor. This would be suitable for imaging a tumor in the brain and for quantifying the iodine using computed tomography [CT] without using conventional contrast enhancement. CT contrast enhancement in malignant gliomas is typically ≈ 2 -20 EMI scale units; 1 EMI scale unit corresponds to $77 \mu g$ I/ml tissue.⁴ It follows that each added EMI scale contrast enhancement unit observed in a tumor image pixel by CT after 95% ^{10}B -enriched IBSSB administration would correspond to an additional $6.7 \mu g$ ^{10}B /ml in the tumor zone corresponding to that pixel. Thus, ^{10}B concentrations useful for BNCT of malignant gliomas should be readily quantified by conventional CT using ^{10}B -IBSSB as a combined radiographic contrast agent and ^{10}B -carrier. Alternatively, iodine-radiolabeled IBSSB would allow visualization and quantification of iodine in the tumor by single photon emission computed tomography [SPECT].

Because boron and iodine are linked covalently in IBSSB, quantification of iodine in the tumor would thereby also quantify boron in the tumor noninvasively using either radiolabeled or nonradiolabeled IBSSB in SPECT or CT, respectively, provided that *in vivo* deiodination of IBSSB, if it occurs at all, is sufficiently slow. In view of the potential clinical advantages of an iodinated boron carrier over an unlabeled boron carrier for BNCT, these results indicate that IBSSB should undergo further preclinical studies.

ACKNOWLEDGEMENTS

This work was supported by U.S. Department of Energy Contract No. DE AC02-76CH00016 and by National Institutes of Health Grant 1R01CA38107-01A2.

REFERENCES

1. D.D. Joel, R.G. Fairchild, J.A. Laissue, S.K. Saraf, J.A. Kalef-Ezra and D.N. Slatkin, *Proc. Natl. Acad. Sci., USA*, **1990**, *87*, 9808-9812.
2. W.H. Knoth, H.C. Miller, J.C. Sauer, J.H. Balthis, Y.T. Chia and E.L. Muetteries, *Inorg. Chem.*, **1964**, *3*, 159-167.
3. M. Miura, P.L. Micca, J.C. Heinrich, D. Gabel, R.G. Fairchild and D.N. Slatkin, *Biochem. Pharm.*, **1992**, *43*, 467-476.
4. M.H. Gado, M.E. Phelps and R.E. Coleman, *Radiology*, **1975**, *117*, 589-593.

**DATE
FILMED**

8 / 18 / 93

END