1960 MAY - AUGUST VOL. 129

THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Founded by
John J. Abel
1909

Official Publication of the American Society for Pharmacology and Experimental Therapeutics, Inc.

BALTIMORE, MARYLAND

PENETRATION OF BRAIN AND BRAIN TUMOR BY AROMATIC COMPOUNDS AS A FUNCTION OF MOLECULAR SUBSTITUENTS[1]

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Received for publication November 20, 1959

The possibility of destroying tumors by neutron capture irradiation was first proposed by Kruger (1940). The most favorable tumor for such therapy was considered by Sweet *et al.* (1952) and Javid, Brownell and Sweet (1952) to be the highly malignant brain tumor, glioblastoma multiforme. The rationale was the existence of the blood-brain barrier (BBB) which was shown (Ehrlich, 1885; Lewandowsky, 1900; Friedemann, 1942; Tschirgi, 1952; Herlin, 1956; Bakay, 1956; Mayer *et al.*, 1959) to restrict the passage of various compounds into the central nervous system (CNS) relative to other tissues. In brain tumors (Moore, 1947) as well as other diseases of the brain (Macklin, 1920) there is a breakdown in this normal barrier phenomenon permitting localization in this lesion of radioactive isotopes (Selverstone *et al.*, 1949; Sweet, 1951).

For neutron capture irradiation of brain tumors, it is necessary to concentrate in the neoplasm an isotope, for example nonradioactive B^{10}, with a high cross-section capture for thermal neutrons. These nonionizing neutrons would be avidly absorbed by the nucleus of the $boron^{10}$ atom producing the following nuclear reaction with the liberation of 2.5 million electron volts:

$$boron^{10} \rightarrow (boron^{11}) \rightarrow lithium^{7} + helium^{4}$$

Tissues containing sufficient boron would be destroyed upon neutron irradiation and thus there is a need for compounds which will be excluded from normal brain and will concentrate in the tumor.

Initial studies by Soloway (1958) showed great differences in penetration of the brain and brain tumor by various substituted phenylboronic acids. There appeared to be a direct correlation between penetration of the brain and a low aqueous-benzene partition coefficient. Those compounds which concentrated in the lipid solvent, benzene, penetrated brain readily, gave poor tumor/brain ratios as measured by boron content and were considered to have a high lipid solubility. Substances which were excluded from the benzene in this partition were considered to have poor lipid solubility. Many of these permeated the brain to a limited degree and gave high tumor/brain boron localizations.

The purpose of this investigation was to determine which substituents on phenylboronic acid restricted and which aided penetration of the CNS. From such a study, information might be obtained about the mechanism of action of the BBB on various compounds and could serve as a guide both to those engaged in therapy based on the exclusion of substances from the CNS (Mark *et al.*, 1960) as well as to those involved in the synthesis of neurotropic drugs.

METHODS. To determine the aqueous-benzene partition coefficient of the various boron compounds, about 10 mg of each substance was distributed between 50 ml of a phosphate-buffered aqueous medium of pH 7.2 and 50 ml of benzene. The compounds were partitioned by shaking in a separatory funnel and the layers were separated. Aliquots of each phase were then analyzed by the method of Ellis *et al.* (1949) for boron content. It was essential for maximum accuracy of this colorimetric method that the samples contain 1 to 5 μg of boron. In the case of the more lipid soluble compounds it was essential to dilute aliquots of the partitioned fluids and to analyze an aliquot of these diluted liquids. Many of those compounds which were largely excluded from the benzene

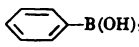
[1] This research was supported by a grant from the U. S. Atomic Energy Commission under contract No. AT(30-1)-1093, and by the National Cancer Institute, U. S. Public Health Service Grant No. C-3174(C2) Rad.

phase required aliquots of 15 ml of this layer to obtain significant readings on the Coleman Junior Spectrophotometer. The results appear in table 1 and are listed as μg of boron per ml of undiluted solution.

The tumor/brain boron ratios were obtained from C3H mice bearing subcutaneous transplanted gliomas.[2] Fresh tumor tissue was ground in normal saline and a cellular suspension was injected in the region of the left scapula in 6- to 8-week-old C3H mice. Within 7 to 10 days the tumors were of sufficient size for use. Each compound was dissolved in dilute base or water, depending upon the solubility of the compound, and was injected intraperitoneally into the glioma mice. At fixed time intervals after injection, the animals were asphyxiated by ether and five 50-mg samples of both tumor and brain were then analyzed by the above method for boron content. In table 2 is recorded the average amount in tissue in μg of boron per g as well as the tumor/brain ratio for each compound. The number of mice used for each dose at each time varied from 1 to 3 and the tissue content listed is an average. Only short sacrifice times are recorded since longer times may permit metabolic alteration in the compound and would therefore not be a true criterion of the compound's immediate localization in tissue. In many cases at times upwards of one hour the tumor/brain ratio approached one. It is also a possibility that this data might be affected by the presence of metabolic breakdown products of the various boron compounds. No evidence was obtained of the metabolism of these compounds in mice since the method of analysis was not for the particular compound in question but for boron in the form of boric acid.

TABLE 1

Aqueous-benzene partition coefficients

	Aqueous	Benzene	Partition Coefficient
p-$Si(CH_3)_3$*‡	0.38	12.6	0.03
2,4,6-tri CH_3†	4.0	9.0	0.4
m-CF_3‡	3.4	7.7	0.4
p-SCH_3*	5.1	7.5	0.7
p-Br	5.1	6.4	0.8
p-OC_2H_5	6.4	6.3	1
p-Cl	6.9	5.6	1
o-CH_3	9.8	6.7	2
m-CH_3	10.9	6.8	2
p-CH_3	8.0	5.2	2
p-OCH_3§	9.4	3.5	3
p-F	11.4	3.5	3
p-H	14.4	2.3	6
o-NO_2	12.6	1.9	7
m-NO_2	13.5	1.1	12
m-$NHCOOC_2H_5$	10.8	0.77	14
p-CHO†	12.4	0.43	29
3-NO_2-4-COOH	9.2	0.18	51
p-COOH	11.4	0.17	67
3-NH_2-4-CH_3	15.4	0.23	67
p-$B(OH)_2$	24.5	0.06	>200
m-NH_2	12.4	0.06	>200
2-NO_2-4-$B(OH)_2$	22.1	0.05	>200
m-COOH	12.1	0.04	>200
p-CH_2CHCOO^- (†) NH_3^+	10.2	0.04	>200
m-OH‡	17.9	0.03	>200
p-OH*	16.8	0.02	>200
2-NO_2-4-COOH	9.8	0	>200
3-NH_2-4-COOH	12.2	0	>200
3-$NHCOCH_2CH_2COOH$	8.9	0	>200
3-$NHCONH_2$	10.9	0	>200

* Dr. H. Gilman, Professor of Chemistry at Iowa State University, kindly supplied this compound.

† Dr. H. R. Snyder, Professor of Chemistry at the University of Illinois, kindly supplied this compound.

‡ These compounds existed prior to partition as trimeric anhydrides.

§ The results reported in Science **128:** 1572, 1958, for this compound were found to be incorrect upon successive analyses.

Results and Discussion. The aqueous-benzene partition coefficients in table 1 are of the various mono- and disubstituted aromatic boronic acids. All compounds with a coefficient less than 29 were toxic at moderate doses producing a depressant action upon the animal's normal activity and response to stimuli. Each compound in this category had a tumor/brain boron ratio of less than 1 as shown in table 2. Those with a coefficient of 2 or less were highly toxic and in small doses (18 to 35/μg/g of mouse) produced death of the animals. Examination of table 1 reveals a definite order for the halogens substituted in the *para* position on phenylboronic acid. The bromo compound concentrated to a greater extent in the benzene phase than did the chloro compound and this in turn had a lower partition

[2] The authors are greatly indebted to Dr. D. M. Perese of the Department of Neurosurgery at the Roswell Park Memorial Institute in Buffalo, New York, for kindly supplying us with the original subcutaneously grown ependymoma. This was produced by implantation in the brain of a methylcholanthrene pellet and the intracranial neoplasm resulting was transplanted subcutaneously.

TABLE 2

*Tumor/brain boron localization factors**

	Dose†	Time of Sacrifice	Tumor‡	Brain‡	Localization Factor§
4-$Si(CH_3)_3$	35	15¶	<3	20	0.1
	35	20¶	6	26	0.2
	35	30	4	31	0.1
2,4,6-tri CH_3	35	10¶	6	37	0.2
	35	15	23	40	0.6
	35	30	29	35	0.8
m-CF_3	35	15¶	7	42	0.2
	18	20¶	12	30	0.4
	35	30	12	14	0.9
p-SCH_3	35	9¶	4	50	0.1
	18	7¶	10	30	0.3
	18	30	11	17	0.6
p-Br	35	15	14	57	0.2
	18	15	9	43	0.2
	18	30	12	31	0.4
p-OC_2H_5	18	15	13	23	0.6
	18	30	11	20	0.6
p-Cl	35	15	23	52	0.4
	35	30	32	59	0.5
o-CH_3	35	15	23	52	0.4
	35	30	32	59	0.5
m-CH_3	35	15	15	47	0.3
	35	30	12	25	0.5
p-CH_3	35	15	18	53	0.3
	35	30	20	44	0.5
p-OCH_3	35	15	29	44	0.7
	35	30	33	40	0.8
p-F	35	15	16	62	0.3
	35	30	13	56	0.2
p-H	35	15	34	51	0.7
	35	30	34	44	0.8
o-NO_2	35	15	25	41	0.6
	35	30	29	41	0.7
m-NO_2	35	15¶	16	40	0.4
	35	15	12	42	0.3
	18	30	16	19	0.8
m-$NHCOOC_2H_5$	35	15¶	18	34	0.5
	18	15	13	20	0.6
	18	30	14	19	0.7
p-CHO	35	15	9	16	0.6
	35	30	26	13	2.0
3-NO_2-4-COOH	70	15	38	15	2.5
	70	30	53	12	4.4
	35	30	17	5	3.4
p-COOH	70	15	30	8	3.8
	140	15	57	10	5.7
	70	30	43	7	6.1
	140	30	63	8	7.9

TABLE 2—*Continued*

	Dose†	Time of Sacrifice	Tumor‡	Brain‡	Localization Factor§
3-NH_2-4-CH_3	35	15	30	33	0.9
	35	30	28	29	1.0
p-$B(OH)_2$	70	15	21	9	2.3
	35	30	18	8	2.2
	70	30	44	12	3.7
	140	30	56	25	2.2
m-NH_2	35	15	25	21	1.2
	70	15	43	39	1.1
	35	30	32	26	1.2
	70	30	60	53	1.1
2-NO_2-4-$B(OH)_2$	35	15	10	4	2.5
	70	30	34	7	4.9
	140	30	43	7	6.1
	200	30	52	9	5.8
m-COOH	140	15	57	10	5.7
	70	30	44	6	7.3
	140	30	63	8	7.9
p-CH_2CHCOO^- | NH_3^+	140	15	34	4	8.5
	140	30	65	10	6.5
m-OH	35	15	26	14	1.9
	70	15	42	38	1.1
	35	30	33	22	1.5
	70	30	60	44	1.4
p-OH	35	15	25	17	1.5
	140	15	133	72	1.8
	35	30	30	19	1.6
2-NO_2-4-COOH	35	15	21	3	7.0
	70	15	42	5	8.4
	70	30	39	7	5.6
3-NH_2-4-COOH	200	15	45	7	6.4
	140	30	49	6	8.2
	200	30	50	7	7.2
3-$NHCOCH_2CH_2COOH$	200	15	62	9	6.9
	140	30	29	4	7.2
	200	30	54	8	6.8
3-$NHCONH_2$	140	15	75	10	7.5
	140	30	74	7	10.5

* In most cases the tumor and brain concentrations were averages of 2 or 3 mice.

† Dose in μg of boron per g of mouse.

‡ Concentrations are in μg of boron per g of tissue.

§ Localization factor is the tumor/brain boron ratio.

¶ The toxicity of the compounds resulted in death of the animals at these times.

coefficient than the fluoro derivative. On this basis it would appear that the higher the atomic weight of the attached halogen the greater the lipid solubility of the compound. As would be anticipated, the *para* ethoxy compound showed a greater relative concentration in the benzene phase than did the methoxy derivative; its longer alkyl chain is known to increase fat solubility. Replacement of the oxygen in the methoxy compound with a sulfur atom appreciably shifted the partition coefficient to a lower value. In this connection it is interesting to note that Mark *et al.* (1958) found that the rate of penetration of the CNS by thiobarbituric acid is greater than that of the oxygen analogue and in agreement with its higher lipid solubility. In general it appears that in a single group of nonmetallic elements in the periodic table, the higher the atomic weight of an element attached to the same organic component, the more lipid soluble the compound becomes.

m-Tolylboronic acid is quite lipid soluble but replacement of the hydrogens on the methyl group with fluorine atoms shifts the partition coefficient to an even lower value. The *m*-trifluoromethylphenylboronic acid corresponds, with regard to aqueous-benzene partition, with the 2,4,6-trimethyl derivative. It is expected that the trimethyl derivative would be more lipid soluble than the monomethyl compound, for increasing the number of alkyl groups increases fat solubility. Evidence of the effect of a methyl substituent on lipid solubility can be seen by a comparison of 3-aminophenylboronic acid with the 3-amino-4-methyl compound and phenylboronic acid with *o*-, *m*- and *p*-tolylboronic acids. In each case the addition of the methyl group results in a lower aqueous-benzene partition coefficient.

For the purpose of neutron capture therapy, it is highly desirable to have compounds which contain a high percentage of boron. If other factors such as toxicity, stability and solubility were the same, those substances with a larger percentage would be the compounds of choice. They would permit large doses of boron to be given and consequently higher levels of B^{10} could be attained in the tumor. Such consideration resulted in a comparison of phenylboronic acid with benzene-1,4-diboronic acid. Introduction of a second boronic acid moiety markedly decreased lipid solubility as shown in table 1. From table 2 it is apparent that such an alteration appreciably improved the tumor/brain boron ratio. It was considered that decreased lipid solubility might be the result of the formation of a diboronic acid anion under physiological conditions, since it is probable that the un-ionized form is the lipid soluble one. Both anions and cations in general show a decreased rate of penetration from plasma into the CNS (Tschirgi, 1952). For the purpose of determining whether the observed difference between phenylboronic acid and benzene-1,4-diboronic acid was due to ionization of the latter under physiological conditions, the pK_a values of both compounds in 50% ethanol was measured. Phenylboronic acid had a pK_a of 10.7 whereas the diboronic acid had one of 10.2. Thus the decreased lipid solubility of the latter compound is not attributable to its ionization.

Three other groups attached to phenylboronic acid were effective in decreasing lipid solubility and at the same time increasing appreciably the tumor/brain boron ratio. They are the aliphatic and aromatic carboxyl groups and the ureido function. Introduction of the carboxyl group in the 4-position of *m*-nitrophenylboronic acid shifted the partition function to higher values, decreased CNS toxicity and gave a larger tumor/brain ratio. Anion formation might also be a factor in the low lipid solubility of the carboxylic acid derivatives and their failure to penetrate the BBB readily. The pK_a of *p*-carboxyphenylboronic acid in 50% ethanol was 5.2 (for the carboxylic acid function) compared to 10.7 for phenylboronic acid. This reasoning of ion formation, however, could not be applied to the ureido group.

Though lipid solubility is an important criterion, it is probably not the only mechanism involved in brain permeability. The problem of explaining active transport of inorganic ions and glucose into the CNS would appear to eliminate solubility in lipid as the sole process for CNS permeability. In this series of boron compounds we have found that those which concentrate well in a lipid solvent show invariably a facile penetration of the brain. However, the converse proposition does not always follow; that is, those compounds which concentrate in the aqueous layer do not necessarily give high tumor/brain ratios and, in certain instances, are quite toxic to the CNS. Two types of compounds are in this category, the amines with the exception of those containing a carboxylic acid function and the phenols. These compounds penetrate the brain nearly as well as the tumor and are moderately

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