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The Potential Development Sulfonylhydrazines for the Treatment of Alzheimer's Disease

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Abstract: The anticancer drug BCNU has striking activity against Alzheimer's disease in humans. This activity is likely due to one of the many reactive electrophiles generated by BCNU. The structure and complex decomposition pathways of BCNU do not allow for the synthesis of analogs that can selectively generate these electrophiles. Therefore, their contributions to the anti-Alzheimer's efficacy and toxicity of these different electrophiles cannot be determined. The sulfonylhydrazine prodrugs (SHP), which readily cross the blood-brain barrier, can be regarded as functional homologs of BCNU but with substantial design flexibility and tolerance for structural modification. Agents have been synthesized, which generate individual or combinations of identical or similar electrophiles to the electrophilic species produced by the BCNU. This should allow for the determination of which electrophilic species are important in the anti-Alzheimer's disease activity and how using SHPs, we can generate the most efficacious of these electrophiles, in a more selective and less toxic manner, to generate an improved anti-Alzheimer's agent.

Keywords: Alzheimer's disease;dementia; BCNU; carmustine; sulfonylhydrazines.

1. Introduction

The concept for this project originated from researchers in the laboratory of the late Professor Alan C. Sartorelli in the Yale Medical School's Department of Pharmacology. Following Professor Sartorelli's death, his laboratory was closed. Thus, a project with the potential to benefit many was also terminated. It is hoped that some of the readers of this article will continue this promising line of work.

A striking reduction of amyloid plaque burden in a murine Alzheimer's disease model was observed after chronic administration of BCNU [1] (a.k.a.,1,3-Bis(2-chloroethyl)-1-nitrosourea, and carmustine). Furthermore, evidence indicates that BCNU has activity against Alzheimer's disease in humans, as a marked improvement from 5 to 2 on the dementia scale test was observed. A score of 5 represents severe dementia, while a score of 0 represents normal cognition [2]. In reference [2], the effects on Alzheimer's disease were assumed to be due to the immunosuppressive and anti-inflammatory actions of BCNU. However, in the previous murine study [1], strong activity was also seen in tissue culture models using CHO cells, where amyloid beta levels were reduced, and immunosuppressive and anti-inflammatory actions could not contribute. These findings lead us to conclude that this striking activity is due to one or more of the many reactive electrophiles generated by BCNU [3] and not an

immunosuppressive or anti-inflammatory action. The electrophiles generated by BCNU vary widely in their cytotoxicities and preferences for different cellular nucleophilic target types. Their nucleophilic targets range from very hard, negatively charged phosphate groups to soft, easily polarizable nitrogen and sulfhydryl groups. The structure and complex decomposition pathways of BCNU do not allow for the synthesis of analogs that can selectively generate these electrophiles individually; therefore, the individual contributions to the anti-Alzheimer's efficacy and toxicity of these different electrophiles cannot be determined. The sulfonylhydrazine prodrugs (SHP), which readily cross the blood-brain barrier [4], can be regarded as functional homologs of BCNU, but with much greater design flexibility and tolerance for structural modification. This flexibility has been exploited in the design of several classes of SHP prodrugs [5-7]. Agents have been synthesized, which generate individual or combinations of identical or similar electrophiles to most of the electrophilic species produced by the BCNU. This should allow for the determination of which electrophilic species are important in the anti-Alzheimer's disease activity. Therefore, studies using various SHPs should determine which of the electrophiles generated by BCNU are responsible for or do not contribute to activity against Alzheimer's disease. Such studies could also point to an SHP that would likely exhibit superior anti-Alzheimer's activity and lower overall cytotoxicity than BCNU. However, it is also possible that unique alkylations, generated by minor decomposition routes exclusive to BCNU [8], are responsible for the anti-Alzheimer's activity, such as the direct DNA alkylation route proposed by Naghipur [8]. If this were the case, no activity would be seen in SHPs that are functional analogs of the nitrosoureas. The electrophiles generated by CNUs and SHPs react with many biological nucleophiles with comparable hardness/softness [9]. Thus, it is likely that the anti-Alzheimer's activity, unlike their anticancer activity, is unrelated to the alkylation of DNA, and corresponds to the alkylation of other target biomolecules/nucleophiles similar hardness/softness to the sites targeted by BCNU in DNA. This, however, would not impact the efficacy of analogous electrophiles derived from SHPs as anti-Alzheimer's agents as they would also target the equivalent non-DNA nucleophilic targets of BCNU. In this manuscript, however, we will discuss the targets of the electrophiles generated with respect to their known target groups in DNA, as this indicates their potential genotoxicity and the chemical moieties that these electrophiles prefer to attack. Let us examine the different alkylating electrophiles produced by BCNU, and their possible roles in the toxicity and anti-Alzheimer's activity of BCNU, and how we could determine which electrophiles are involved in their efficacy. Using SHPs, we could produce the most efficacious of these electrophiles more selectively to generate an improved anti-Alzheimer's agent.

2. Materials and Methods

Laromustine(1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(methylamino) carbonyl] hydrazine) and 90AC, (1-acetyl-1,2-bis(methylsulfonyl)-2-(2-chloroethyl)hydrazine) were synthesized in this laboratory as previously described [10,11] (structures given Fig 1 panels A-C).

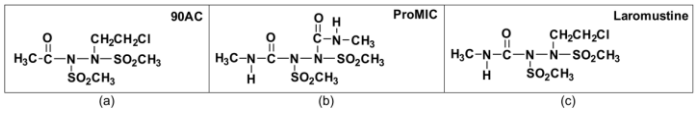

Figure 1. The structures of some key sulfonylhydrazines. (a) 90AC (90CE prodrug agent with chloroethylating activity only); (b) ProMIC (methyl isocyanate prodrug with carbamoylating activity only); (c) Laromustine a dual prodrug generating 90CE, and methyl isocyanate, hence having both chloroethylating, and carbamoylating activities.

ProMIC 1,2-bis(methylsulfonyl)-1,2-bis(methylaminocarbonyl)hydrazine was synthesized using a procedure similar to that described for laromustine, but substituting 1,2-bis(methylsulfonyl)hydrazine for 90CE. The structures of these bis(sulfonyl)hydrazine prodrugs are given in Figure 1.

3. Results and Discussion

The generation of electrophiles during the decomposition of the BCNU and other CNUs has been thoroughly investigated [12-15] and compared with those generated by the SHPs [16]. Let us first examine the electrophiles produced by BCNU illustrated in Figure 2.

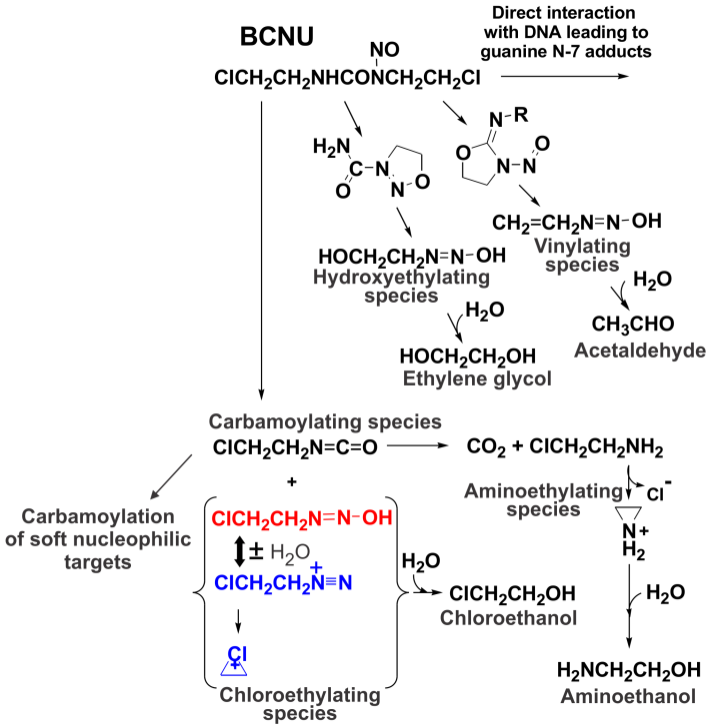

Figure 2. The major BCNU decomposition routes.

BCNU can decompose via several routes, generating an array of electrophiles including carbamoylating, aminoethylating, vinylating, and hydroxyethylating species not strongly associated with anticancer activity. In addition, three chloroethylating species can be formed, two of which are charged (shown in blue) and are likely to be the hardest electrophiles generated and favor the alkylation of the hardest nucleophilic sites in DNA, i.e., the charged DNA backbone phosphates. The 2-chloroethyldiazohydroxide shown in red is likely to favor the alkylation of the O-6 position of guanine and largely be responsible for the anticancer activity and MGMT cytotoxicity dependence of BCNU. The soft carbamoylation species 2-chloroethylisocyanate will attack thiol groups and soft nitrogen centers. These adducts can subsequently be involved in transcarbamoylating reactions. These two actions are responsible for the covalent inhibition of several important enzymes [17].

The major electrophiles generated by 90CE and other BSHs are illustrated in Figure 3. These BSHs can be converted into prodrugs which not only greatly improve their biodistribution but can also allow them to generate a carbamoylating activity, as seen in 101m (a.k.a. Cloretazine, Laromustine, and 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(methylamino)carbonyl]hydrazine) and 101 (1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(2-chloroethylamino)carbonyl]hydrazine) which generate methyl isocyanate, and 2-chloroethyl

isocyanate during prodrug activation, respectively. Thus, 101 is a close analog of BCNU in terms of the reactive electrophiles it produces. Additionally, prodrugs can be produced by adding an acetyl moiety to the 2- position of a BSH. This similarly improves biodistribution but generates acetic acid on activation rather than a soft electrophilic isocyanate. The activation/decomposition of the BSHs is illustrated below in Figure 3.

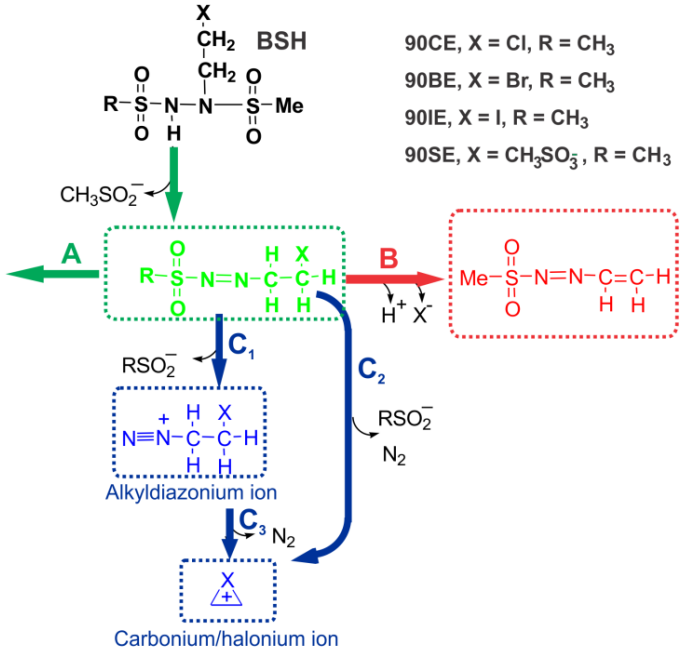

Figure 3. The major decomposition routes open to 2-haloethyl BSHs and their prodrug forms.

Pathway A (Fig 3) results in alkylation by a hard oxophilic therapeutic electrophile. This hard oxophilic electrophile is believed to be largely responsible for the therapeutic alkylation of DNA guanine O-6, both 'R' and 'X' will influence the yields by two mechanisms: 1) by altering the decomposition pathway preference and 2) by altering the electrophile's preference for nucleophiles. Pathway B, Brønsted-Lowry base-catalyzed pathway, generates a very soft thiophilic electrophile with little or no anticancer activity. Very soft thiophilic electrophiles favor the alkylation of thiols and soft nitrogen centers [9]. Pathways C_1, C_2, and C_3 generate very hard, charged electrophiles. The alkyldiazonium and halonium ions are extremely hard electrophiles favoring the alkylation of charged nucleophiles; most likely DNA targets are the DNA backbone phosphate residues. Increased flux along the B and C pathways diverts flux from pathway A, responsible for the anticancer activity and the bulk of the observed cytotoxicity.

Both BCNU and the SHPs generate their anticancer activity by producing the G-C ethane crosslink (1-(N3-cytosinyl)-2-(N1- guaninyl)ethane) [17], Figure 4.

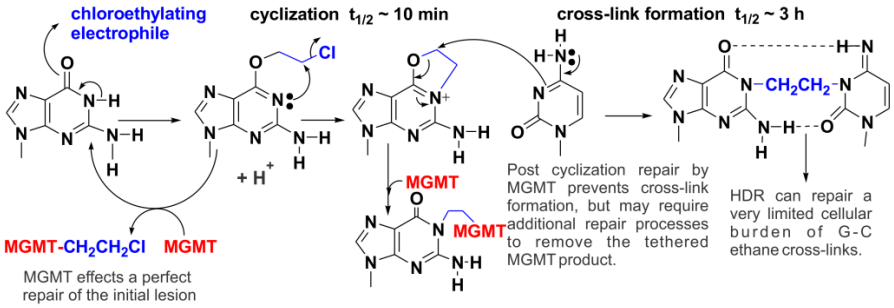

Figure 4. Chloroethylation of the O-6 position of DNA guanine and its sequelae.

Chloroethylation of DNA guanine O-6 results in the formation of O^6-(2-chloroethyl)guanine, which rapidly rearranges to form the N^1,O^6-ethanoguanine crosslink precursor. This lesion slowly transitions, $t_{1/2}$ ~ 3 hours [7], into highly cytotoxic G-C ethane crosslinks. Points of repair /crosslink precursor quenching by MGMT are indicated in Figure 4. A limited number of G-C ethane crosslinks can be repaired via homology-directed repair (HDR). Tumor selectivity arises predominantly from tumor deficits in one or more of these repair processes, with MGMT insufficiency likely being the foremost factor in most cases.

Let us now consider each of the electrophiles generated by BCNU, in turn, and how we could investigate their role in BCNU's anti-Alzheimer's effect. The electrophile produced from BCNU that favors the 2-chloroethylation of the O-6 position of guanine, and is responsible for its anticancer activity, is most likely $ClCH_2CH_2N{=}NOH$. In the case of the 2-chloroethylating BSHs, two possible electrophiles likely attack this position ($ClCH_2CH_2N{=}NOH$ and $ClCH_2CH_2N{=}NSO_2CH_3$), and these are generally thought to be responsible for their anticancer activity. The 2-chloroethylating BSHs give a higher net yield of G-C ethane crosslink and lower toxicity, giving them a much higher therapeutic index [4,16,17]. If it is the O-6 position of DNA guanine-targeting electrophiles that are responsible for the anti-Alzheimer's effect, this would be the worst-case scenario as these two activities could not be divorced. The higher therapeutic indices of BSH prodrugs would produce a less toxic agent, but one could not justify using a chemotherapeutic agent in the absence of cancer. Suppose the anti-Alzheimer's effect was due to the attack of very hard anionic sites like the DNA backbone phosphates (or equivalent in other biomolecules). In that case, the two therapeutic activities could easily be separated. In the case of BCNU, CNUs, and the 2-chloroethylating BSHs, it is believed that the very hard halonium cation is largely responsible for attacking this DNA position [9,15,17-19]. Phosphate alkylations are believed to be almost non-toxic while being at least 20-fold more numerous than the O-6 position of DNA guanine assaults [9,15,17-19]. Prodrugs of 90IE (1,2-bis(methylsulfonyl)-1-(2-iodoethyl)hydrazine) could easily accomplish this feat. The relatively high stability of the iodonium ion (iodine-containing halonium cation) means that N_2 is rapidly eliminated from $ICH_2CH_2N{=}NSO_2CH_3$ and $ICH_2CH_2N{\equiv}N^+$, producing this very hard phosphate alkylating moiety. As a consequence of this action, 90IE generates an insignificant number of G-C ethane crosslinks while generating abundant phosphate alkylations [16,17].

The most interesting and exploitable possibility is that it is, in fact, the 2-chloroethylisocyanate, generated by the decomposition of BCNU that is responsible for the observed anti-Alzheimer's activity. 2-Chloroethyl isocyanate (CEIC) is highly unstable, and its half-life in water is only around 17s [20]. However, it forms relatively stable conjugates with many soft cellular nucleophiles, which via transcarbamoylation reactions, can inhibit glutathione reductase and a variety of other enzymes [20-26] Figure 5. These conjugates are very stable, with 75% of their glutathione reductase inhibitory activity surviving a 100 min incubation at 37°C [21]. Suppose enzyme carbamoylation is the cause of the anti-Alzheimer's activity of BCNU. In that case, this could be mimicked by relatively non-toxic SHPs such as 101DCE (1,2-bis(methylsulfonyl)-1-[[(2-chloroethyl)amino]carbonyl]hydrazine), or conjugates of CEIC with simple thiols such as 1-thioglycerol. This approach (utilization of 101DCE and conjugates of CEIC with simple thiols) could also have therapeutic utility in the treatment of *Plasmodium falciparum* infections since it has been observed that BCNU has activity in this disease, and this activity was attributed to BCNU's inhibition of glutathione reductase [27,28].

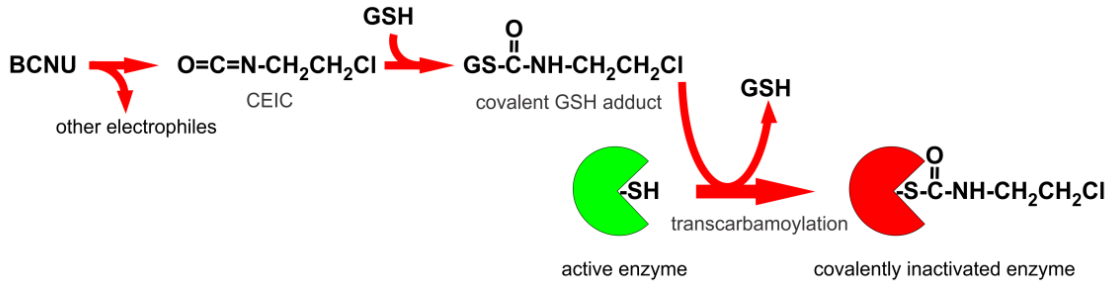

Figure 5. Carbamoylation and transcarbamoylation reactions.

This glutathione reductase inhibition results from the formation of an inhibitor, produced when low molecular weight soft nucleophiles (such as glutathione) react with the 2-chloroethyl isocyanate (CEIC) generated during the decomposition of BCNU to form an inhibitory adduct. This adduct can react via a transcarbamoylation reaction with an essential thiol in an enzyme's active site, such as in glutathione reductase, rendering it inactive. The glutathione moiety in this adduct likely gives it added selectivity as an inhibitor of glutathione reductase [28].

4. Conclusions

BCNU has potent activity as an anti-Alzheimer's agent. Its activity is due to one of the many reactive electrophiles it generates during decomposition. These electrophiles vary greatly in their toxicity. Unfortunately, the activation/decomposition of BCNU is complex, and its structure does not allow for the production of analogs that selectively generate these electrophiles. SHPs can be engineered to generate most of these electrophiles in a segregated manner and may mimic the anti-Alzheimer's activity of BCNU in a much less toxic manner.

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Conflicts of Interest

The authors declare no conflict of interest.

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