A Neutron Dynamic Therapy with a Boron Tracedrug UTX-51 Using a Compact Neutron Generator

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Abstract. *Background/Aim: We are developing a neutron dynamic therapy (NDT) with boron tracedrugs for a new mechanical-clearance treatment of pathotoxic misfolded, aggregated, and self-propagating prion-associated disease proteins. We present a compact neutron generator-based NDT using a boron tracedrug UTX-51. Our NDT is based on the weak thermal neutron-bombarded destructive action of UTX-51 on bovine serum albumin (BSA) using the neutron beams produced from a compact inertial electrostatic confinement fusion (IECF) neutron generator. Results: BSA as an NDT molecular target was subjected to thermal neutron irradiation for eight hours using a compact neutron generator. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern showed no protein band when 2 nmoles of BSA were irradiated with more than 100 nmoles of UTX-51, while BSA was not affected when irradiated without UTX-51. Conclusion: For the first time, we have succeeded in the molecular destruction of a prion-disease model protein, BSA, by NDT with a boron tracedrug, UTX-51, using a compact neutron generator.*

We are developing a neutron dynamic therapy (NDT) (1-3), as shown in Figure 1, using boron tracedrugs for a new mechanical-based protein quality control clearance treatment of pathotoxic misfolded and aggregated, and self-propagating proteins such as those found in age-related neurogenerative diseases, prion-associated diseases, cardiac diseases and cancer (4-8). We previously examined our NDT with boron tracedrugs with model proteins (9-11). A major advantage of NDT is that it requires nothing but neutron beams and boron elements. Boron neutron capture therapy (BNCT) provides the basis for NDT. BCNT is an innovative form of radiotherapy for the treatment of head and neck and recurrent glioma which uses neutrons generated from a nuclear reactor, and recently using room-based (still large) powerful linear accelerators (12, 13). However, its method of immobilization is frame-based (*i.e.* it requires a rigid invasive head-frame screwed to the head and neck of a cancer patient's skull). This is in contrast to a frameless and mobile configuration such as the one provided by the frameless robotic radiosurgery system Cyberknife™ and a recently commercially-available radiosurgery system Novalis Tx™ used for recent radiotherapy (14). We present herein a demonstration of our NDT procedure by evaluating the destructive effects of weak thermal neutron-bombardment of the difluoroboron-curcumin boron tracedrug UTX-51 (9) interacting with bovine serum albumin (BSA), using neutron beams produced from a compact or desktop/mobile neutron generator. The major reason for selection of difluoroboron-curcumin UTX-51 chosen from amongst available boron tracedrugs (1, 9, 11) is based primarily on the multiple interactions of its scaffold curcumin molecule with biological molecular targets (15, 16). Thus, UTX-51 has the potential to function as a general drug (one molecule, multiple targets), not as a more common local/specific drug (one molecule, one target). In addition, the relatively high metabolic stability (17) of the boron complex in difluoroboron-curcumin UTX-51 is an indispensable property for our NDT drugs. In our experiments, we employed a compact or desktop/mobile neutron generator consisting of a discharge-driven D-D fusion neutron source, called inertial-electrostatic confinement fusion (IECF) device (18, 19). This replaces the use of neutron beams produced by a nuclear reactor or by the recently developed room-based powerful linear accelerators.

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Key Words: Neutron dynamic therapy, NDT, compact neutron generator, boron tracedrug, UTX-51, curcuminoid.

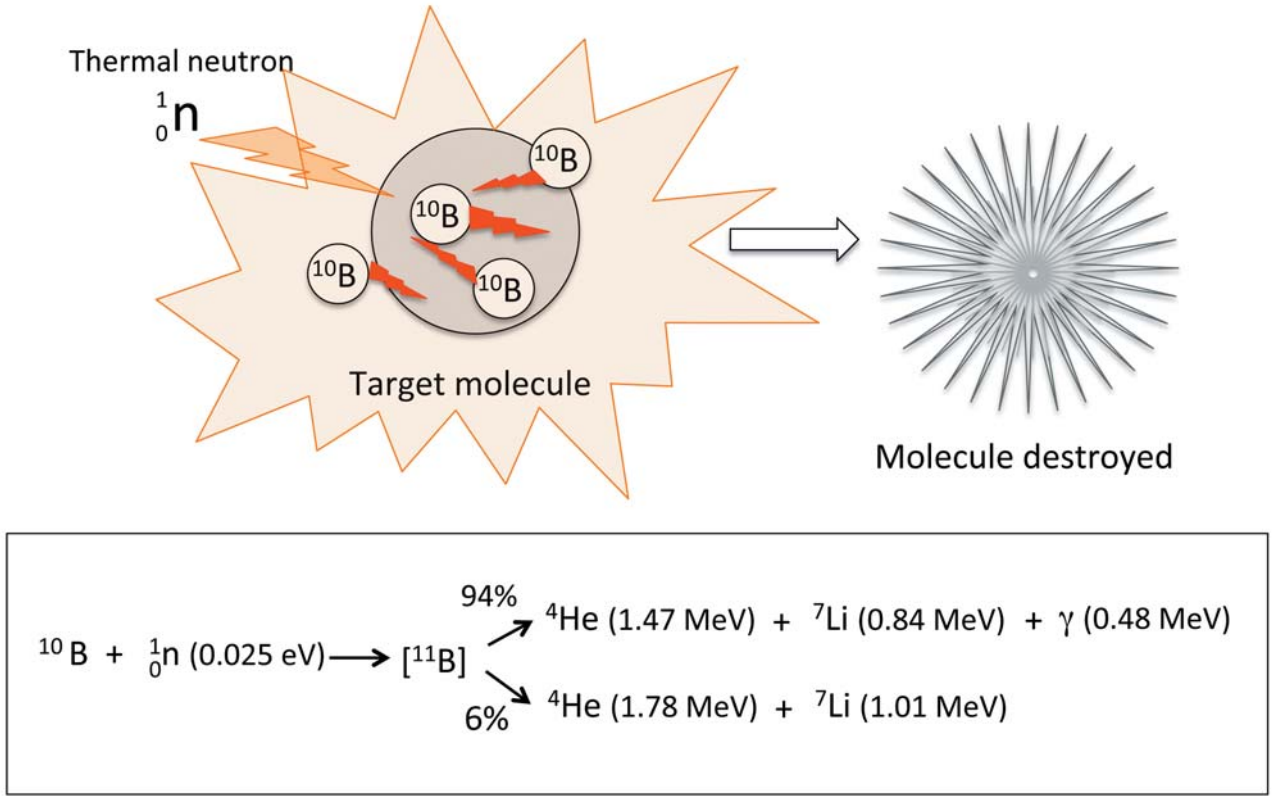

Figure 1. *Neutron dynamic therapy overviews: Neutron attack the B-10 atom of boron tracedrugs bound to, or surrounding, macromolecules such as proteins, nucleic acids, saccharides, lipids, to produce tremendous energy by which boron tracedrugs themselves and their surrounding macromolecules are destroyed. The equation shows the nuclear reaction B-10 (n, α).*

Materials and Methods

Materials. All chemicals including BSA were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan), Sigma-Aldrich Japan Co (Tokyo, Japan).

Synthesis of UTX-51. The boron tracedrug UTX-51 (Figure 2), originally designed for NDT based on molecular orbital calculations, was synthesized in our laboratory (9).

Neutron irradiation. The eight-hour thermal neutron irradiation of BSA (MW: 66 kDa) with stoichiometric doses of UTX-51 was performed on five samples located at 35 cm directly under a compact discharge-driven deuterium-deuterium (D-D) fusion neutron source (2.45 MeV) as shown in Figure 3.

NDT evaluation. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to detect protein decomposition resulting from thermal neutron irradiation of BSA treated with UTX-51.

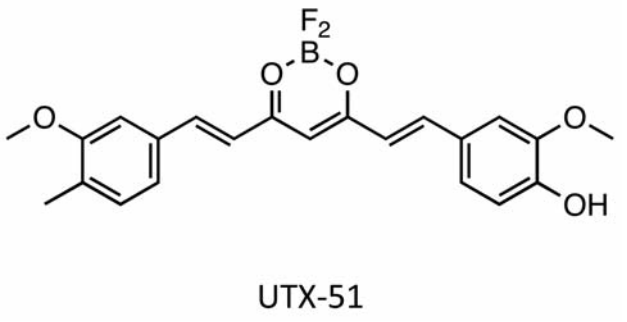

Figure 2. *A boron tracedrug UTX-51.*

Results

Thermal neutron irradiation using a compact discharge-driven D-D fusion neutron source was performed for eight hours on BSA as an NDT molecular target. Our compact discharge-driven D-D fusion neutron source produced D-D neutrons stably with its neutron energy spectrum of the flux density (/cm^2/s/erg) *versus* the neutron energy (eV) at the sample position (at 35 cm directly under the device) shown in Figure 4. The SDS-PAGE electrophoretic pattern showed no band when 2 nmoles of BSA were irradiated in the

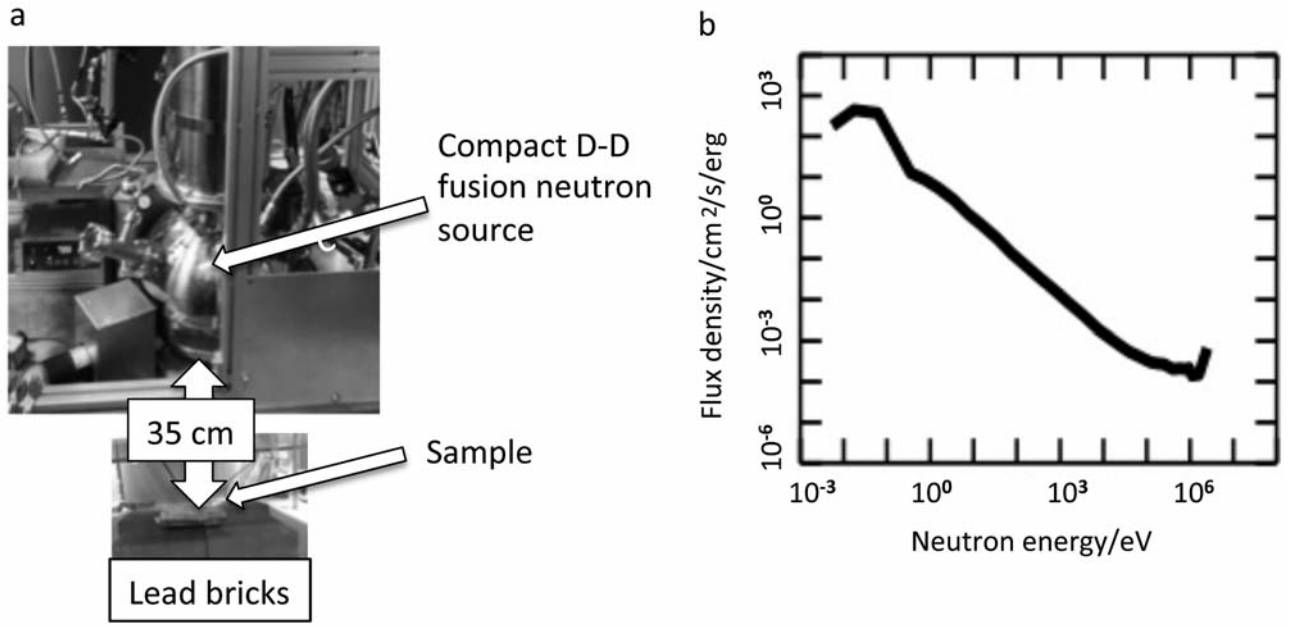

Figure 3. *a: A compact inertial electrostatic confinement fusion neutron generator showing the compact D-D fusion neutron source (2.45 MeV), and the sample position; (b) the neutron energy spectrum at the sample position.*

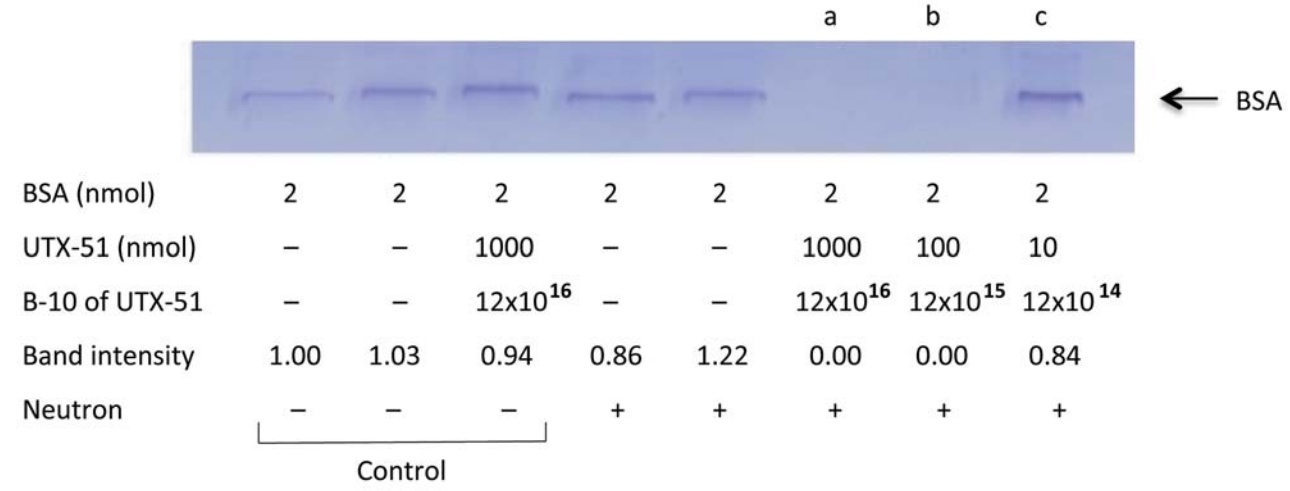

Figure 4. *SDS-PAGE obtained from NDT with a boron tracedrug UTX-51 to BSA using a compact IECF neutron generator: Dynamic effects of a boron tracedrug UTX-51 to BSA under eight hours neutron irradiation using a compact IECF neutron generator using a compact discharge-driven D-D fusion neutron source; UTX-51: 10, 100, 1000 nmole; BSA: 2 nmol. The quantity of B-10 atoms of UTX-51 (nmol) used are also shown. Ten-fold diluted BSA treated with UTX-51 on SDS-PAGE gel was stained with Coomassie Brilliant Blue. Band intensities were measured with SWEDAY Just TLC soft (control=1.00). a: B-10:BSA=100:1; b: B-10:BSA=10:1; c: B-10:BSA=1:1.*

presence of more than 100 nmoles of UTX-51, while BSA was not decomposed when irradiated in the absence of UTX-51. We ascribe these results to the neutron-capturing activity of UTX-51 interacting with BSA.

Discussion

Our present NDT neutron generator, a compact discharge-driven D-D fusion neutron source, called an IECF device, is an

extremely compact and simple neutron generator as shown in Figure 4, operating on an electrical discharge using D-D fuel gases (15). It basically consists of a hollow cathode at the center of a spherical vacuum chamber, serving as the anode, filled with a fuel gas. A glow discharge tales place between the anode and cathode, and many of the fuel molecules penetrating the hollow cathode wire undergo fusion reactions through beam-beam collisions, or beam-background gas collisions. A compact IECF neutron generator has several benefits, differing from other sources such as nuclear reactors or accelerators. As a compact neutron generator, the neutron beams can be focused in arbitrary directions and applied to a variety of patients with intractable diseases, such as those suffering from prion-associated diseases, as well as a variety of cancer types (19).

Conclusion

We have demonstrated the first successful "anti-molecule gun-like" destruction of a boron tracedrug (UTX-15) using a compact discharge-driven D-D fusion neutron source as a compact or desktop neutron generator suitable for our NDT system. Neutron capture by UTX-51 resulted in the destruction of BSA, employed in the experiments as a model of pathotoxic misfolded, aggregated, and self-propagating proteins such those found in as prion diseases, We are further studying compact neutron generator-based NDT for its preclinical application to a variety of intractable human diseases, such as prion-associated diseases, as well as a variety of cancer types. Our SDS-PAGE electrophoretic results, showing the neutron beam-mediated destruction of BSA when 2 nmoles of BSA are irradiated with more than 100 nmoles of UTX-51, convinces us of the unexpected power of a compact discharge-driven D-D fusion neutron source as a neutron generator. We feel that the use of this neutron source will greatly enhance the clinical applications of NDT in the future.

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References

1 Hori H, Uto Y and Nakata E: Boron tracedrugs challenge for neutron dynamic therapy. Anticancer Res *32*: 2235-2239, 2012.
2 Hori H, Uto Y and Nakata E: Medicinal bricolage design of hypoxia-targeting drugs and invention of boron tracedrugs as next-generation universal drugs. SHIKOKU ACTA MEDICA *67*: 7-14, 2011.
3 Hori H, Uto Y and Nakata E: Medicinal electronomics bricolage design of hypoxia-targeting antineoplastic drugs and invention of boron tracedrugs as innovative future-architectural drugs. Anticancer Res *30*: 3233-3242, 2010.
4 Jucker M and Walker LC: Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature *501*: 45-51, 2013.
5 Prusiner SB: Biology and genetics of prions causing neurodegeneration. Annu Rev Genet *47*: 601-623, 2013.
6 Chhangani D and Mishra A: Protein quality control system in neurodegeneration: a healing company hard to beat but failure is fatal. Mol Neurobiol *48*: 141-156, 2013.
7 Willis MS and Patterson C: Proteotoxicity and cardiac dysfunction–Alzheimer's disease of the heart? N Engl J Med 368: 455-464, 2013.
8 Rangel LP, Costa DC, Vieira TC and Silva JL: The aggregation of mutant p53 produces prion-like properties in cancer. Prion *8*: doi10.4161/pri.27776, 2014 [Epub ahead of print].
9 Nakata E, Koizumi M, Yamashita Y, Onaka K, Sakurai Y, Kondo N, Ono K, Uto Y and Hori H: Design, synthesis and destructive dynamic effects of BODIPY-containing and curcuminoid boron tracedrugs for neutron dynamic therapy. Anticancer Res *31*: 2477-2481, 2011.
10 Nakata E, Koizumi M, Yamashita Y, Uto Y and Hori H: Boron tracedrug: design, synthesis and pharmacological activity of phenolic BODIPY-containing antioxidants as traceable next generation drug model. Adv Exp Med Biol *737*: 301-306, 2012.
11 Hori H, Nazumi Y and Uto Y: Boron tracedrug design for neutron dynamic therapeutics for LDL. Adv Exp Med Biol *789*: 385-389, 2013.
12 Moss RL: Critical review, with an optimistic outlook, on boron neutron capture therapy (BNCT). Appl Radiat Isot *88C*: 2-11, 2014.
13 Barth RF, Vicente MG, Harling OK, Kiger WS 3rd, Riley KJ, Binns PJ, Wagner FM, Suzuki M, Aihara T, Kato I and Kawabata S: Current status of boron neutron capture therapy of high grade gliomas and recurrent head and neck cancer. Radiat Oncol *7*: 146, 2012.
14 Gevaert T, Levivier M, Lacornerie T, Verellen D, Engels B, Reynaert N, Tournel K, Duchateau M, Reynders T, Depuydt T, Collen C, Lartigau E and De Ridder M: Dosimetric comparison of different treatment modalities for stereotactic radiosurgery of arteriovenous malformations and acoustic neuromas. Radiother Oncol *106*: 192-197, 2013.
15 Esatbeyoglu T, Huebbe P, Ernst IM, Chin D, Wagner AE and Rimbach G: Curcumin–from molecule to biological function. Angew Chem Int Ed Engl *51*: 5308-5332, 2012.
16 Goel A, Kunnumakkara AB and Aggarwal BB: Curcumin as "Curecumin": from kitchen to clinic. Biochem Pharmacol *75*: 787-809, 2008.
17 Bai G, Yu C, Cheng C, Hao E, Wei Y, Mu X and Jiao L: Syntheses and photophysical properties of BF_2 complexes of curcumin analogues. Org Biomol Chem *12*: 1618-1626.
18 Yoshikawa K, Masuda K, Takamatsu T, Shiroya S, Misawa T, Hotta E, Ohnishi M, Yamauchi K, Osawa H and Takahashi Y: Research and development of a compact discharge-driven D-D fusion neutron source for explosive detection. Nucl Instr Meth Phys Res B *261*: 299-302, 2007.
19 Nakai Y, Kazuyuki N, Takeuchi Y, Kasada R, Yamamoto Y and Konishi S: A feasibility study of an application of fusion neutron beam source based on cylindrical discharge device for cancer therapy. Fusion Sci Technol *64*: 379-383, 2013.

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