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Synthesis and biological evaluation of radioiodinated 2NUBTA as a cerebral ischemia marker

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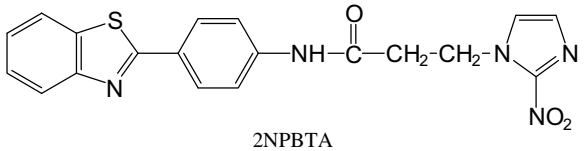
ABSTRACT

N-[4-(Benzothiazol-2-yl)phenyl]-11-(2-nitroimidazole-1-yl)undecanamide (2NUBTA) was synthesized and radiolabeled with iodine-131. In vitro evaluation of the [^{131}I]2NUBTA using murine sarcoma S180 cells showed increase in radioactivity in hypoxic cells up to 4 h, while it was not in aerobic cells. Its potential as a cerebral ischemia marker was evaluated using gerbil stroke models that had been subjected to right common carotid artery ligation to produce cerebral ischemia. The uptake in the right cerebral hemisphere decreased slowly than that of the left and the right/left hemisphere uptake ratios increased with time going on. It indicated that [^{131}I]2NUBTA might be a possible cerebral ischemia marker.

Stroke is the third cause of mortality and the first cause of disability in adults and until recently there is no efficacious treatment for acute cerebral ischemic stroke. To help the stroke sufferers, optimal management now requires delineation of infarction prior to intervention.[1] Nuclear medicine which can offer a non-invasive method for demonstrating cerebral ischemia will receive more and more attention.[2] However, the marker of stroke ischemia is scarce up to now because of the difficulty for it to permeate across the intact blood–brain barrier (BBB) into the brain and for it to retain in the cerebral ischemia.[3]

It is well known that the 2-nitroimidazole derivatives have the property of selective accumulation in hypoxic/ischemic tissue.[3,4] In order to design the molecule easy to permeate across the blood–brain barrier (BBB), 2-nitroimidazole was conjugated to BTA (derivative of Thioflaven-T, developed by Mathis et al. for imaging amyloid in Alzheimer's disease),[5,6] then it could be possibly used as cerebral ischemia marker. *N*-[4-(benzothiazol-2-yl)phenyl]-3-(2-nitroimidazole-1-yl) propanamide (2NPBTA, see Fig. 1) has been synthesized.[7] Biodistribution results showed that [^{131}I]2NPBTA could locate in the cerebral ischemia, but it cleaned fast.[8] In order to improve the biological properties of the cerebral ischemia marker, a new derivative with higher partition coefficient (*P*) *N*-[4-(benzothiazol-2-yl)phenyl]-11-(2-nitroimidazole-1-yl)undecanamide (2NUBTA) was synthesized and radiolabeled with iodine-131 in this study. Its biological evaluation in hypoxic cells and gerbil cerebral ischemia models was also explored.

Synthesis, radiolabeling, determination of P value. 11-Bromoundecanoic acid (0.70 g, 2.64 mmol) and thionyl chloride (2.0 mL, 27 mmol) were mixed and heated slightly in the fume hood. When there was no bubble, 11-bromoundecanoyl chloride (**1**) was obtained (Fig. 2). The excess thionyl chloride was removed under vacuum. BTA (**2**, 0.60 g, 2.65 mmol) was dissolved in a 3 mL solution of DMF and triethylamine (50/50, v/v), then poured into the vial of 11-bromoundecanoyl chloride. The mixture was stirred at 0 °C for 1 h. After adding diluted HCl solution, the resulting precipitate was filtered and washed with deionized water and ethyl acetate. The residue was recrystallized from methanol to give 0.50 g of *N*-[4-(benzothiazol-2-yl)phenyl]-11-bromoundecanamide (**3**), yield 40%, mp 159–160 °C. ^{1}H NMR δ (*DMSO-d*$_6$): 10.20 (s, 1H),



2NPBTA

Figure 1. Structure of *N*-[4-(benzothiazol-2-yl)phenyl]-3-(2-nitroimidazole-1-yl) propanamide.

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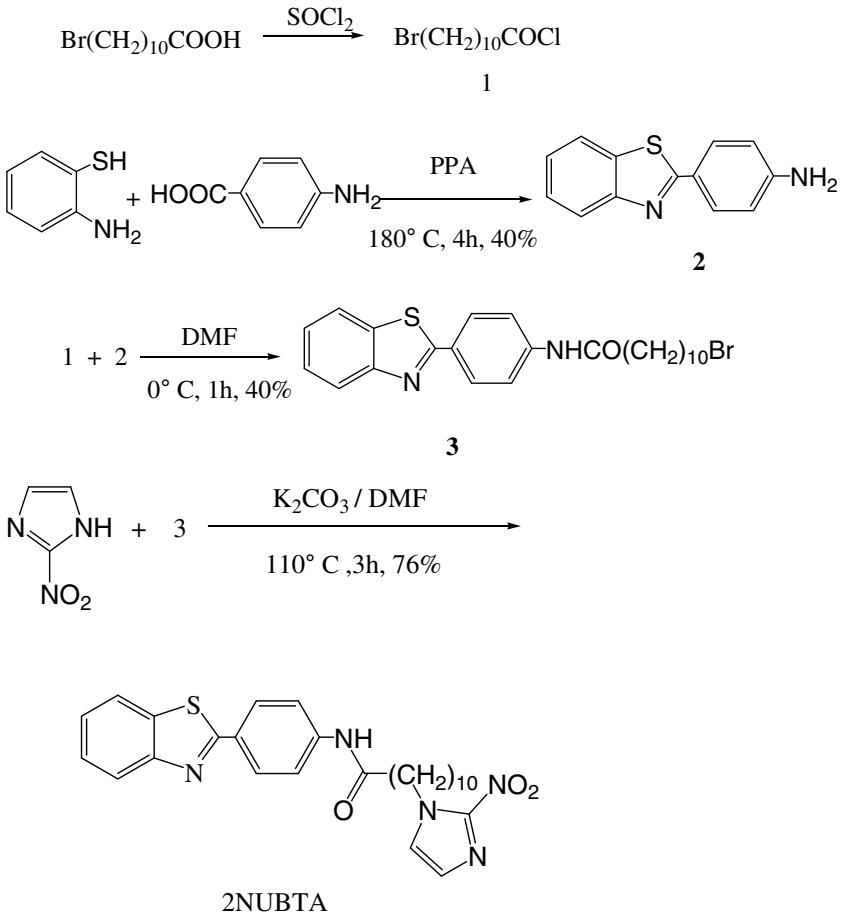

Figure 2. Synthesis of *N*-[4-(benzothiazol-2-yl)phenyl]-11-(2-nitroimidazole-1-yl)undecanamide.

8.13 (d, 1H), 8.04 (d, 2H), 8.01 (d, 1H), 7.80 (d, 2H), 7.53 (t, 1H), 7.43 (t, 1H), 3.51 (t, 2H), 2.35 (t, 2H), 1.78 (m, 2H), 1.61 (m, 2H), 1.32 (m, 12H). EI-MS calculated for $C_{24}H_{29}BrN_2OS$ (M^+): *m/z* 472. Found: 472. Analytical data calculated for $C_{24}H_{29}BrN_2OS$: C, 60.88; H, 6.17; N, 5.92. Found: C, 60.81; H, 6.21; N, 5.81.

The mixture of 2-nitroimidazole (0.051 g, 0.45 mmol), *N*-[4-(benzothiazol-2-yl)phenyl]-11-bromoundecanamide (**3**, 0.281 g, 0.59 mmol), and K_2CO_3 (0.082 g, 0.59 mmol) in 2 mL of DMF was stirred at 110 °C for 3 h. After cooling and adding the deionized water, the resulting precipitate was filtered and washed with deionized water several times. The residue was recrystallized from methanol, and 0.173 g (0.34 mmol) of *N*-[4-(benzothiazol-2-yl)-phenyl] -11-(2-nitroimidazole-1-yl)undecanamide (2NUBTA), as pale yellow crystals, was obtained. mp 155–156 °C. Yield: 76%. ^{1}H NMR δ (*DMSO-d*$_6$): 10.20 (s, 1H), 8.12 (d, 1H), 8.05 (d, 2H), 8.02 (d, 1H), 7.79 (d, 2H), 7.69 (d, 1H, imi-H), 7.52 (t, 1H), 7.43 (t, 1H), 7.17 (d, 1H, imi-H), 4.35 (t, 2H), 2.34 (t, 2H), 1.75 (t, 2H), 1.60 (t, 2H), 1.26 (s, 12H). EI-MS calculated for $C_{27}H_{31}N_5O_3S$ (M^+): *m/z* 505. Found: 505. Analytical data calculated for $C_{27}H_{31}N_5O_3S$: C, 64.14; H, 6.18; N, 13.85. Found: C, 64.27; H, 6.25; N, 13.22.

The carrier-free $Na^{131}I$ (10 μL, 7.4 MBq) and 20 μL of 5 mg/mL chloramines-T were added to 100 μL of 1 mg/mL 2NUBTA in ethanol/pH 7.4 PBS (50/50, v/v) solution. Standing at room temperature for 10 min, 20 μL of 5 mg/mL $Na_2S_2O_5$ was added in to terminate the reaction. A polyamide column chromatography (ethanol as eluent) was used to separate [^{131}I]2NUBTA from $^{131}I^-$. The radiochemical yield was 40% and the specific activity was 1.5×10^{10} Bq/mmol. The product was determined by TLC in which a polyamide strip was used as the fixed phase and ethanol as the developer. The radioiodinated product migrated to the top half of the strip ($R_f = 0.8 \sim 0.9$) while the insoluble radiochemical impurities and $^{131}I^-$ remained near the origin ($R_f = 0 \sim 0.2$). The radiochemical purity was >98%. The ethanol eluent was concentrated and diluted with an isotonic saline solution to a 10% ethanol (v/v) solution for

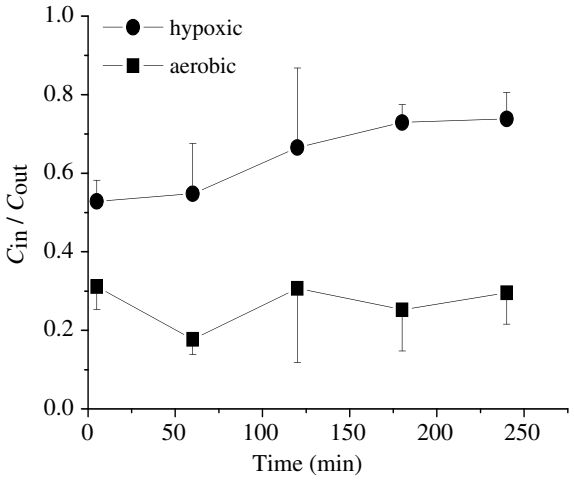

Figure 3. Accumulation (C_{in}/C_{out}) of [^{131}I]2NUBTA in hypoxic (—●—) and aerobic (—■—) S180 cells as a function of time (means ± standard deviation of five independent parallel experiments).

Table 1
Uptake of [^{131}I]2NPBTA[8] and [^{131}I]2NUBTA in the gerbil ischemic and normal brain hemisphere

Brain	[^{131}I]2NPBTA[8]			[^{131}I]2NUBTA		
	4 h	8 h	12 h	4 h	8 h	12 h
Right	0.042 ± 0.005	0.034 ± 0.006	0.025 ± 0.004	0.233 ± 0.021	0.174 ± 0.015	0.127 ± 0.008
Left	0.036 ± 0.004	0.025 ± 0.003	0.014 ± 0.002	0.151 ± 0.029	0.097 ± 0.017	0.049 ± 0.012
Right/left	1.18 ± 0.13	1.39 ± 0.10	1.76 ± 0.10	1.57 ± 0.29	1.82 ± 0.28	2.76 ± 0.96

Each value is mean ± standard deviation.

further partition coefficient determination and in vitro and in vivo study. Being kept at room temperature for one week, the radiochemical purity of the product was still >95%. Five milliliters of 1-octanol, 5 mL of water, and 0.1 mL of radioiodinated product were mixed by vigorously vortexing at room temperature for 5 min. Then it was centrifuged at 4000 rpm for 5 min, and the phases were separated. Sample (0.1 mL) was taken from each phase and counted. The octanol/water partition coefficient (P) was the ratio of the radioactivity of octanol phase to that of aqueous phase. Then the water layer was removed and the same volume of fresh water was added until consistent P values were obtained. This measurement was repeated for three times. The log$P$ of [^{131}I]2NUBTA was 1.99, higher than that of previously reported cerebral ischemia marker [^{131}I]2NPBTA (log$P$ = 1.56).[7,8] Obviously, by adding the length of the linker between 2-nitroimidazole and BTA, the lipophilicity of new cerebral ischemia marker [^{131}I]2NUBTA increased.

Cellular accumulation. S180 cells were suspended in 20 mL Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) of fetal bovine serum at a concentration of (1–2) × 10^6 cells/mL and incubated at 37 °C. The hypoxic and aerobic conditions were generated by stirring the cell suspension under an atmosphere of nitrogen or air, respectively (both containing 5% CO_2). In the hypoxic conditions, after pre-equilibrated for 40 min, the oxygen sensor of the Dissolved Oxygen Meter read 0.00–0.01 mg/L. In the aerobic conditions, no pre-equilibration was performed, the oxygen meter read ~6.00 mg/L. [^{131}I]2NUBTA (0.2 mL, 148 kBq, 13.5 nmol) was added to the suspension and aliquots (200 μL, in pentaplicate) were pipetted using a syringe through rubber plug to keep the oxygen out of the cell suspension at 5, 30, 60, 90, 120, 150 min, and centrifuged at 1500 rpm for 5 min. A 90 μL sample of each supernatant was removed for counting (A), each tube containing cells and 110 μL medium with resuspending before counting was also counted (B). The accumulation ratio, C_{in}/C_{out}, was defined as concentration of radioactivity within the cells divided by that in the external medium, and calculated as $[(B - A)/A]$, because the volume of cells in 200 μL of cell suspension is about 20 μL. The final results were expressed as means ± standard deviation (SD) of five independent parallel experiments. During the whole procedure, the cells maintained >90% viability.

The C_{in}/C_{out} ratios under hypoxic and aerobic conditions were plotted as a function of time as showed in Figure 3. The accumulation of [^{131}I]2NUBTA steadily increased with time in hypoxic cells, but fluctuated with time and had no fixed trend in aerobic cells. The C_{in}/C_{out} values at 5 min for hypoxic and aerobic cells were 0.52 and 0.31, respectively, for [^{131}I]2NUBTA, while at 4 h the C_{in}/C_{out} was 0.74 for hypoxic cells and 0.29 for aerobic cells, representing a 2.6-fold hypoxic/aerobic difference. At 4 h, the difference between hypoxic cells and aerobic cells was very significant and the P values (two-tailed) <0.01 (P value was determined using Student's t-test). The results indicated [^{131}I]2NUBTA could retain in hypoxic/ischemic tissue but flow out from normal tissue with time going, when it was injected intraperitoneally (ip) into animals.

Evaluation in gerbil cerebral ischemia models. The gerbils did not have a posterior communicating artery, that is, the circle of Willis was incomplete. Therefore, the blood supply of each hemisphere was isolated from the contralateral carotid and basilar arteries. Ligation of one carotid artery caused ischemia in the ipsilateral hemisphere, while the other side was unaffected, providing neighboring normal tissue as an internal control. The unique anatomical feature of the gerbil made them widely used as a model in global ischemia.

Adult mongolian gerbils (male, 80 g) were used for stroke models. They had been subjected to right common carotid artery ligation to produce cerebral hypoxia–ischemia (HI) as initially described by Levine and Payan[9] and the stroke index described by Ohno et al.[10] was calculated. Animals with total stroke indices of >10 were used for injection. [^{131}I]2NUBTA (0.5 mL, 1.0 × 10^5 Bq) was injected intraperitoneally (ip) into the gerbils. At the time of sacrifice, animals with total stroke indices of >10 were killed (no anesthesia) by cervical dislocation in groups of three at 4, 8, and 12 h after injection. The whole brain was removed, placed on dry ice for 2 min, and then cut in half along the cerebral longitudinal fissure. The right and left halves were weighed and radioactivity counted. The percent injected dose per gram of tissue, that is, % ID/g, was determined for the right and left hemispheres. The right/left hemispheral uptake ratios were calculated. All experiments were carried out following the principles of laboratory animal care and the China law on the protection of animals.

The final results were expressed as means ± standard deviation (SD) as listed in Table 1. The uptake of [^{131}I]2NUBTA in the right hemisphere was higher than that in the left at 4, 8, and 12 h post-injection. The results indicated that the clearance from ischemic brain tissue was slower than that from normal brain tissue. The right/left uptake ratios, that is, the uptake ratios of ischemic to normal brain tissues were gradually increasing for 2NUBTA, from 1.57 at 2 h to 2.76 at 12 h. The difference between the uptake of the right hemisphere and the left hemisphere was very significant (P value <0.01) at 8 and 12 h.

And the results of the uptake of [^{131}I]2NPBTA, with lower lipophilicity (log$P$ = 1.56) reported previously[8] were also listed in Table 1 for comparison. It was found that the uptakes of [^{131}I]2NUBTA (log$P$ = 1.99) in both the right hemisphere and the left hemisphere were higher than those of [^{131}I]2NPBTA, and especially the right/left uptake ratio of [^{131}I]2NUBTA was also higher than that of [^{131}I]2NPBTA, such as 2.76 for [^{131}I]2NUBTA, 1.76 for [^{131}I]2NPBTA at 12 h post-injection, respectively.

Therefore, after adding the length of the linker between 2-nitroimidazole and BTA to increase the lipophilicity, [^{131}I]2NUBTA may be a more suitable cerebral ischemia marker than [^{131}I]2NPBTA.

Acknowledgments

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