Published in final edited form as:

Bioorg Med Chem Lett. 2017 March 15; 27(6): 1425–1427. doi:10.1016/j.bmcl.2017.01.093.

Radiolabeling and initial biological evaluation of [^{18}F]KBM-1 for imaging RAR- α receptors in neuroblastoma

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Abstract

Retinoic acid receptor alpha (RAR- α) plays a significant role in a number of diseases, including neuroblastoma. Children diagnosed with high-risk neuroblastoma are treated *13-cis-retinoic acid*, which reduces risk of cancer recurrence. Neuroblastoma cell death is mediated via RAR- α , and expression of RAR- α is upregulated after treatment. A molecular imaging probe that binds RAR- α will help clinicians to diagnose and stratify risk for patients with neuroblastoma, who could benefit from retinoid-based therapy. In this study, we report the radiolabeling, and initial *in vivo* evaluation of [^{18}F]KBM-1, a novel RAR- α agonist. The radiochemical synthesis of [^{18}F]KBM-1 was carried out through KHF_2 assisted substitution of [^{18}F] from aryl-substituted pinacolatoesters-based retinoid precursor. *In vitro* cell uptake assay in human neuroblastoma cell line showed that the uptake of [^{18}F]KBM-1 was significantly inhibited by all three blocking agents (KBM-1, ATRA, BD4) at all the selected incubation times. Standard biodistribution in mice bearing neuroblastoma tumors demonstrated increased tumor uptake from 5 min to 60 min post radiotracer injection and the uptake ratios for target to non-target (tumor: muscle) increased 2.2-fold to 3.7-fold from 30 min to 60 min post injection. Tumor uptake in subset of 30 min blocking group was 1.7-fold lower than unblocked. These results demonstrate the potential utility of [^{18}F]KBM-1 as a RAR- α imaging agent.

Keywords

Retinoic acid receptor alpha (RAR- α); All-trans retinoic acid (ATRA); Biodistribution; Neuroblastoma

Retinoic acids (RA) exhibit various important biological functions in the control of normal growth, differentiation and fetal development.^{1,2} Their activity is mediated through retinoic acid receptors (RAR), which are ligand activated nuclear receptors.^{3,4} This receptor family consists of α , β , and γ subtypes; each subtype has multiple isoforms. Recent studies have demonstrated that both all-trans retinoic acid (ATRA) and 13-*cis*-retinoic acid (isotretinoin) promote morphological differentiation and inhibit growth of neuroblastoma cells, mainly through activation of RAR- α subtypes.^{5,6} Human neuroblastoma cell lines treated with

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ATRA differentiate in culture, forming neurites and exhibiting growth arrest. Haber et al. demonstrated that RAR- α expression was specifically induced in neuroblastoma cell lines by ATRA and other differentiating agents tested.⁷ RAR- α expression appears to be necessary for ATRA to have therapeutic effects on neuroblastoma cells *in vitro*.⁷ Furthermore, reduced expression of RAR- α *in vivo* may contribute to the process of neuroblastoma tumorigenesis and resistance to ATRA therapy.⁸ Although attempts have been made to measure RAR- α activity using neuroblastoma cell lines *in vitro*, no reliable *in vivo* clinically relevant probe exists to measure and quantify RAR- α expression.⁷ A RAR- α binding PET radiotracer could be a key tool to measure real-time RAR- α expression and elucidate effects of RAR- α on the pathogenesis of neuroblastoma.

Das et al. developed a novel boron-based RAR- α agonist (BD4) specifically for treatment of kidney diseases.⁹ They analyzed structure-activity relationships (SAR) derived from *in vitro* binding assays and demonstrated that 10-fold concentration of ATRA was required to induce a 50% competitive inhibition of BD4 binding to a GST-tagged RAR- α . This confirms that BD4 binds to RAR α with a higher affinity than ATRA.^{9,10} Using BD4 as a lead structure, we designed an F-18 based PET tracer by replacing the boronic ester group with a borontrifluoride group.¹¹ Here we report for the first time a novel radiolabeling procedure, and initial *in vitro* and *in vivo* biological evaluation of [¹⁸F]**KBM-1** as a potential imaging probe for RAR- α in a murine model of neuroblastoma.

The precursor **BD4** for the radiochemical reaction was synthesized following the previously reported method from aryl aldehyde derivative using Michael-Aldol and Wittig reactions.^{9,12–14} The non-radioactive standard **KBM-1** was obtained from the precursor aryl bornic ester moiety BD4 using KF/KHF₂ chemistry as previously reported.^{9,13,15} Radiochemical synthesis of [¹⁸F]**KBM-1** was achieved by adding the precursor **BD4** (200 mM) to the azeotropically dried [¹⁸F[−]] residue followed by addition of 2 M HCl (6 μ L) and 0.4 M KHF₂ (1.0 μ L) at a pH of ~3, using published methods (Scheme 1).^{15–17} The reaction mixture was heated in a closed vial setup at 40 °C for 130 min. The final product was purified on silica gel chromatography using EtOH:NH₄OH (95:5) as the eluent. The radiotracer [¹⁸F]**KBM-1** was formulated with 0.9% NaCl solution and filtered through a 0.22 μ m pyrogen-free Millipore filter. [¹⁸F] **KBM-1** was produced in 6–8% decay corrected radiochemical yield in a total synthesis and formulation of 2.5 h (n = 6). The overall radiochemical purity was ~98% and the specific activity of the [¹⁸F]**KBM-1** was ~1800–2000 mCi/ μ mol (decay corrected to end of synthesis).

We conducted *in vitro* cell uptake assays using SH-SY5Y cell line, a human neuroblastoma cell line previously reported to express RAR- α with and without the blocking agents to evaluate the specificity and binding efficiency of [¹⁸F]**KBM-1**.^{7,18–21} The precursor molecule, **BD4** has been reported to demonstrate high and specific binding towards RAR- α over ATRA. The fluorometric binding assay showed that **BD4** binds specifically to RAR α with an affinity of 14 ± 5 nM (K_D) over ATRA.⁹ We used nonradioactive standard **KBM1**, ATRA, and precursor **BD4** as blocking agents in our *in vitro* cell uptake assay. The uptake of [¹⁸F]**KBM-1** was ~62% and 60% inhibited by non-radioactive **KBM-1** as blocking agent, ~37% and 32% inhibited by ATRA as blocking agent, and ~58% and 51% inhibited by precursor **BD4** as blocking agent after 15 min and 60 min incubation times respectively (Fig.

1). These findings indicate that [^{18}F]**KBM-1** uptake was significantly decreased in neuroblastoma cell lines with all three blocking agents (1) non-radioactive standard **KBM-1**, (2) ATRA, the widely used RAR- α targeting agent and (3) **BD4**, the previously published highly selective RAR- α ligand; thus demonstrating binding of [^{18}F]**KBM-1** to RAR- α .

We then conducted a biodistribution study with [^{18}F]**KBM-1** in male BALB/c mice with neuroblastoma tumors. Mice were grouped into three groups ($n = 4$) based on 5 min, 30 min and 60 min post injection (p.i). [^{18}F]**KBM-1** displayed rapid clearance from most of the major organs from 5 min to 60 min post injection (Fig. 2). The initial uptake at 5 min p.i in blood, kidney and liver was 4.37 ± 1.131 , 26.22 ± 2.351 and 2.92 ± 0.209 (%ID/g) respectively. The uptake had mostly cleared by 60 min, with blood levels at 0.93 ± 0.512 , kidney at 10.50 ± 1.509 and liver at 1.95 ± 0.233 (% ID/g) respectively. However, from 5 min to 60 min, bone uptake was constant or slightly increased-%ID/g of 1.65 ± 0.781 (5 min), 2.65 ± 0.112 (30 min) and 2.48 ± 0.131 (60 min), suggesting minor metabolic *in vivo* defluorination.

Tumor uptake remained stable, while the uptake ratios for target to non-target (tumor: muscle) increased from 2.2-fold to 3.7-fold at 5 min to 60 min p.i respectively (Fig. 3). To demonstrate specific binding, we performed a blocking experiment in a subset of mice from the 30 min group ($n = 2$). The blocking agent was the nonradioactive **KBM-1** (15 mg/kg) and was administered 15 min before the radiotracer injection. Tumor uptake in the 30 min blocking group was 1.7-fold lower than in the unblocked ones i.e., 0.80 ± 0.23 (%ID/g) versus 1.46 ± 0.81 in the unblocked group, demonstrating specificity for RAR- α receptors in the tumor (Table 1).

In conclusion, [^{18}F]**KBM-1** was synthesized for the first time using $\text{ArBF}_3^-/\text{KHF}_2$ chemistry with high radiochemical purity and specific activity. The *in vitro* cell uptake assay indicated that [^{18}F] **KBM-1** has the potential to serve as a RAR- α imaging agent. Initial *in vivo* biodistribution studies in BALB/c tumor-bearing mice demonstrate the potential of [^{18}F]**KBM-1** as a radiotracer for imaging neuroblastoma. Although we demonstrate novel radiochemistry and initial evaluation of [^{18}F]**KBM-1** binding *in vitro* and *in vivo* towards RAR- α , we are currently characterizing the complete receptor specificity, *in vivo* metabolic profile, kinetics and its performance in microPET imaging studies.

Acknowledgments

This work was supported by a pilot from Roena B. Kulynych Center for Memory and Cognition Research, the National Institute of Health (NIH) grant #1R01CA179072-01A1 (Mintz), P30 CA012197, the American Cancer Society Mentored Research Scholar grant # 124443-MRSG-13-121-01-CDD (Mintz), and the Translational Imaging Program (TIP) of the Wake Forest CTSA (UL1TR001420). We acknowledge the editorial assistance of Karen Klein, MA in the Wake Forest Clinical and Translational Science Institute.

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Fig. 1.

In vitro cell uptake of [^{18}F]KBM-1 by at baseline and blockade conditions at 15 min and 60 min incubation time points. The data were expressed as % injected dose (ID)/mg of protein present in each well with p values < 0.05 considered statistically significant ($n = 6$). All the blocking sections received 60-fold excess concentration of the blocking agent including, KBM-1, ATRA, and BD4 five min prior to the radiotracer addition.

Fig. 2. Biodistribution of [^{18}F]KBM-1 in tumor-bearing mice ($n = 4$) after 5 min, 30 min and 60 min post injection. Results are expressed in % injected dose (ID)/g $\pm$ standard deviation, with p values < 0.05 considered statistically significant.

Fig. 3. Ratio of target (tumor): nontarget (muscle) at 5 min, 30 min and 60 min post injection of [^{18}F]KBM-1 in tumor bearing mice (n = 4).

Scheme 1.
Radiochemical synthesis of $[^{18}\text{F}]\text{KBM-1}$.

Table 1

Biodistribution of [^{18}F]**KBM-1** in tumor bearing mice (n = 3) at 30 min post injection, with and without blocking agent. Results are expressed in % injected dose (ID)/g $\pm$ standard deviation.

Organs	30 min-nonblockade	30 min-blockade
Brain	0.23 $\pm$ 0.09	0.15 $\pm$ 0.07
Heart	1.91 $\pm$ 1.12	1.70 $\pm$ 0.75
Blood	4.01 $\pm$ 2.31	2.91 $\pm$ 1.19
Lung	2.78 $\pm$ 0.77	2.88 $\pm$ 0.11
Liver	2.32 $\pm$ 0.22	2.44 $\pm$ 0.84
Pancreas	2.03 $\pm$ 1.23	2.13 $\pm$ 0.65
Kidneys	24.33 $\pm$ 2.36	20.43 $\pm$ 2.78
Tumor	1.46 $\pm$ 0.81	0.81 $\pm$ 0.23
Muscle	0.49 $\pm$ 0.11	0.40 $\pm$ 0.07
Bone	2.65 $\pm$ 1.12	3.18 $\pm$ 1.65