



Radioiodination of 5-aminoisoquinoline for Poly (ADP-ribose) Polymerase Targeting: An *In vitro* Evaluation in Colorectal Carcinoma Cells

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THE ISOQUINOLINE derivative, 5-aminoisoquinoline (5-AIQ), is a potent inhibitor of poly (ADP-ribose) polymerase (PARP) activity; the DNA repairing protein frequently overexpressed in various carcinoma cells. The current study is dedicated for developing a highly specific radioiodinated PARP-1 binding tracer namely, [¹³¹I] 5-aminoisoquinoline. The radioiodination of 5-aminoisoquinoline with ¹³¹I was carried out via electrophilic substitution reaction. In order to achieve the maximum radioiodination, the parameters including 5-aminoisoquinoline concentration, oxidizing agent, reaction time, temperature and type of solvent were optimized. The radiochemical purity was determined by high performance liquid chromatography (HPLC) using Reversed phase lichrosorb column 250×4.6 mm, C-18 5 μm. The maximum radiochemical yield was 80% with radiochemical purity reached 98%. The log P value for [¹³¹I] 5-aminoisoquinoline was found to be 2.08 ± 0.79. Data presented from Cell viability (MTT) cytotoxicity assay on Caco-2 cells showed matched IC₅₀ values with the selected drug concentrations profile for either labeled or unlabeled 5-aminoisoquinoline. Likewise, the radioiodinated 5-aminoisoquinoline elucidated a higher cell uptake compared to free I-131.

Keywords: 5-aminoisoquinoline, Colorectal adenocarcinoma (Caco-2) cell line, PARP, Radiolabeling.

Introduction

Among plentiful advances in molecular medicine that have been elucidated, poly (ADP-ribose) polymerase (PARP) family has been revealed as a necessary temple in different cellular events and signaling pathways including; cell surviving and proliferation, DNA single strands repair and genomic maintenance, mitochondrial biogenesis, modification of apoptotic and necrotic cascades during cell death, regulation of the immune system and inflammatory response (McGlynn & Lloyd, 2002). Such function diversity emphasizes the idea of considering PARP as a pharmacological target in many pathological conditions. Overexpression

of PARP has been reported in various human malignancies; malignant lymphoma (Tomoda et al., 1991), breast carcinoma (Bieche et al., 1996), Ewing's sarcoma (Soldatenkov et al., 1999), hepatocellular carcinoma (Shimizu et al., 2004) and endometrial carcinoma (Ghabreau et al., 2004). Thus, various PARP-1, the main family member, inhibitors were designed based on their specific competitive inhibition ability to the enzyme-catalytic domain, such as PJ-34 and 5-AIQ, or non-specifically substitute its NAD⁺ substrate, such as 3-aminobenzamide and nicotinamide (Graziani & Szabó, 2005; Wiggans et al., 2015). Due to the fact that PARP inhibitors have the priority in treatment of several forms of cancer and recently

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turned into well-recognized market products, e.g. Lynparza, the need to determine the PARP levels for cancer patients is clinically essential prior starting the treatment regimen. Despite such significant impact, inadequate number of reports have been recorded for developing newly single photon emission computed tomography (SPECT) or positron emission tomography (PET) radiotracers for PARP biomarker evaluation. Isoquinoline derivatives have been identified as potent inhibitors of PARP-1 activity upon examining in different biological systems and experimental models (Wayman et al., 2001; Mota-Fillipe et al., 2002). Predominantly, 5-aminoisoquinoline (5-AIQ) has a supreme function group among different PARP inhibitors, reflecting its structure correlation with PARP activity and the inhibiting potency (Vinod et al., 2010). The current work aims at developing a new specific radioiodinated PARP-1 binding tracer, [¹³¹I] 5-aminoisoquinoline, targeting molecular imaging of colorectal adenocarcinomas depending on their high expressing ability to the nuclear enzyme, PARP-1.

Experimental

Material

All chemicals used in this study were of the highest purity grade from Sigma-Aldrich and Pierce Chemical Company and were used without further purification. In all cases, double distilled water was used. Na¹³¹I (200 MBq/5 mL) in diluted NaOH, pH 7–11, was obtained from Egypt Second Research Reactor, Inshas. Mass spectrometry was performed using Hewlett Packard 5988 spectrometer. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz for ¹H and at 75.46 MHz for ¹³C. HPLC system (Sykam) was used for separation and purification. The γ -counter (Nucleus Model 2010) connected with a well-type NaI (Tl) crystal was used for radioactivity measurements.

Iodination of 5-AIQ by ¹³¹I/¹²⁷I

For preparation of [¹³¹I]5-AIQ, an appropriate concentration (0.06 – 1 μ mol) of the 5-AIQ in ethanol was added to iodogen which deposited on the wall of the brown glass vial as a thin film and about 15 MBq Na¹³¹I was added. The total volume was completed to 0.5 mL with ethanol. The reaction was proceeded at different temperature for variable periods (Kandil et al., 2017).

The iodination reaction was performed on larger scale (5 fold) using Na¹²⁷I for MS and NMR analysis. The product was isolated using HPLC on

discovery[®] Bio Wide Pore column (RPC18, 50 x 10 mm, 10 μ m) at 2.5 mL/min with the same mobile phase used for the labeled compound. ¹H NMR (DMSO-d₆): δ 7.28(dd, J = 8.9 HZ, 1H), 7.37(dd, J = 8.9 HZ, 1H), 8.12(dt, J = 5.3 HZ, 1H), 8.6(dd, J = 5.2, 1H), 8.9(dt, J = 1.96 HZ, 1H). ¹³C NMR (DMSO-d₆): 90.7, 117, 118.8, 125.5, 128.4, 138.5, 142.6, 143.5, 150.8. MS (M +H), 270.45 (found) and 270.17 (calculated).

Determination of the radiochemical yield and purity

The radiochemical yield and purity of [¹³¹I]5-AIQ were determined by HPLC (Yuan et al., 2017) with little modification. Briefly, the reaction mixture was injected on the column (RPC18, 250 x 4.6 mm, 5 μ m) and eluted by acetate buffer pH 5: acetonitrile (3:7 v/v) at 1 mL/min. The wavelength of the U.V detector was adjusted to 254 nm. Each 0.5 mL fraction was collected and counted using γ -counter.

Determination of the partition coefficient of radiolabeled 5-AIQ

The Lipophilicity was determined according to a previously described method (Aglan et al., 2016). Briefly, 2 mL of labeled 5-AIQ solution (50 μ M in phosphate buffer solution, pH7.4) was mixed with 2 mL n-octanol for 3 min that was performed in triplicate. The mixture was vortexed at room temperature for 1 min and then centrifuged at 5000 rpm for 5 min. The two layers were allowed to separate and counted. P was calculated as the ratio of activities (n-octanol/buffer) and its logarithm was determined.

Cell culture and treatment protocol

Human colorectal adenocarcinoma (Caco-2) cells were purchased from the Cell Bank of VACSERA (Cairo, Egypt) and maintained in high glucose DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO₂. They were passaged at 80-90% confluency after trypsinization with pre-warmed trypsin-EDTA solution. Cells were incubated (4h, at different concentrations) with either the PARP inhibitor (5-AIQ) or its iodinated analogue before evaluation of cytotoxicity (El-Hamoly et al., 2017).

Cell viability (MTT) assay

Confluent cells (10⁵ cells/mL) were inoculated in 96-well plate with either 5-AIQ or its iodinated product (R) four hours at 37°C. At the end of the experiment, 20 μ l of 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) solution

was added to each well and then incubated for one extra hour. The absorbance of living cells was measured at 560 nm (El-Hamoly et al., 2017).

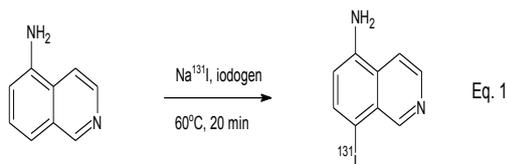
In vitro evaluation of [¹³¹I] 5-AIQ uptake

In culture flasks, Caco-2, cells were seeded and treated with 10 μ Ci of NaI-131 and [¹³¹I]5-AIQ diluted in culture media for 60 min (37 $^{\circ}$ C, 5% CO₂). The cells were rapidly washed twice with 1 mL cold fresh phosphate buffer saline (PBS). Afterward, they were incubated in 1 mL 95% ethanol at room temperature for 20 minutes. The flasks were counted in a gamma radiation counter. The counts per minute were normalized to 10⁶ cell number. Untreated cells were used as a sham operated control (Seddik et al., 2018).

Results and Discussion

Radiochemistry

The structure of [¹³¹I]5-AIQ via iodine-131 hydrogen exchange in the presence of iodogen as an oxidizing agent is shown in Eq. 1.



In order to achieve the maximum radioiodination of 5-AIQ with ¹³¹I, a previously described protocol (Kandil et al., 2017) was used toward that goal. The parameters namely, 5-AIQ concentration, oxidizing agent, reaction time, temperature and type of solvent were optimized to achieve the maximum radiochemical yield.

The used concentration range of 5-AIQ varied from 0.06 to 1 μ mol. As shown in Fig. 1, the radiochemical yield (RCY) of [¹³¹I] 5-AIQ increased as the concentration of 5-AIQ increased. It reached 80% at a concentration of 0.5 μ mol. Any excess of 5-AIQ concentration had no effect on the RCY.

The influence of oxidizing agents, namely, Chloramine-T (CAT) and iodogen concentrations on the radioiodination of 5-AIQ were studied. As shown in Fig. 2, the data reveal that using iodogen the RCY reached 80 % at 0.5 μ mol. compared to that obtained using CAT (53%). This may be attributed to the ability of iodogen in radioiodination which is 4-folds as compared to CAT with less oxidative damage effect (Petzold & Coenen, 1981).

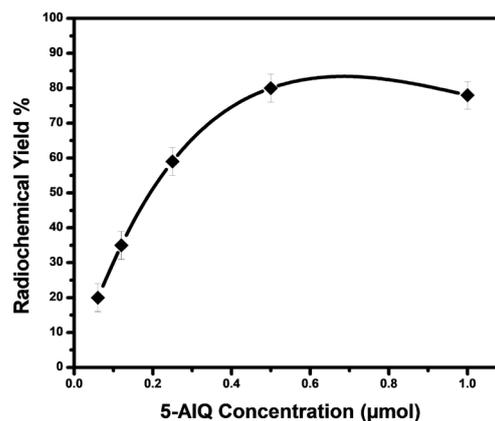


Fig. 1. Variation of the radiochemical yield of [¹³¹I]5-AIQ as a function of substrate concentration.

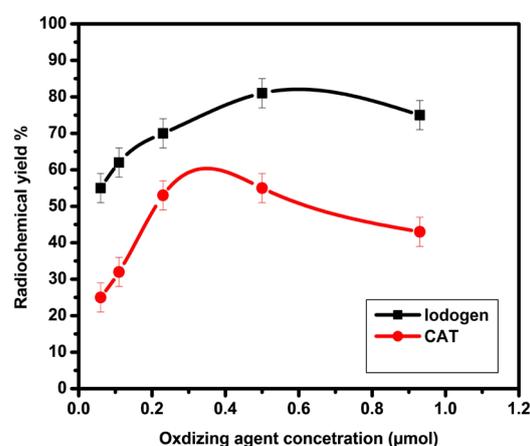


Fig. 2. Variation of radiochemical yield of [¹³¹I]5-AIQ as a function of oxidizing agent.

The radioiodination is actually dependent on the reaction time. The RCY was tested at different time intervals between 5 and 60 minutes using 0.5 μ mol. 5-AIQ and 0.5 μ mol. iodogen (Fig. 3). The reaction was completed after 20 minutes. These conditions were applied to study RCY at different reaction temperatures (Fig. 4). At ambient temperature up to 60 $^{\circ}$ C, the RCY did not exceed 80%. However, above 60 $^{\circ}$ C up to 100 $^{\circ}$ C, the low RCY may be attributed to the thermal decomposition of the labeled compound or distortion of the oxidizing agent (Amin et al., 2015).

The reaction was treated in different solvents such as methanol, acetonitrile, ethanol, Dimethylformamide and Dimethyl sulfoxide. Ethanol gave the highest radiolabeling yield. The optimized radioiodination procedures to obtain [¹³¹I]5-AIQ was applied in ethanol using 0.5 μ mol. 5-AIQ, 0.5 μ mol. iodogen

and 15 MBq Na^{131}I at 60°C for 20 min. For radiochemical yield determination, the reaction mixture was subjected to HPLC. Reversed phase lichrosorb column 250×4.6 mm, C-18 $5\mu\text{m}$ was used for this purpose. Fig. 5 shows the HPLC radiochromatogram and UV absorbance profile for the reaction. As shown in this Figure, the peak refers to iodonium ion appeared at 2 min, and the substrate (5-AIQ) peaks appeared from 4 – 6.5 min, while the peak represents the labeled compound, ^{131}I 5-AIQ, separated far at 9 min. The ^{131}I 5-AIQ was collected using fraction collector, evaporated under reduced pressure, dissolved in saline solution, filtered by Millipore ($0.22\mu\text{m}$) and its activity was counted. The radiochemical purity of the radiotracer was determined using HPLC, and was found to be 98% after 24 h. For authentication of identity of the radiotracer, the cold and hot products were mixed together and then injected in HPLC. They were both identified at the same retention time.

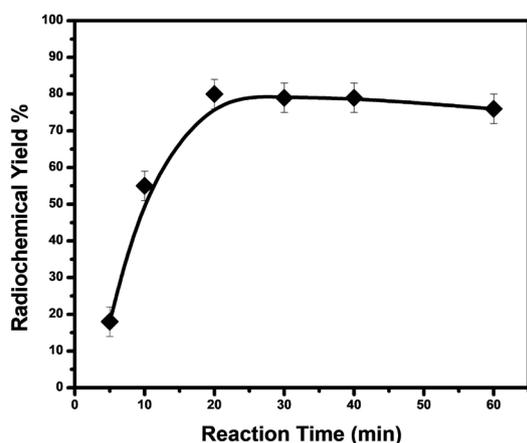


Fig. 3. Variation of radiochemical yield of ^{131}I 5-AIQ as a function of time.

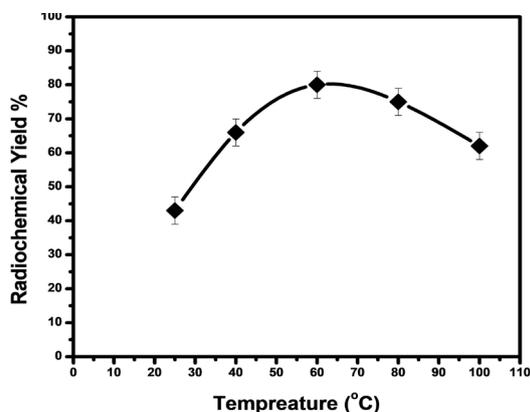


Fig. 4. Variation of radiochemical yield of ^{131}I 5-AIQ as a function of temperature.

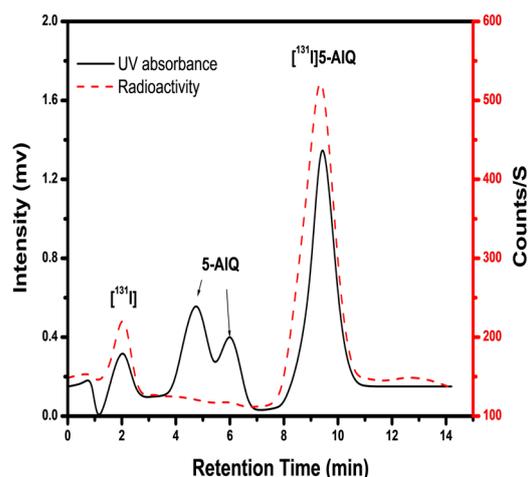


Fig. 5. HPLC elution profile of ^{131}I 5-AIQ, separated on reversed phase lichrosorb column 250×4.6 mm, C-18, $5\mu\text{m}$

Partition coefficient of radiolabeled ^{131}I 5-AIQ

Compound lipophilicity plays a pivotal role in the absorption, distribution, metabolism, and elimination (ADME) of therapeutic drugs. A higher lipophilicity ($\log P$) may enhance tissue penetration to increase its tumor's uptake; it simultaneously would increase the non-target tissue uptake and lead to a higher nonspecific uptake in target and in non-target tissues. The measured $\log P$ value for ^{131}I 5-AIQ was found to be 2.08 ± 0.79 .

Effect of iodinated 5-AIQ on colorectal carcinoma (Caco-2) cell line cytotoxicity

Caco-2 cells showed a high expression of the DNA repair enzyme; Poly (ADP-ribose) polymerase-1 (PARP-1). 5-AIQ (PARP-1 inhibitor) pre-incubation revealed inversely loss of cell viability upon decreasing the dose levels. Interestingly, iodination of 5-AIQ did not interfere its IC_{50} values as data illustrated in Fig. 6 and Table 1. Encouraged by these results, it could be concluded that the structure-activity relationship (SAR) of labelled 5-AIQ did not affect the iodination.

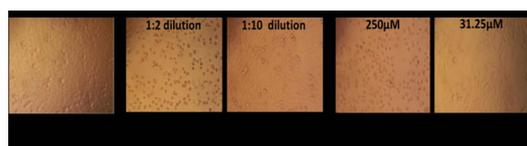


Fig. 6. Effect of iodinated 5-AIQ on Caco-2 cytotoxicity [The Caco-2 cells were incubated with 5-AIQ and iodinated 5-AIQ for an hour, cell viability was determined with MTT assay].

TABLE 1. Effect of 5-aminoisoquinoline (5-AIQ) of iodinated analogue on cytotoxicity of Caco-2 cell line.

	Concentration (μM) - Dilution ratio	Cell viability (% of control)
5-AIQ	250	4.4 ± 0.0001
	125	5.3 ± 0.002
	62.5	98.3 ± 0.004
	31.25	100 ± 0.003
	15.625	101 ± 0.001
Iodinated-5-AIQ	---	4.003 ± 0.0005
	1:2	3.008 ± 0.0008
	1:4	97.5 ± 0.0006
	1:8	100.01 ± 0.001
	1:10	101.6 ± 0.002

Evaluation of [^{131}I]5-AIQ uptake in Caco-2 cell line

A recent study suggested that advanced patients suffering from colorectal carcinoma underlining a recruitment of PARPs and their conjugated metabolites; the phenomena that interrogates the mechanism behind the resistance to chemotherapy and may contribute to a higher number of cancer recurrent patients (Jarrar et al., 2019). In the present study, the authors investigated a radiotracer for determination of PARP-1 levels based on the impact of PARylation in such cancer cell lines. As shown in Fig. 7, Caco-2 cells showed a significant higher [^{131}I] 5-AIQ uptake, around seven folds greater than that of radioiodine-131. Such finding confirms the selectivity of radioiodinated-5-AIQ on PARP-1-expressing cancer cells.

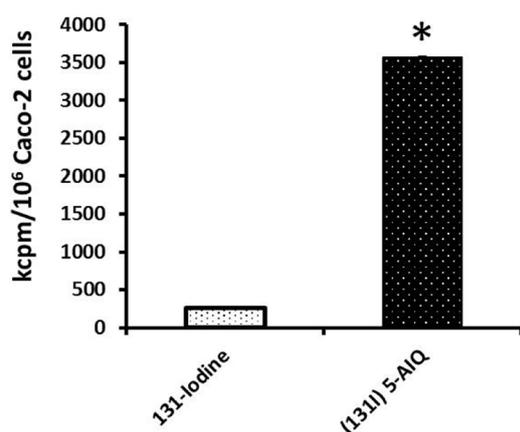


Fig. 7. Uptake of Radioiodinated 5-AIQ in Caco-2 cells [Each uptake was measured in kcpm/10⁶ cells. Data are expressed as mean \pm SEM of three independent experiments, (*) indicates significant difference versus free iodine-131 (* $P < 0.05$)].

Conclusions

The overall data presented in the current study might be of utmost importance in demonstrating an approach towards molecular mapping of PARylation pathway by using a novel radiotracer, tracking the propagation of carcinomas. Further preclinical studies are mandatory in future.

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