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BIODISTRIBUTION OF LABELED IODO-AZATHIOPRINE IN EAC TUMOR BEARING MICE

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ABSTRACT

Azathioprine (Aza) is an antimetabolite drug, could be labeled with the Auger emitters iodine-125. Aza could be used as an ideal vehicle to deliver radioactive decay energy to DNA of tumor cells causing DNA double strand break, thus stop DNA synthesis. In this study, the process of labeling was done via direct labeling technique using chloramine-T as an oxidizing agent and heating to 75 °C for 30 minutes at pH 7 using 0.5 M phosphate buffer. The radiochemical purity of the labeled compound, at the above conditions, was determined using electrophoresis technique and was above 90%. About 2.5×10^6 of Ehrlich Ascites Carcinoma (EAC) was injected intraperitoneally (i.p) to produce ascites and intramuscularly (i.m) in the right thigh to produce solid tumor in female mice. Biodistribution studies were carried out by injecting solution of ^{125}I-Aza in normal and tumor bearing mice. The uptake in ascites was over 40% of the injected dose at 12h post injection and above 20% in solid tumor. The data revealed localization of the cancer in the tumor tissues with high percentage sufficient to give radiotherapeutic effect as well as promising uses for diagnosis.

INTRODUCTION

It was reported that many delivery systems are able to deliver chemotherapeutic drugs or radioisotopes to the specific tumor site with decreased toxicity to other proliferating tissues as well as neighboring tissues[1]. Antimetabolites are compounds that prevent biosynthesis or utilization of normal cell metabolites. They are usually closely related in structure to the metabolites that they antagonize. Many antimetabolites block enzymes involved in nucleic acids synthesis[2]. Auger electron emitters are widely used in cellular radiation studies. The most frequently used Auger-emitters are iodine-125 and iodine-123[3]. They decay by electron capture with the emission of Auger-electrons which deposit a sizable energy at the site designated for tumor therapy[4]. Moreover, radioisotopes such as iodine-131 and iodine-123 can also facilitate tumor imaging[5]. The radiotherapeutic effectiveness of these radionuclides can be achieved by the incorporation of these radionuclides into cellular DNA leading to the breakage of DNA double strand[6]. The ideal vector for delivering Auger-electron emitters should be specifically accumulated in the target cells and concentrated in the cell nucleus in close proximity to the DNA. For treatment of cancer, one of the routes that had been studied widely is the use of an agent that will be taken up inside the cancer cells, several chemotherapeutic and antiviral agents are based on such agents[7]. Previous work was done for labeling of many antimetabolites with iodine-125 for using in cancer radiotherapy such as ^{125}I-iododeoxyuridine (IUdR, thymidine analogue)[8]. This was manifested by a high degree of lethality, double strand break in bacteria, bacteriophage and diminished survival of tumor cells[9]. The toxicity of ^{125}IUdR, a potential therapeutic agent is unrelated to chemical toxicity or to the radiosensitizing effect of the halogenated deoxyribonucleoside, but it may be attributed to the Auger effect that takes place inside the nucleus[10]. The radioactive iodine alone gives no lethal effect even by locoregional use because it can't cross cell membrane[11].

Other antimetabolite, such as cytarabine was also labeled with iodine-125 and suggested as a potential radiotherapeutic agent[12]. Its biodistribution reflects localization in target tissues with high percentage sufficient to produce radiotherapeutic effect[13].

Azathioprine, a prodrug, is converted in the body to 6-mercaptopurine, in order to protect it from catabolic reaction[14]. Although azathioprine has antitumor activity, it has also an important role as an immunosuppressive agent in organ transplants and in a variety of autoimmune diseases as rheumatic arthritis[15].

This study was conducted to find a model for labeling azathioprine as a vehicle to carry iodine-125 to a tumor cells. This was achieved by injecting EAC to mice either intraperitoneally to induce ascites or intramuscullary in the right thigh to produce solid tumor. In addition *in vitro* stability of ^{125}I-Azathioprine and its *in vivo* biodistribution was also carried out.

MATERIALS AND METHODS

Drugs and chemicals:

- Azathioprine was purchased from ICN Chemical Co. USA. It was dissolved in 0.5 M phosphate buffer pH 7 (1:3).
- Iodine-125 was purchased from Nordion Co. Belgium as a no carrier added dissolved in diluted NaOH.
- Chloramine-T (CAT) was purchased from Sigma Chemical Company, USA.
- Ehrlich ascites carcinoma (EAC) supplied from National Cancer Institute, Cairo, Egypt.

Animals:

Female Swiss Albino mice weighing 20-25 gm were purchased from the Institute of Eye Research Cairo, Egypt. The animals were kept at constant environmental and nutritional conditions throughout the experimental period and kept at room temperature (22 ± 2°C) with a 12 hr on/off light schedule. Animals were kept with free access to food and water all over the experiment.

Labeling procedure and requirement:

Iodo-Azathioprine was prepared by electrophilic substitution of hydrogen with iodonium ion using chloromine-T as oxidizing agent [16].

Azathioprine was dissolved in 0.5 M phosphate buffer (1:3) pH 7, with heating and stirring. CAT was added to Aza solution, followed by the addition of a specified volume of 0.5M phosphate buffer pH 7 and approximately 18-37 MBq (0.5-1 μCi) carrier free Na ^{125}I. After a specified interval of time and temperature, the reaction was stopped using 0.2 N $Na_2S_2O_3$ solutions (100 μl) to ensure that the un-reacted iodine is reduced before chromatographic analysis [16]. The yield of the reaction and the radiochemical purity were determined by paper electrophoresis.

Factors affecting % labeling yield:

This experiment was conducted to study the different factors that affect labeling yield such as:

(1) Oxidizing agent,
(2) Substrate content,
(3) Reaction temperature,
(4) pH of the reaction, and
(5) Reaction time.

In the process of labeling, trials and errors were performed for each factor under investigation to obtain the optimum value. The experiment was repeated with all factors kept at optimum changing except the factor under the study until the optimal conditions achieved[17].

Electrophoresis conditions:

Electrophoresis was done with EC 3000 p-series 90 programmable power and chamber supply units using cellulose acetate strips (45 cm). These stripes were moistened with 0.05 M phosphate buffer pH 7 and then introduced into the chamber. Samples were applied at a distance of 10 cm from cathode. Standing time and applied voltage were continued for one and half-hours. Developed strips were dried and cut into 1cm segments then counted by a well-type NaI scintillation counter. The radiochemical yield is calculated as the ratio of the radioactivity of the labeled product to the total radioactivity[7].

$$\% \text{ radiochemical yield} = \frac{\text{Peak activity of } ^{125}\text{I-azathioprine} \times 100}{\text{Total activity}}$$

Induction of tumor in mice:

The parent tumor line (Ehrlich Ascites Carcinoma) was withdrawn from 7 days old donor female Swiss albino mice and diluted with sterile physiological saline solution to give 12.5 x 10^6 cells/ml. 0.2 ml solution was then injected in mice intrapritoneally to produce ascites, or intramuscularly in the right thigh to produce solid tumor. The animals were maintained till the tumor development was apparent for about 10 to 15 days[18]. Female mice were used in this study because of their high susceptibility to Ehrlich ascites carcinoma than male mice[19].

***In-vitro* stability:**

This experiment was conducted to determine the stability of ^{125}I-Aza after labeling and the impact of time on that compound. The yield was measured at different time intervals (1, 4, 12, 24 and 48 hours) after labeling[20].

***In-vivo* biodistribution:**

Biodistribution of ^{125}I-Aza was carried out in two groups of animals each group consists of 24 mice, one was ascites bearing group and the other was solid tumor bearing mice. Each mouse was injected in the tail vein with 0.2 ml solution containing 5-10 KBq of ^{125}I-Aza two weeks post inoculation. Each group subdivided to 4 subgroups 6 mice each. Mice in each group were kept in metabolic cages to be sacrified, after 15 min, 1 h, 12 hour and 24 hour post injection of the labeled drug. Mice were killed by cervical dislocation and the organs or tissues of interest were isolated, weighted and counted for its uptake of radioactivity. Ascites fluid was drained and counted as a whole. The counting tubes, including a standard equivalent to 1% of the injected dose, were assayed in a well type NaI (TI) gamma counter and the results were calculated as percentages of injected dose (I.D) per gram tissue. The final results were expressed as mean ± one standard error[21].

The weights of whole blood, bone and muscles were assumed to be 7, 10 and 40% of the total body weight, respectively. Correction was made for background radiation and physical decay during the experiment [7, 21].

Statistical analysis:

The results are expressed as means ± SEM for the indicated number of different experiments. The statistical significance of differences was assessed by unpaired Student's t-test $P < 0.05$.

RESULTS AND DISCUSSION

Treatment of cancer is constantly changing and moving to molecular levels. Currently, targeted delivery is becoming a reality in cancer treatment. Target specific molecules and novel delivery systems are able to deliver chemotherapeutic drugs or radioisotopes to the specific tumor sites with decreased toxicity to other proliferating tissues (gut and bone marrow). For treatment of cancer, one of the routes that have been studied widely is the use of chemotherapeutic agent that could be taken inside the cancer after being labeled with iodine-125 in order to combine the chemo- and radiotherapeutic effects[16].

Electrophoresis analysis:

Figure (1) illustrates the analysis of the fractions that produced from the reaction by electrophoresis. Three peaks were formed, one corresponding to the free iodide that moved towards the anode with 16 cm distance at the condition mentioned before. The second peak remained at the point of spotting while, the third fraction was also migrated towards the anode to a lesser extent equal to 11cm. The species that stayed at the point of spotting was found to be identical to that of ^{125}I-UdR under the same electrophoretic conditions[16].

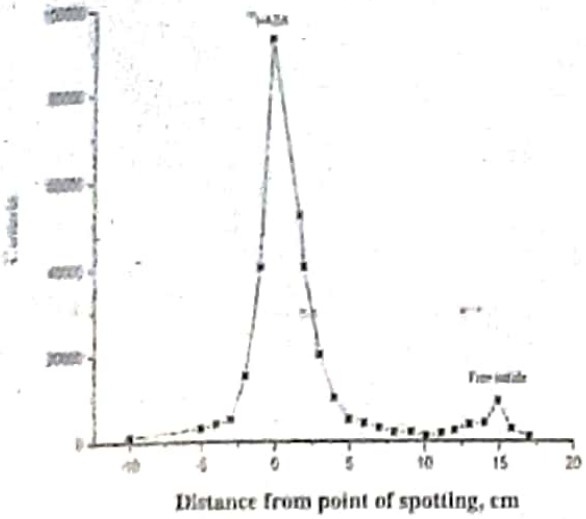

Figure (1): Electrophoresis pattern of the radio-iodinated Azathioprine (^{125}I-Aza)

Factors affecting labeling yield:

1- Effect of oxidizing agents:

Results obtained in this study revealed that the electrophilic substitution of the Iodonium ion [$^{*}I^{+}$] onto Aza molecule afforded a high radiochemical yield by utilizing CAT as an oxidizing agent (table 1). It was observed that the radiochemical yield significantly increased by increasing the amount of CAT from 25 µg to 100 µg (optimum content) at which maximum labeling yield was obtained. By increasing the amount of CAT above 100 µg, the yield significantly decreased. A significant reduction in the labeling yield was noted by decreasing the concentration of CAT below 100 µg as well as increase the concentration above 100 µg. A possible explanation for this observation that at low concentrations of CAT, not all iodide converted to iodonium ion and thus, the yield was decreased. However, high concentration of CAT may affect the sites that facilitate the process of substitution (labeling)[22].

Table (1): Effect of chloramines-T (CAT) content on the radiochemical yield of ^{125}I azathioprine

CAT (µg)	% labeled compound	% free iodide
25 µg	80.3 ± 0.4*†	20 ± 0.4
50 µg	85.5 ± 0.5*†	14.5 ± 0.50
100 µg	94.3 ± 0.04*†	5.7 ± 0.04
200 µg	88.5 ± 0.32*†	11.5 ± 0.32

Values represent the mean ± SEM (n = 6)
*Significantly different from the initial values using student's t- test ($p < 0.05$)
†Significantly different from the previous values using student's t- test ($p < 0.05$)

2- Effect of substrate content:

The influence of Aza content as a substrate on the labeling yield using CAT as an oxidizing agent was shown in table (2). The increase of the concentration of Aza was accompanied by a significant increase in the labeling yield, where it reached above 90 % at 100 µg of Aza. Increasing the amount of Aza above 100 µg produced no significant increase in the labeling yield. Increasing the concentration of starting material is usually increases the total incorporation of radioiodine, since there is a minimum limit to the volume used [23]. 100µg of Aza was required to obtain maximum labeling yield, below this concentration there is a significant decrease in the yield. On the other hand, using higher concentration did not significantly affect labeling yield.

Table (2): Effect of Azathioprine (Aza) content on the labeling yield

Aza (µg)	% Labeled compound	% Free Iodide
25	88.4 ± 0.31	11.6± 0.31
50	89.0 ± 0.36	11.0 ± 0.36
75	89.7 ± 0.18*	10.3 ± 0.18
100	94.5 ± 0.40*†	5.5 ± 0.40
200	94.5 ± 0.26*	5.5 ± 0.26

Values represent the mean ± SEM (n = 6).
*Significantly different from the initial values using unpaired student's t-test ($p < 0.05$)
†Significantly different from the previous values using unpaired student's t-test ($p < 0.05$)

3- Effect of reaction temperature:

The radio-iodination was carried out at different temperatures to attain the optimum one at which maximum labeling yield is obtained (table 3). Increasing the reaction temperature was accompanied by significant increase in the radiochemical yield, where it reached maximum (94.3%), at 75°C within 30 min. At 100°C, the reaction yield was significantly decreased to 73.6% within 30 minutes. This observation could be attributed to the enhancement of substitution process and increased activity of oxidizing agent. With increasing temperature to 100°C the efficiency of oxidizing agent may be decreased and thus, a decrease in the labeling yield or the substrate itself may be affected[24].

Table (3): Effect of emperature on the labeling yield of ^{125}I-azathioprine

Temperature °C	% Labeled compound	% Free Iodide
25	20.3 ± 0.8	79.7 ± 0.1
50	54.3 ± 2.0*†	45.7 ± 2.0
75	94.5 ± 1.2*†	5.5 ± 0.5
100	73.6 ± 0.7*†	26.4 ± 0.7

Values represent the mean ± SEM (n = 6)
*Significantly different from the initial values using unpaired student's t-test ($p < 0.05$)
†Significantly different from the previous values using unpaired student's t-test ($p < 0.05$).

4- Effect of pH:

In order to percolate a suitable pH value for maximum radiochemical yield, radioiodination of Aza was carried out at different pH values ranging from 1-11. The test was performed using 100 µg of Aza, 300 µl of 0.5 M phosphate buffer of pH7 at 30-minute reaction time. The experiment was repeated using 300 µl of each buffer at different pH values. As shown in table 4, pH 7 is the optimum pH at which the maximum yield was obtained (94.8%). Also, it was observed that at pH 1 or 3, the yield was 8.4%, and 12%, respectively, while at pH values 9.8 and 11, the yield was 56.0%, 89.5%, respectively. There was a significant difference between all pH values of the reaction mediums. The observation of this study demonstrates that the optimum pH is 7, using phosphate buffer is coincide and similar with other previous work, which reported that CAT showed optimum efficacy at pH 7[22]. Around pH 7, iodide is oxidized to iodonium cation, which is involved in substitution process (labeling), while at other pH values different oxidizing states are obtained and labeling yield decreases.

Table (4): Effect of pH of the reaction medium on the labeling yield of ^{125}I-azathioprine

pH value	% Labeled compound	% Free iodide
1	8.40 ± 0.11	91.6± 0.11
3	12.0 ± 0.30*	88.0 ± 0.30
7	94.5 ± 0.30*†	5.5 ± 0.30
9.8	56.0 ± 0.44*†	33.9 ± 0.44
11	89.0 ± 0.20*†	11.0 ± 0.20

Values represent the mean ± SEM (n = 6).
*Significantly different from the initial values using unpaired student's t-test ($p < 0.05$).
†Significantly different from the previous values using unpaired student's t-test ($p < 0.05$).

5- Effect of reaction time:

Table (5) shows the relationship between the reaction time and the yield of ^{125}I-Aza. Radiochemical yield was significantly increased from 56.9% to 94.8% with increasing reaction time from one minute to 30 minutes. Extending the reaction time to 60 minutes, produced significant decrease of the radiochemical yield. The efficiency of oxidizing agent may be affected by heating for long time and thus yield decreased[15].

Table (5): Effect of reaction time on the % labeling yield of ^{125}I-azathioprine

Time/minute	% Labeled compound	% Free Iodide
1	56.9 ± 0.36	43.1 ± 0.36
5	76.7 ± 0.29*	23.3 ± 0.29
15	81.3 ± 0.21*†	18.7 ± 0.21
30	94.8± 0.35*†	5.2 ± 0.35
60	89.3 ± 0.14*†	10.7 ± 0.14

Values represent the mean ± SEM (n = 6).
*Significantly different from the initial values using unpaired student's t-test ($p < 0.05$).
†Significantly different from the previous values using unpaired student's t-test ($p < 0.05$).

***In-vitro* stability of ^{125}I-azathioprine:**

In the present experiment, a significant decrease in the stability of ^{125}I-Aza from 94.8% to 93% at 12 hour post labeling was observed. Further significant reduction was observed at 24 hour post labeling, as the yield was 91%. The labeled compound produced yield about 90 % at 48 hour post labeling. This indicates in vitro stability of the labeled compound (table 6).

Table (6): Effect of time on the stability of ^{125}I-azathioprine

Time (hour)	% Labeled compound	% Free Iodide
1	94.8 ± 0.09	5.2 ± 0.09
4	94.5 ±0.13	5.5 ±0.13
12	93.0 ±0.24*†	7.0 ± 0.24
24	91.0 ±0.40*†	9.0 ±0.40
48	90.0 ±0.48*	10.0 ±0.48

Values represent the mean ± SEM (n = 6)
*Significantly different from the initial values using unpaired student's t-test ($p < 0.05$)
†Significantly different from the previous values using unpaired student's t-test ($p < 0.05$)

Biodistribution of ^{125}I-Azathioprine:

a- In ascites bearing mice:

The results of this experiment showed that the sites of greatest uptake of ^{125}I-Aza after 15 minutes post injection were the blood, heart and lung (16.5, 8 and 7.5), respectively. Table 7 shows that the concentration of ^{125}I-Aza was the lowest in thyroid, muscle and spleen at 15 minutes post injection. The uptake of ^{125}I-Aza in ascitic fluid was rapidly take place as each ml of ascitic fluid received 3.3% of total activity. The uptake of ascitic fluid was significantly increased after one hour and 12 hours to reach 5.2% and 6.5% per 1 ml; respectively. No significant change in the uptake of ^{125}I-Aza at 24 h post injection was observed when compared to its previous value. The data also showed that some organs exhibit significant increase of uptake at one hour post injection like stomach, ascitic fluid, bone and thyroid. On the other hand, significant decrease in ^{125}I-Aza uptake was observed in blood, heart, kidney and lung at the same time. At 12 hour post-injection, the majority of organs showed significant decrease in uptake of ^{125}I-Aza. Significant increase was only observed in ascitic fluid and thyroid at 12 hour post-injection. Similarly, at 24 hour post-injection, the majority of organs showed additional significant decrease in ^{125}I-Aza uptake. The results of biodistribution study of ^{125}I-Azathioprine in ascites bearing animal revealed that ascites was one of the most site of uptake of ^{125}I-Aza and this was clear at 1 h and lasted to 24 h post injection. ^{125}I-Azathioprine uptake in ascites was about 40% of the injected dose at 12h post injection before reflecting the uptake per gram tissue. The uptake of each ml of ascites was 5.2, 6.5 and 6.3 at 1, 12 and 24 hours, respectively. It was also observed that ascites was the site of highest uptake considering the average volume of ascites (8.2 ± 0.7). This result suggests the use ^{125}I-Azathioprine in imaging of tumor. The high uptake of ^{125}I-Aza in kidney may reflect the excretion of the drug via urine[3]. The observation that % ^{125}I-Aza concentration in the thyroid was significantly less than in other tissues indicates that less free iodide is associated with iodo-azathioprine, since free iodide is rapidly captured by thyroid[25]. However, thyroid uptake was increased by time from 4% at one hour to 6.2% at 24 hour post-injection due to *in-vivo* deiodination of ^{125}I-Aza[7].

Table (7): Biodistribution of ^{125}I-azathioprine in ascites bearing mice

Organs and body fluids	% ^{125}I-Aza /gram organ time post -injection			
	15 minutes	1 hour	12 hours	24 hours
Blood	16.5 ± 1.10	6.1 ± 0.4*	4.1 ± 0.1*	3.2 ±0.15*
Bone	3.00 ± 0.15	3.3 ± 0.15*	2.4 ± 0.15*	1.8 ± 0.17*
Muscle	1.25 ± 0.09	1.4 ± 0.02*	1.1 ± 0.01*	0.7 ± 0.04*
Liver	3.70 ± 0.25	3.4 ± 0.2*	1.8 ± 0.06*	1.5 ± 0.07*
Lung	7.50 ± 0.10	6 ± 0.04*	3 ± 0.1*	0.5 ± 0.1*
Heart	8.00 ± 0.30	4 ± 0.40*	2 ± 0.1*	1.4 ± 0.12*
Stomach	6.20 ± 0.30	12.4 ± 0.9*	10.1 ± 0.6*	6.0 ± 0.5*
Intestine	4.60 ± 0.50	3.7 ± 0.07*	3.40 ± 0.1*	2.90 ± 0.2*
Kidney	6.10 ± 0.40	3.4 ± 0.1*	2.01 ± 0.1*	1.2 ± 0.06*
Spleen	2.50 ± 0.10	1.5 ± 0.01*	0.7 ± 0.01*	0.3 ± 0.0*
Thyroid	1.00 ± 0.02	4 ± 0.04*	6.1 ± 0.06*	6.2 ± 0.05
Ascitic fluid	3.30 ± 0.30	5.2 ± 0.4*	6.5 ± 0.1*	6.3 ± 0.05

Values represent the mean ± SEM (n = 6).
*Significantly different from the initial value of each organ using unpaired student's t-test ($p < 0.05$).

b- In solid tumor bearing mice:

Biodistribution of ^{125}I-Aza in solid tumor bearing mice was found to be greatest in blood, heart and stomach (22.8, 12 and 11.1, respectively) at 15 minutes post injection and lowest in left leg, bone and thyroid (0.8, 1.2 and 2, respectively) (table 8). The biodistribution of ^{125}I-Aza in the right thigh (inoculated) was greater than that of left one. The uptake of ^{125}I-Aza in right thigh was significantly increased with time at one hour and 12 hour post-injection, as it was 5.5 and 7% per g, respectively.

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Table (8): Biodistribution of ^{125}I-azathioprine in solid tumor bearing mice

Organs and body fluids	% ^{125}I-Aza /gram organ time post-injection			
	15 minutes	1 hour	12 hour	24 hour
Blood	22.8 ± 1.8	14 ± 1.2*	7.5 ± 0.2*	4.6 ± 1.3*
Bone	1.2 ± 0.1	1.8 ± 0.1*	2.4 ± 0.1*	1 ± 0.02*
Liver	4.4 ± 0.27	3.7 ± 0.25*	2.17 ± 0.1*	1.6 ± 0.1*
Lung	5.5 ± 0.1	6.5 ± 0.2*	4.0 ± 0.2*	3.1 ± 0.3*
Heart	12 ± 0.8	3 ± 0.2*	2 ± 0.1*	2 ± 0.01
Stomach	11.1 ± 0.6	16.1 ± 0.8*	10.8 ± 0.5*	8.0 ± 0.6*
Intestine	2.9 ± 0.2	6.0 ± 0.5*	4 ± 0.22*	3.5 ± 0.15
Kidney	2.3 ± 0.9	7 ± 0.2*	4.6 ± 0.08*	2. ± 0.16*
Spleen	2 ± 0.1	3.0 ± 0.2*	2.3 ± 0.2*	1 ± 0.05*
Thyroid	2 ± 0.02	4 ± 0.22*	6.5 ± 0.4*	6.4 ± 0.3
Left leg	0.8 ± 0.05	0.9 ± 0.03*	1.1 ± 0.07*	0.5 ± 0.3*
Right leg	2.7 ± 0.2	5.5 ± 0.5*	7.0 ± 0.04*	7.3 ± 0.4

Values represent the mean ± SEM (n = 6)
*Significantly different from the initial value of each organ using unpaired student's t-test ($p < 0.05$)

Liver showed significant increase in % iodo-aza uptake at 15 minute, one hour and 12 hour post-injection, when compared to ascetic bearing animals. In addition, iodo-aza uptake in the stomach of solid tumor mice was significantly increased at 15 minute, one hour and 24 hour post-injection when compared to ascetic bearing mice.

In the present study, the increase in % of iodo-Aza in the blood of solid tumor bearing mice may be due to the large volume of ascetic fluid that are formed in ascetic bearing animals[7]. Significant increase in iodo-aza uptake in bone of ascites bearing mice may be due to high vascularities to ascetic fluid that may lead to destruction of blood cells. This may activate bone marrow and increase uptake of iodo-aza in the bone[24].

CONCLUSION

Incorporation of Auger emitters (^{125}I) to a tumor site was achieved by labeling of azathioprine with iodine-125. The appropriate conditions for labeling of Aza (94% yield) were 100 µg CAT as oxidizing agent, 100 µg Aza as substrate, at pH 7, temperature of 75°C and 30 minute reaction time. The great incorporation of ^{125}I-Aza in tumor sites (asites or solid tumor) facilitates tumor imaging. ^{125}I-azathioprine was found to be highly localized in tumor sites which considered an ideal victor to carry iodine-125 to the nucleus of tumor cells[25, 26]. In conclusion, this study demonstrates a hopeful approach for cancer remedy with a local cytotoxic activity[27].

REFERENCES

1- Unak, T. **Curr. Pharm**; 11: 1127-42 (2000).
2- Montgomery J. Drugs for neoplastic diseases. IN burger, A. (Ed.). Medicinal Chimistry. New York, Wiley-Interscience; 3: 680 (1970).
3- Kassis AI, Annick D, Vanden A, Patrick YC and Wen, Adelestein SJ. **Cancer Res**; 50: 5199-5203 (1990).
4- Desombre ER. **Cancer Res**; 52: 5752-5658 (1992).
5- Tujvajev JGJ. **Nucl. Med.**; 35: 1407-1417 (1994).
6- Hofer KG, Van Loon N, Schnederman MH and Charlton DE. **Radiat. Res.**; 130: 121-124 (1992).
7- Korde A, Venkatesh, M. and Sarma, HD. Int. Symposium on modern trends in radiopharmaceuticals for diagnosis and therapy, Lisbon, Portugal; IAEA, SM-355/13 (1998).
8- Krisch, RE. **Int. J. Radiat. Biol.**; 21: 167-189 (1972).
9- Sedelnikova OA, Rogakou EP, Panyutin IG and Bonner WM. **Radiat Res.**; 4: 486-492 (2002).
10- Bradley EW, Chan PC and Adiestein SJ. **Radiat. Res.**; 64: 555-563 (1975).
11- Roelvid TA, Horenblas S, Moonen LM, Te Velde A, Meinhard TW and Bartilink H. **Ned Tijdschr Geneskd**; 3: 171-2 (1997).
12- El-Ghany EA, Mahdy MA, Attallah K and Ghazy FS. **J. of Radioanalytical and Nuclear Chemistry**; 1: 156 - 169 (2002).
13- Kassis AI, Sastry KSR and Adelestein SJ **Rad. Res.**; 109: 78- 89 (1987).
14- El-Ghany EA, Mahdy MA, Attallah K and Ghazy FS. **Arab J. Nucl. Sci. Appl.**; 37(1): 1-13 (2004).
15- Wilson CO and Gisvold O. The Textbook of Organic and Pharmaceutical Chemistry. Ninth edition. Antineoplastic agents; 313-348 (1992).
16- Seevers RH and Counsell RE. **Chem. Rev.**; 82: 575-590 (1982).
17- Yamada A, Trabouisi A, Dittert LW and Hussain AA. **Anal. Biochem.**; 2: 232-5 (2000).
18- Rayudu GVS and Colombettil G. Radiotracer for Medical Applications, CRC series in Radiotracers, in Biology and Medicine; 3: 105-109 (1983).
19- Abu-Zeid M, Hori H, Nagasawa H, Uto Y and Inayama S. **Biol. Pharm. Bull.**; 2: 195-8 (2000).
20- Richard J, Flangan F, Peter Charlson, Eyvindl Synnes and Wiebe LI. **J. Nucl. Med.**; 27: 1165-1171 (1986).
21- Chan PC, Lisco E, Lisco H and Aldstein SG. **Radiat. Res.**; 67: 332-43 (1976).
22- Osman AM, Sayed Ahmed MM, Khayyal MT and Merzabani MM **Tumori.**; 79: 268. (1993).
23- Taiwei Chu, Shaowen HU, Bing Wei and Wang WI. **Letters.**; 14: 747-749 (2004).
24- Adliestein SJ, Kassis AL, Bodel L and Mariani G. **Radiopharm.**; 3: 301- 316(2003).
25- Dupertuis YM, Buchegger F and Balancea PC. **Eur. J. Nucl. Med.**; 29: 499-505 (2002).
26- Igorg Panyutin and Neumann RD. **Acta Oncologica.**; 7: 8227-823 (1996).
27- Raina R, Agarwal A, Goyal KK, Jackson C, Ulchaker J, Angermeier K, Klein E, Ciezki J and Zippe CD. **Urology**; 6: 1103-1108 (2003).

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التوزيع البيولوجى للأزاثيوبرين المرقم باليود المشع فى الفئران المصابة بأورام الإرليخ

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يعتبر الأزاثيوبرين من مضادات الإستقلاب الذى تم ترقيمه باليود ١٢٥ المشع. وتم استخدامه كعامل للمادة المشعة إلى داخل النواة مما يؤدى إلى كسر انفصال شريطى الحامض النووى DNA وإيقاف تخليقه. وتم فى هذا البحث الترقيم بنجاح باستخدام كلورامين-ت كعامل مؤكسد عند درجة حرارة ٧٥ مئوية لمدة ٣٠ دقيقة باستخدام محلول الفوسفات المنظم عند أس هيدروجينى رقم ٧(متعادل). تم اختبار المادة المرقمة باستخدام جهاز الفص الكهربائي وإتضح أن نسبة نقائها حوالى ٩٠ بالمائة. وقد تم حقن الفئران بخلايا إرليخ السرطانية إما في التجويف البريتوني أو في عضلة الساق (للحصول على ورم سائل أو صلب). وبعد ذلك تم دراسة التوزيع البيولوجى للمركب المرقم فى كل من الفئران السليمة غير المصابة وكذلك فى المصابة بالخلايا السرطانية السائلة أو الصلبة. وكانت نتيجة هذه الدراسة أن نسبة تلقى الورم في التجويف البريتونى للمادة المشعة كان أكثر من ٤٠% فى حين كان فى حدود ٢٠% في الأورام الصلبة ، وذلك بعد ١٢ ساعة من الحقن ويتضح من هذه الدراسة أن المادة المشعة يمكن تمركزها فى الخلايا السرطانية بتركيزات كافية لإمكانية استخدامها فى علاج وتصوير الخلايا السرطانية.