

Cell culture models: Time course of cellular accumulation of ^{99m}Tc -HMPAO

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Abstract. The accumulation of ^{99m}Tc -HMPAO was studied and compared at two different temperatures. This experiment shows besides temperature, time and efficiency of gama detector also give a significant affect to the result. Overall, the radioactivity rate is decrease as time goes in the supernatant samples, however the accumulation of ^{99m}Tc -HMPAO within the cells increases. In addition, in both the supernatant and the cell pellets, the values of the activity were lower at 4oC compared to 37oC showing they had a reduced ability to accumulate the ^{99m}Tc -HMPAO.

1. Introduction

^{99m}Tc -HMPAO (Tchnetium-labeled hexamethylpropyleneamineoxime) is a lipophilic radiotracer which diffuses freely through facilitated diffusion crosses brain barrier [1][2][3]. The blood-brain barrier (BBB) is an important role to localise radiopharmaceutical tracer within the brain. In order to enter the cells, ^{99m}Tc -HMPAO should be transported through the proteins channel in phospholipid bilayer [4]. When ^{99m}Tc -HMPAO went inside the brain tissue, it is converted to hydrophilic species which are unable to exit the brain[2]. Neirinckx et al. published that interaction with glutathione (GSH) caused intracellular conversion of lipophilic ^{99m}Tc -HMPAO into hydrophilic form[4]. Besides that, ^{99m}Tc -HMPAO also has six hours half-life [2][5]. However, several factors such as temperature and metabolism of the brain tissue affect the uptake of ^{99m}Tc -HMPAO. According to Mustafa et al (2007), the elevation of body temperature decreased cerebral blood flow significantly, hence reduction of ^{99m}Tc -HMPAO uptake [1][6]. Due to that, this experiment is conducted to measure how the difference temperature effects the accumulation of ^{99m}Tc -HMPAO within the cells.

2. Methods

6ml aliquots of cell suspension ($\sim 1 \times 10^6$ cells/mL) were transferred into 4 tubes. Two tubes were placed into lead pots and incubated in a water bath at 37°C while the other two tubes were incubated at 4°C. Those four tubes were incubated at that certain temperatures for approximately 15 minutes and stirred all those tubes occasionally. In this experiment, HMPAO kit reconstituted with $\sim 50\text{MBq}$ ^{99m}Tc -pertechnetate in 5ml saline was used in order to introduce the radiotracer. $\sim 1\text{MBq}$ (0.1ml) of ^{99m}Tc -HMPAO was added into the previous tubes (stock solution). After 1, 15, 30, 45, and 60 minutes the tubes were swirled to suspend the cells evenly. Moreover, 0.5ml aliquots from each tube and the duplicates were pipetted and transferred to a micro-centrifuge tubes that contained 0.5ml ice-cold saline. Those micro-centrifuge tubes was placed in a micro-centrifuge and spun at 12000g within 1 minute. After that, 0.1 ml of the supernatant from each tube was transferred into a gamma counting tube and



then placed into a gamma counter set for ^{99}Tc to do the measurement and then recorded. After centrifugation, supernatant was aspirated with a syringe and then the pellets (cells) were placed in the gamma counter and its radioactive counts were measured per ten second. Standards were prepared by adding 0.1ml of the $^{99\text{m}}\text{Tc}$ to a tube containing 6ml saline. This was done in triplicate of 0.1ml standard and was added to the gamma ready counter. Accumulation of $^{99\text{m}}\text{Tc}$ -HMPAO as a cellular radioactivity was expressed as charts. The supernatants measurements were expressed as percentage activity; the standard being halved due to dilution was considered as the 100% value.

3. Results

In this experiment we calculated the total counts expected in each 0.5mL aliquot, percentage of expected count, accumulation of ^{99}Tc and calculated the efficiency of the gama counter.

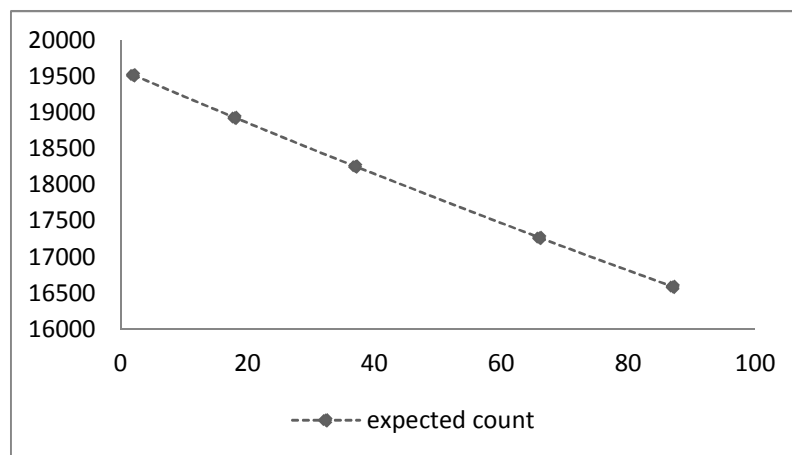


Figure 1. Expected counts of radioactivity in time profiles.

Figure 1 shows a negative linear decay as the result of interaction between total counts and times. Measuring the expected counts means measuring the radioactivity rate. In order to predict that measurement, the first equation below was used. The decay constant (λ) was found to be 0.0019158 by dividing $\ln 2$ with half-life of ^{99}Tc (equation 2). A_0 is the initial quantity of the substance that will decay, A is the quantity that still remains and has not yet decayed after a certain time (t), and t is the difference in time between the time when ^{99}Tc was introduced to the cell culture and the time when the sample (0.5 mL) was collected from the stock solution.

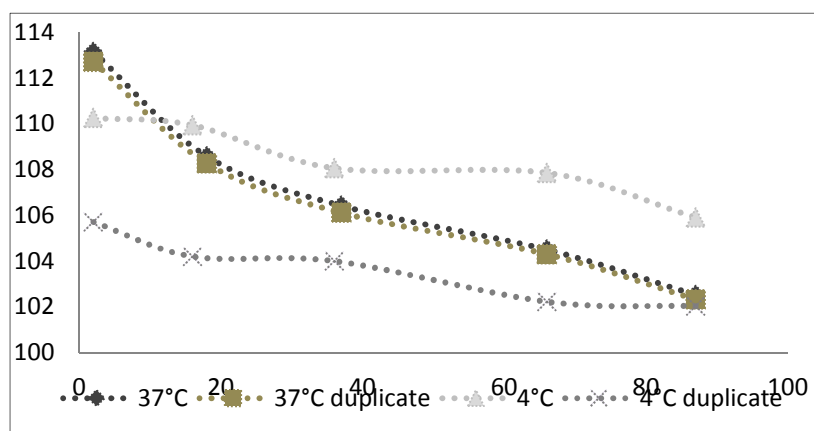


Figure 2. Percentage of expected counts against time profiles.

It shows the counts of the supernatant expressed as a percentage (added) expected counts against time profiles. However, there are several factors that will influence the results. The reason those values above 100% will be taken into the discussion part. Samples from stock solution with its duplicate which were incubated at 37°C shows a similar radioactivity rate, however there are a massive gap of radioactivity rate of sample (from 4°C) with its duplicate. In general, it shows a reduction value of radioactivity rate both in stock at 37°C and 40°C.

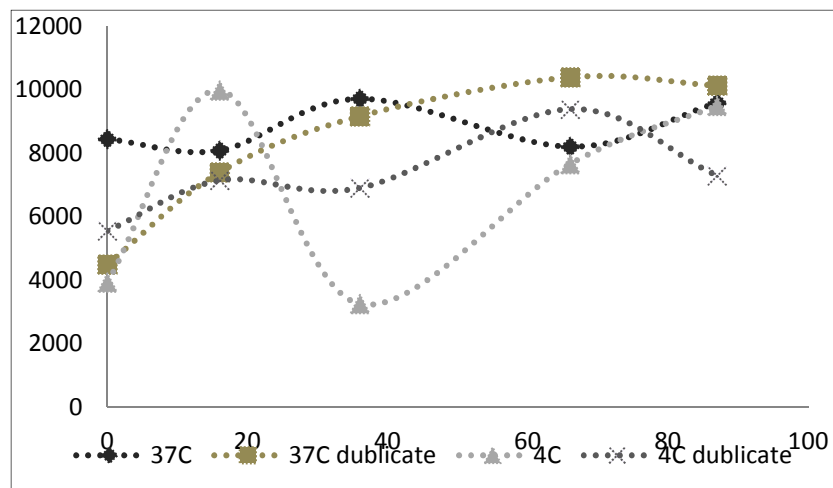


Figure 3. The accumulation of ^{99}Tc within cells which were incubated at 37°C and 4°C.

Overall, Figure 3 shows there were a steady increase in accumulation as time increases. However, the value of tracer within the cell at 4°C increased sharply from 3935.5 to 9987 after 16 minutes. Moreover, it reduced to be 3258 after 37 minutes. The higher temperatures tend to accumulate more of the tracer over a prolonged period of time. From the initial addition of the tracer to around the 1000s mark, is the sharpest rise. There is a steady increase after the 1000s mark from both temperatures. As these were an abnormal condition there were some issues that will be taken into account in the discussion.

Table 1. The value of standard, radioactivity, disintegrations in order to measure efficiency of gamma detector.

Average of Standard (k Bq/m)	Activity/6ml Saline (M Bq)	Activity/0.1ml Aliquot (M Bq)	Disintegrations/Minute	Counting Efficiency
235182	1	0.016667	1000020	23.518%

Table 1 shows the different values obtained prior to measure out the counting efficiency of gamma detector. It shows quite a low counting efficiency of 23% but potential reasons for this can be found in the discussion.

4. Discussion

Figures 1 shows the expected counts we should have. It shows a strong linear decrease as time goes. This experiment shows $^{99\text{m}}\text{Tc}$ -HMPAO accumulation in cells and it slightly increases as linear of time. The reason why the plateau of $^{99\text{m}}\text{Tc}$ -HMPAO comes up is due to diffusion of the tracers from supernatant into the cells. There are several factors that influenced the result of this experiment which are temperature and incubation time [7]. If the cells live in higher temperature than normal, it would give a several impact such as increase the metabolism system and change the permeability of the membrane cell, it makes easier for substrate intracellular transport cross the membrane cell. The protein channel for passive diffusion always active due to gradient temperature between inter and intracellular

environment. When those proteins opened, ^{99m}Tc -HMPAO transports inside the cells through those proteins which will increase the accumulation of this tracer within the cells [8].

On the other hand, low temperature gives the opposite impact. In order to keep the heat of the cells still equilibrium, when the intracellular temperature decrease, the volume of the cell reduces and the membrane cells becomes more rigid. It decreases permeability of membrane cells and only small amount of ^{99m}Tc -HMPAO will be transferred into the cells. Also with a lower temperature it means that the tracer has less kinetic energy of the tracer. However, from this results we can there is no significant different of ^{99m}Tc -HMPAO accumulation in the cell that were incubated at 37C and 4C. It causes of when we did the measurement with the gama counter, temperature of the sample slowly come back to room temperature, thus the condition of the cell recover to their normal condition. On the contrary, the values of expected counts were corrected to the standard value and halved due to the dilution factor, however all recorded counts were significantly higher. A lack of experimental dilution between sample (cell culture) and saline could give a contribution to the high values due to potential mishandling of the cell tubes. If the the sample and saline was not correctly mixed, this would essentially mean less of tracer would be accumulated into the cells. Figure 3 shows there are unreasonable results when the accumulation of ^{99}Tc increased sharply to 9987 and It reduced to be 3258. It is happening as a result of mishandling the samples. It increased due to there was supernatant left when the cells were read and it was reduced due to there were cells wasted when the supernatant was aspirated. In addition, low efficiency of the gama counter could be caused by the size of the gama tracer, and also the calibration.

5. Conclusion

In summary these experiments have shown that temperature gives a significant impact in the uptake of ^{99m}Tc -HMPAO within cells. It also shows that although the half-life for Tc is six hours, it needs a considerable amount of time to build up in cells. The result also showed how crucial management of sample such as the cells suspended in the supernatant cause a notatable effect to the result, especially in in vitro studies. In addition to that, calibration for the gama tracer also important to get high efficiency result.

References

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