Study of chemical kinetics on labeling of ^{99m}Tc-N-ethyl-N_2S_2-Memantine

CAO Guoxian[1,2,3,*] ZHOU Xingqin[1,2,3] LIU Yingtao[1,2,3]
KONG Yanyan[1,2,3] ZHANG Jiankang[1,2,3]

[1]*Key Laboratory of Nuclear Medicine, Ministry of Health, Wuxi 214063, China*
[2]*Key Laboratory of Molecular Nuclear Medicine of Jiangsu, Wuxi 214063, China*
[3]*Jiangsu Institute of Nuclear Medicine, Wuxi 214063, China*

Abstract In this work, a calculation method of chemical kinetics was established for labeling reaction of ^{99m}Tc-N-ethyl-N_2S_2-memantine, a potential NMDA receptor imaging agent prepared in our laboratory. Four groups of vials (3 vials per group) were added with 0.02 mL (1 mg/mL) N-ethyl-N_2S_2- Memantine, 0.08 mL (40 mg/mL) GH, 0.05 mL (10 mg/mL) EDTA-2Na, 0.035 mL (2 mg/mL) SnF_2, 0.8 mL phosphate buffer(1 mol/L, pH 6.5) and 37 MBq $Na^{99m}TcO_4$. The vials were incubated at 70°C, 80°C, 90°C or 100°C. Samples were taken with capillary from the vials at 2, 5, 10, 20, 30, 40 and 60 min. Labeling yields were determined by TLC. Order of reaction n, rate constant k, activation energy E_a and half life $t_{1/2}$ of labeling reaction were calculated with the kinetics software we compiled. Mean labeling yields of ^{99m} Tc-N-ethyl-N_2S_2-memantine at 2, 5, 10, 20, 30, 40 and 60 min were (1) 13.5, 15.7, 34.0, 64.8, 81.9, 91.4 and 95.4 at 70°C; (2) 13.2, 20.5, 40.1, 70.0, 88.2, 94.5 and 95.6 at 80°C; (3) 15.6, 22.9, 43.7, 74.3, 87.2, 93.4 and 96.1 at 90°C; and (4) 20.5, 25.8, 45.3, 81.1, 92.2, 95.6 and 96.0 at 100°C. The other parameters were; n =1; k=0.053, 0.061, 0.063 and 0.076 L/min at 70°C, 80°C, 90°C and 100°C, respectively; E_a=12.38 kJ/L; $t_{1/2}$=13.11, 11.45, 11.05 and 9.07 min at 70°C, 80°C, 90°C and 100°C, respectively. The mean labeling yield increased with temperature and time, optimized at 100°C and 40–60 min. The concentration of ^{99m}Tc-N-ethyl-N_2S_2-Memantine was larger than that of $Na^{99m}TcO_4$, so n=1. The k increased with reaction, hence the accelerated reaction rate at higher temperatures. The labeling reaction was not so difficult because of the low E_a. The $t_{1/2}$ decreased with increasing reaction temperature, hence the acceleration of labeling reaction.

Key words ^{99m}Tc-N-ethyl-N_2S_2-Memantine, NMDA receptor, Labelling, Chemical kinetics

1 Introduction

At the present time, nervous and mental diseases have been ranked the first one among total social burdens due to them causing a serious impact on human health and social development. It is very important to study the relationship between N-methy-D-aspatrate receptor (NMDAR) and the diseases. As a major receptor of ligand-gating ionotropic glutamate receptors, NMDAR is dual regulated by ligand and membrane potential. NMDAR has more than one special binding sites, such as H^+, Zn^{2+}, Mg^{2+}, non-competitive antagonist, glutamic acid, glycine, polyamine, redox sites, etc and can also incur modifications such as phosphorylation[1]. Calcium conductance is one of characteristics of NMDAR, and also the cause of close relation between NMDAR and Glu excitotoxicity, long-term potentiation (LTP) effect or the formation of learning memory. NMDAR is a main regulator of the synaptic plasticity and LTP of cerebral cortex and hippocampus[2]. The sustained depolarization of excitatory neurons resulted from NMDAR's over-activation may lead to Ca^{2+} influx, and intracellular calcium overload cause cell death. This may play an important role during the development of

Supported by the Jiangsu Natural Science Foundation (BK2008111, BK2010157) and the National Natural Science Foundation of China (30770602)
* Corresponding author. *E-mail address:* jswxcgx@sina.com
Received date: 2011-07-18

the neurological diseases such as Parkinson's disease, Alzheimer's disease, Huntington's, pain, stroke and the mental illnesses such as schizophrenia, bipolar disorder[3,4]. With irreplaceable advantages to other research methods, brain receptor imaging of nuclear medicine is today's hot research and topics. As to detect the variance in distribution, the number (density) and function (affinity) of NMDAR in specific regions of nervous system, NMDAR imaging can show the physiological and pathological states of brain at the molecular level, make early diagnosis of schizophrenia, observe the disease progress, guide the rational use of drugs and fulfill the efficacy evaluation and prognosis. Therefore, NMDAR imaging has important theoretical and practical value.

We used NMDAR antagonist memantine as the lead compound to design and synthesize a new NMDAR ligand 1-N-[N-[2-(S-thioethyl)]-N-[2-[N-[2-(S-thioethyl) amino]ethyl] aminoethyl] amino-3,5-dimethyladamantane(N-ethyl-N_2S_2-Memantine), which was connected a chelating group S_2N_2 and remained active groups of memantine[5]. ^{99m}Tc-N-ethyl-N_2S_2-Memantine was obtained by ligand exchange method labeled with ^{99m}Tc under the action of SnF_2. During the research process of labeling, condition of reactions were optimized by the study of chemical kinetics.

The order of reaction n, rate constant k, activation energy Ea and reaction half life $t_{1/2}$ of labeling reaction were calculated with chemical kinetics software compiled by ourselves according to theory of chemical kinetics[6], and the results were used to quantitatively study the labeling reaction and decide the optimal condition of reaction.

2 Materials and methods

2.1 Materials

The N-ethyl-N_2S_2-Memantine was synthesized in our lab. Sodium Glucoheptonate (GH) was provided by Jiangyuan Pharmaceutical Factory. SnF_2, EDTA-2Na, Na_2HPO_4, KH_2PO_4 and other reagents were of analytical grade. Polyamide thin-film was from Taizhou Luqiao Sijia Biochemical Plastic Factory, China.

2.2 Instruments

Wizard 1470 γ-automatic counting device (the United States Perkin Elmer Instrument, Inc), pH Ø72 meter (Beckman, California, USA), AC2105-type analytical balance (Sartorius Company, Germany), ^{99}Mo-^{99m}Tc generator (HTA CO., LTD. Beijing, China) and Thermostat water bath (Jintan honghua instrument Co., Jintan, China) were used in experiments.

2.3 Chemical kinetics

To prepare ^{99m}Tc-N-ethyl-N_2S_2-Memantine, 0.02 mL (1 mg/mL) N-ethyl-N_2S_2-Memantine, 0.08 mL (40 mg/mL) GH, 0.05 mL (10 mg/mL) EDTA-2Na, 0.035 mL (2 mg/mL) SnF_2, 0.8 mL phosphate buffer(1 mol/L, pH 6.5) and 37 MBq Na^{99m}TcO$_4$ were added into 4 groups of vials(3 vials per group), respectively. These 4 groups of vials were incubated at 70, 80, 90 and 100°C, respectively. Samples were taken with capillary from vials at 2, 5, 10, 20, 30, 40 and 60 min, respectively. Labeling yields were determined by thin-layer chromatography (TLC). Polyamide thin-film was used as stationary phase and mobile phase solution was methanol:ethylether:ammonia = 2:5:0.25 (*V*/*V*). Films were dried and cut into 10 sections evenly, then counted with γ-counter. The n, k, E_a and $t_{1/2}$ of labeling reaction were calculated with chemical kinetics software we compiled.

2.4 Calculation methods

According to theory of chemical kinetics[6], we compiled a software named CHEMKIN to calculate chemical kinetics parameters of labeling reaction of ^{99m}Tc-N-ethyl-N_2S_2-Memantine.

2.4.1 Order of reaction

Rate constants k corresponding to each kind of order of reaction(n=0,1,2,3) were calculated at first by using experimental data of chemical kinetics at different temperature and different time according to formulas listed below[6], and order of reaction n was finally confirmed by minimum variance of rate constants k corresponding to n.

first-order reaction (n=1, A→product):$\ln[a/(a-x)] = k_1 t$

second-order reaction (n=2, A+B→product, $a = b$) : $1/(a-x) - 1/a = k_2 t$

second-order reaction (n=2, A+B→product, $a \neq b$) :$[1/(a-b)] \ln\{b(a-x)/[a(b-x)]\} = k_2 t$

third-order reaction (n=3, A+B+C→product, a=b=c) : $[1/(a-x)^2 - 1/a^2]/2 = k_3t$

zero-order reaction (n=0): $x = k_0t$ (1)

where a is initial concentration of the reactant A, b is initial concentration of the reactant B, c is initial concentration of the reactant C, and x is the concentration of product at time t.

2.4.2 Rate constant

Rate constants k at different temperatures was obtained at the same time when order of reaction n was calculated with above formulas.

2.4.3 Activation energy

Activation energy of reaction E_a was evaluated according to the modified Arrhenius' equation[6]:

$$\ln(k_2/k_1) = (E_a/R)\ (1/T_1 - 1/T_2) \quad (2)$$

where k is the rate constant, T (in K) is temperature and R=8.314 J·mol^{-1}·K^{-1} is Rydberg constant.

2.4.4 Reaction half life

Reaction half life $t_{1/2}$ was calculated with the following equations[6]:

first-order reactions (n=1, A → product) : $t_{1/2} = \ln 2/k_1$

second-order reactions (n=2, A+B→product, a=b) : $t_{1/2}=1/(k_2a)$

second-order reactions (n=2, A+B→product, $a \neq b$) : $t_{1/2}(A)=\{\ln[b/(2b-a)]\}/[k_2(a-b)]$, $t_{1/2}(B)=\{\ln[(2a-b)/b]\}/[k_2(a-b)]$

third-order reactions (n=3, A+B+C→ product, a=b=c) : $t_{1/2}= 3/(2k_3a^2)$

zero-order reactions (n=0): $t_{1/2}= a/(2k_0)$ (3)

3 Results

3.1 Experimental data of chemical kinetics

Table 1 shows labeling yields(%) of $^{99}Tc^m$-N-ethyl-N_2S_2-memantine at 70°C–100°C and 2–60 min.

Table 1 Labeling yields(%) of $^{99}Tc^m$-N-ethyl-N_2S_2-memantine (x±SD, n=3)

Time / min	70°C	80°C	90°C	100°C
2	13.5±0.50	13.2±0.32	15.6±0.22	20.5±0.13
5	15.7±0.62	20.5±0.50	22.9±0.24	25.8±0.15
10	34.0±0.85	40.1±0.72	43.7±0.36	45.3±0.27
20	64.8±0.60	70.0±0.90	74.3±0.43	81.1±0.30
30	81.9±0.77	88.2±0.83	87.2±0.78	92.2±1.05
40	91.4±1.00	94.5±0.78	93.4±0.69	95.6±0.97
60	95.4±1.12	95.6±0.95	96.1±0.94	96.0±1.03

3.2 Order of reaction

The rate constants k corresponding to the first, second, third and zero order were evaluated according to Eq.(1) by using experimental data listed in Table 1. The order of reaction was automatically confirmed by our software with the method of minimum variance of rate constants, and result showed that n was 1.

3.3 Rate constant

The rate constants k at different temperatures and minutes are listed in Table 2.

Table 2 Rate constants k (min^{-1}) at different temperature and different time

Temperature / °C	2 min	5 min	10 min	20 min	30 min	40 min	60 min	Mean
70	0.073	0.034	0.042	0.052	0.057	0.061	0.051	0.053
80	0.071	0.046	0.051	0.060	0.071	0.073	0.052	0.061
90	0.085	0.052	0.057	0.068	0.069	0.068	0.040	0.063
100	0.115	0.060	0.060	0.083	0.085	0.078	0.054	0.076

3.4 Activation energy

Activation energy E_a was calculated according to formula (2), and the results were 11.87, 10.05, 11.60 and 16.01 kJ/mol at temperature 70°C, 80°C, 90°C and 100°C, respectively, and mean activation energy was 12.38 kJ/mol.

3.5 Reaction half life

The results of reaction half life $t_{1/2}$ calculated with formulas (3) were 13.11, 11.45, 11.05 and 9.07 min at temperature 70°C, 80°C, 90°C and 100°C, respectively.

4 Discussion

Labeling reaction is a very important step in the process of radiopharmaceuticals preparation. For a long time, people often paid more attention to studying experiment of the reaction than studying theory of the reaction, as well as paid more attention to studying chemical thermodynamics of the reaction than studying chemical kinetics of the reaction. For instance, we often care whether or not a chemical or biological molecule could be labeled with radionuclide? What extent (i.e. labeling yield) the reaction could reach to? All these questions only belong to category of chemical thermodynamics. But activation energy, rate constant and reaction half life of labeling reaction, which belong to category of chemical kinetics, have rarely been studied. When a molecule cannot be labeled with radionuclide at a certain condition, we often experientially rise reaction temperature or use ligand exchange labeling to try to solve the problem, but did not comprehend the real reason. Although some of labeling reactions could be carried out on the view of chemical thermodynamics, experimental data did not show the fact. It could be explained by chemical kinetics that the rate was so slow that the labeling reaction could not be observed taking place. In fact, the optimal condition of labeling reaction could be rapidly ascertained by analysis of influence factors (such as reaction temperature) with chemical kinetics. When some molecules cannot be directly labeled with radionuclide, on the other hand, the method of ligand exchange labeling was often used. Its hypostasis actually is to reduce activation energy E_a so as to accelerate labeling reaction, and E_a can be easily evaluated by chemical kinetics. Sometimes, ligand exchange labeling might be abused while those molecules, in fact, could be directly labeled. The aftereffect was that impurity was mistakenly introduced into the reaction system and, worse and worse, the chelate of exchange ligand might be falsely considered as product. The problem can be solved by comparing both activation energies adding labeled molecule or not into the system of ligand exchange labeling. If both activation energies were obviously different, ligand exchange labeling would just be necessary. Therefore, it can get twice the result with half the effort to apply chemical kinetics studying labeling reaction.

At first, we used the method of direct labeling to label N-ethyl-N_2S_2-Memantine with ^{99m}Tc at room temperature, and the result showed that N-ethyl-N_2S_2-Memantine did not be labeled by ^{99m}Tc at all. Then, we changed reaction temperature to 100°C or added GH at room temperature respectively, but results were also unsuccessful. At last, labeling reaction was performed by adding GH and reacting at 100°C, and samples were took with capillary from vials at different time to inspect progress of the reaction by TLC. It was found that labeling yield increase with reaction time. The result illumed us to study labeling reaction with chemical kinetics.

Labeling yields of $^{99}Tc^m$-N-ethyl-N_2S_2-memantine at different temperatures and different minutes were listed in Table 1. In addition to labeling yield increasing with reaction time prolonging, labeling yields at the same time were also increasing with reaction temperature rising. According to data of Table 1, optimal reaction time was finally chose as 40−60 min when labeling yields were all larger than 90% at temperature 70°C, 80°C, 90°C or 100°C. Due to favorable thermal stability of N-ethyl-N_2S_2-memantine and for convenient in experiment operation, the optimal reaction temperature was finally chose as 100°C when labeling yields were larger than 95% within 40−60 min. By the way, the selection of reaction temperature is very important especially for that bad thermal stability of molecule, so the study of chemical kinetics is authentically necessary.

The order of reaction n was 1 decided by calculation. Properly, the n ought to be called apparent order of reaction. Because the concentration of ^{99m}Tc-N-ethyl-N_2S_2-Memantine was very larger than that of $Na^{99m}TcO_4$, the concentration of ^{99m}Tc-N-ethyl-N_2S_2-Memantine was nearly unaltered during the process of labeling. Then, n=1 implied that the labeling reaction was actually a second-order reaction, i.e. one molecule of N-ethyl-N_2S_2-memantine chelated one ion of ^{99m}Tc.

Rate constants k at different temperatures and minutes are listed in Table 2. The k value increased with reaction temperature, which means that rise of temperature is in favor of accelerating reaction rate.

Alinear relation was found between logarithm of mean rate constant (ln$k$) and reciprocal of reaction temperature (1/T): ln$k$=–1442/T + 1.2054, R^2=0.9342. The result accorded with the Arrhenius formula that ln$k$ linearly related to 1/T[6].

Magnitude of activation energy Ea decided the difficult degree of reaction. The higher E_a was, the more difficulty reaction carried out. We used ligand exchange labeling to decrease E_a and rose reaction temperature to provide energy. The calculated mean activation energy was 12.38 kJ/mol. This indicated that labeling reaction was not so difficulty on the selected reaction condition. Therefore, high labeling yields were obtained.

Reaction half life $t_{1/2}$ expressed the time when the main reactant had been consumed to half of incipient quantity. Evaluated $t_{1/2}$ were 13.11, 11.45, 11.0 and 9.07 min at 70°C, 80°C, 90°C and 100°C, respectively, indicating accelerated labeling reaction at higher temperatures.

5 Conclusion

Labeling of radiopharmaceuticals usually adopts some experiential methods, especially labeling with ^{99m}Tc. In general, these labeling reactions are fast and efficient. Therefore, chemical kinetics is hardly to be used to study these reactions. But labeling of some radiopharmaceuticals especially those new compounds which characters remain unclear, often due to reason of chemical kinetics, it will be mistakenly considered that they cannot be labeled with radionuclide. Furthermore, it is frequent that labeling yields of some radiopharmaceuticals cannot be increased to a perfect level such as >90% or labeling yields are precarious, sometimes high and sometimes low. Although other influencing factors maybe exist, the effect of chemical kinetics ought to be a very important factor. N-ethyl-N_2S_2-Memantine is a new compound, which needs farther researches. Although N_2S_2 as a chelate group has been applied in some radiopharmaceuticals, labeling reaction of N-ethyl-N_2S_2-Memantine with ^{99m}Tc is different from those radiopharmaceuticals because molecular structures are different to each other. The chemical kinetics study demonstrates the hypostasis of labeling reaction of N-ethyl-N_2S_2-memantine, which is a guide of an experiment. This method can be applied to study labeling reactions of other radiopharmaceuticals.

References

1 Paoletti P, Neyton J. Curr Opin Pharmacol, 2007, **7:** 39−47.

2 Liu Y T, Wong T P, Aarts M, *et al.* J Neuroscience, 2007, **27:** 2846−2857.

3 Lau C G, Zukin R S. Nat Rev Neurosci, 2007, **8:** 413−426.

4 Labrie V, Lipina T, Roder J C. Psychopharmacology, 2008, **200:** 217−230.

5 Liu Y T, Zhou X Q, Cao G X, *et al.* Nucl Sci Teth, 2010, **21:** 171−176.

6 Fu X C, Shen W X, Yao T Y. Physical chemistry. Fourth edition, Beijing: Higher Education Press, 1990, 700−755.