A study of ^{131}iodine labeling of indomethacin, its in vivo biological distribution in Lewis-bearing lung cancer, and its induction of apoptosis in lung cancer

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ABSTRACT

الأهداف: دراسة التركيب الكيميائي لمركب يود الاندوميثاسين هيستامين (Histamine–indomethacin) وكيفية انتشاره في أنسجة الفئران من فصيلة لويس، بالإضافة إلى دراسة مدى تأثير هذا المركب على كبت نمو الخلايا السرطانية في الرئة وتحفيز عملية الموت الخلوي.

الطريقة: أُجريت هذه الدراسة في مركز الأبحاث بمستشفى شينغ جينغ التابع لجامعة الصين الطبية، شينيانغ، الصين وذلك خلال الفترة من ديسمبر 2008م إلى أكتوبر 2009م، وشملت هذه الدراسة 95 فأراً من فصيلة لويس (C57) وتم تقسيمها إلى 12 مجموعة. لقد قمنا بالتصنيع الكيميائي لمركب الاندوميثاسين هيستامين في المختبر، ومن ثم أجرينا مجموعة من الاختبارات، وتبع ذلك اكتشافنا لكيفية الانتشار الحيوي لمركب يود الاندوميثاسين هيستامين في الخلايا السرطانية. لقد قمنا بتقييم مدى تأثير الاندوميثاسين و مركب يود الاندوميثاسين هيستامين على الخلايا السرطانية وذلك من خلال الاختبارات التالية: اختبارات كبت نمو الخلايا السرطانية، ومقياس التدفق الخلوي، وتحليل معامل تنخر الأورام.

النتائج: أشارت النتائج إلى أن نسبة النوكليد المشع وهو يود الاندوميثاسين هيستامين قد زادت كثيراً مع مرور الوقت في مناطق الخلايا السرطانية والمناطق المحيطة، كما أن وقت تثبيت هذا المركب كان أطول في الخلايا السرطانية بالمقارنة مع الخلايا السليمة. ولقد كان للجرعات المختلفة من مركب يود الاندوميثاسين هيستامين (3.0 ملغ/كلغ و3.5 ملغ/كلغ) وما يعادلها من جرعات الاندوميثاسين تأثيراً واضحاً على كبت نمو الخلايا السرطانية وتحفيز الموت الخلوي، غير أن جرعة 3.5 ملغ/كلغ من يود الاندوميثاسين هيستامين كان لها التأثير الأكبر من الناحية الإحصائية مقارنة بالمجموعات الأخرى.

خاتمة: أثبتت هذه الدراسة أن مركب يود الاندوميثاسين هيستامين لا يقوم فقط بتثبيت خاصية الاندوميثاسين المضادة للورم السرطاني، ويعمل هذين المركبين معاً من أجل تفعيل عمليتي كبت نمو الخلايا السرطانية وتحفيز الموت الخلوي.

Objectives: To study the synthesis of ^{131}iodine (I) labeled histamine-indomethacin (His-IN), its in vivo distribution in Lewis-bearing mice, and its effects on suppression of Lewis lung cancer growth and induction of apoptosis.

Methods: The present study was carried out in the Experimental Research Center, Sheng Jing Hospital of China Medical University Hospital, Shenyang China between December 2008 and October 2009. Chemical synthesis of His-IN was carried out. Ninety-five C57 mice were allocated into 12 groups, and a series of experiments including the in vivo biological distribution of ^{131}I-His-IN in C57 mice bearing Lewis lung cancer was explored, and the therapeutic effects of IN and ^{131}I-His-IN in lung cancer-bearing mice were assessed through tumor suppression experiments, flow cytometry, and detection of tumor necrosis factor.

Results: The ^{131}I-His-IN radionuclide count ratio of the tumor site and surrounding region significantly increased with time, namely, the retention time of ^{131}I-His-IN radionuclide was longer in the tumor site. A 3.0 mg/kg and 3.5 mg/kg ^{131}I-His-IN, as well as 3.0 mg/kg and 3.5 mg/kg IN all had tumor suppression and apoptosis induction effects on tumors, among which the 3.5 mg/kg ^{131}I-His-IN group had significant differences compared with all other groups.

Conclusion: The ^{131}I-His-IN not only retains the tumor-affinity property of IN, the synergistic effect of these 2 also enhances the tumor suppression and pro-apoptotic function.

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Lung cancer is one of the most common malignant tumors. For nearly 30 years, the mortality rate and incidence pose a rising tendency in the world, and traditional chemotherapy and radiotherapy do not bring ideal effect.[1] Therefore, seeking a relatively cheap, safe, and effective drug has become a hotspot in the study of lung cancer treatment. As a traditional non-steroid anti-inflammatory drug (NSAIDs) with anti-rheumatic, antipyretic, and analgesic effects, indomethacin (IN) has been proven by many studies to be effective in preventing colon cancer, stomach cancer, lung cancer, throat cancer, leukemia, and other malignant cancers.[2-7] It was reported that IN has a relative strong affinity to tumor tissue and can be accumulated at tumor tissue selectively.[8] The amount of IN in the tumor tissue is relative more than those in other tissues with a longer retention time.[8,9] Furthermore, it reveals obvious effect of tumor suppression. In recent years, seed implantation therapy has been a hotspot in tumor radiation therapy. Radioactive (^{131}I) shows that with ideal nuclear physical characteristics and low cost can be labeled on IN so that ^{131}I can be accumulated at tumor tissue with IN through the high tumor affinity effect of IN to synergize anti-tumor effects of both molecules. Our study investigated the synthesis of histamine-indomethacin (^{131}I-His-IN), in vivo biological distribution and its anti-tumor effect through induction of mice lung cancer apoptosis.

Methods. ***Animal.*** The present study was carried out in the Experimental Research Center, Sheng Jing Hospital of China Medical University Hospital, Shenyang, China between December 2008 and October 2009. The study was approved by the Experimental Animal Committee of Sheng Jing Hospital of China Medical University Hospital. Mice Lewis lung cancer cells were obtained from the Chinese Academy of Medical Sciences, Institute of Materia Medica, Beijing, China. Ninety-five C57 male mice (age from 6-8 weeks, weight: 20±2 g) were purchased from the Experimental Animal Center, Sheng Jing Hospital of China Medical University. The IN was obtained from Shanxi Yupeng Pharma Co. with 98.5% purity. The His-IN was synthesized by Shenyang Pharmaceutical University with a purity of >95%. Dimethyl sulfoxide was purchased from Sigma-Aldrich Co (St. Louis, MO, USA).

Main reagents and instruments. The IN from Shanxi Yunpeng Pharmaceutical Company with a purity of 98.5%, and other organic reagents are from domestic company with analytical purity. The ^{131}I was provided by Chendu Zhonghe Gaotong Corp. Ltd., Chengdu, China, and the GF254 thin-layer silica gel plate was by Jiangsu Hanbang Tech. Ltd., Nanjing, China, the C431200-P-screen scanning imaging system was from PerkinElmer Inc., Waltham, USA, radioactive thin-layer chromatography (TLC) scanner by Bioscan Inc., Washington DC, USA, and the SN-695 automatic y counter instrument by Shanghai Rihuang Guangdian instruments, Shanghai, China. The tumor necrosis factor (TNF)-α radioimmunoassay kit was purchased from Beijing Kemeikongya Biotech Co. Ltd., Beijing, China, and the SE Facsvantage flow cytometry (FCM) was purchased from Becton Dickinson, Franklin Lakes, NJ, USA.

Synthesis of His-IN. The mixture of IN and dichlorosulfoxide (obtaining a concentration of 200 mmol/l) were heated and refluxed for one hour. Then, acetone was added after the vacuum concentration to obtain IN chloride solution of acetone spare. Alternatively, histamine dihydrochloride was added into the acetone and stirred with ice bath. Then, anhydrous potassium carbonate was added and stirred for one hour at 5°C. At a temperature no higher than 10°C, the IN chloride solution was dripped into the above solution within 60 minutes, and was stirred for one hour after the temperature reached room temperature, keeping reflux for 3 hours. Then vacuum concentration was conducted to get rid of the acetone. Chloroform was applied for repeated extraction, drying, and re-crystallization to obtain the final product (the synthesis route is shown in **Figure 1**)

^{131}I labeling on His-IN. 1) Using chloramine-T method to label. A 10 µL His-IN derivative dissolved in anhydrous ethanol, 6 µL Na ^{131}I (11-22 Mbq) and 10 µL chloramine-T (1-5 mg/ml, 50 mmol/L phosphate buffered saline (PBS [pH 7.3-7.6]) were added into a label tube and vortexed for 1-10 minutes at room temperature. Then 100 µL $Na_2S_2O_3$ (5-10 mg/ml, 50 mmol/L PBS [pH; 7.3-7.6]) was added to stop the reaction. Complete control group was a mixture of 6 µL Na ^{131}I (11-22 Mbq), 10 µL chloramine-T (1-5 mg/ml, 50 mmol/L PBS [pH; 7.3-7.6) with 1-10 minutes vortex at room temperature, and addition of 100 µL

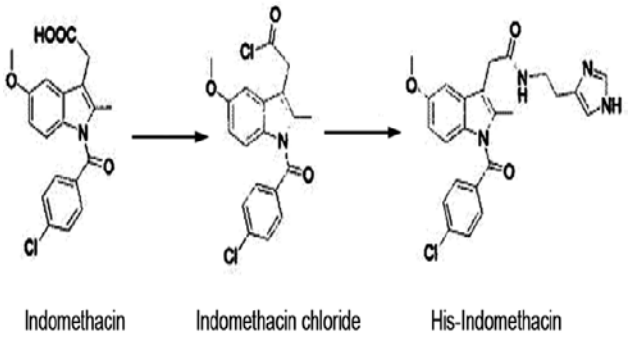

Figure 1 - Synthesis of histamine (His)-indomethacin.

sodium thiosulfate (5-10 mg/ml, 50 mmol/L PBS [pH 7.3-7.6]) for stopping the reaction.[9]

In vivo distribution of ^{131}I-His-IN in Lewis-bearing mice. Lewis-bearing lung cancer mice was executed when the tumor size reaches one cm in diameter, and 5 g of tumor tissue was taken for grounding, and diluted into the cell suspension by adding 15 ml physiological saline. Thirty-five C57 mice were inoculated at hypodermic axillary skin of right forelimb with 0.2 ml cell suspension containing 1x10^6 cells each. When the tumor reaches a maximum of one cm in diameter after inoculation, 35 Lewis-bearing C57 mice were taken and divided into 7 groups with 5 mice in each group. Within 3 days before inoculation, each mouse was injected intravenously with 2 ml 0.1% potassium iodide at the tail in order to block the thyroid gland each day. Three days after, ^{131}I-His-IN was injected intravenously at the tail of the mice at a dosage of 185 Kbq each, and after 2, 4, 8, 12, 16, 24, and 48 hours, eye blood samples were collected before killing the mice by breakneck. Then the heart, liver, spleen, lung, kidney, stomach, thyroid, and tumor tissues were taken, washed by physiological saline, and dried by filter paper. Those samples were weighed and radioactive counting was conducted through the R-well type counter instrument. The percentage of radioactive counts per gram of the tissue among total counts (%ID) and ratio of tumor tissues and other tissues were calculated.[10]

Preparation of Lewis-bearing lung cancer model and tumor suppression test. The preparation of tumor mice model is the same as in vivo distribution experiment. When the tumor size reaches one cm in diameter, 60 mice were randomly grouped into 5 groups including physiological saline control group, IN group (3.0 mg/kg, 3.5 mg/kg) and ^{131}I- His-IN group (3.0 mg/kg, 3.5 mg/kg). Intragastric infusion method was applied for administration every other day, and continuous observation lasted for 10 days.

Tumor suppression test. Tumor long diameter (a) and short diameter (b) were measured by Vernier caliper each time before administration and tumor volume will be calculated based on the equation: $V=1/2ab^2$. One ml blood of each mouse was taken before and after treatment, and serum was placed in -20°C freezer after centrifugation. The second day after the end of a 10-day intragastric infusion treatment, the mice were killed by breakneck, and the whole tumor tissue were taken for weighing. Tumor suppression rate was calculated according to volume method for each group. The equation is as follows: tumor suppression rate (%)=(1-average volume of experimented group/average volume of control group)×100%

Apoptosis rate by flow cytometry (FCM). Mice were killed and tumor tissues were taken and washed by physiological saline for washing. Tissues were grounded with 2 ml physiological saline on 200 mesh stainless steel strainer to obtain a single cell suspension with cell density of 5×10^5-1×10^6/ml. One ml cell suspension was taken for centrifugation at 335 g, and 4°C for 10 minutes. The supernatant was discarded, and one ml cold PBS buffer was added (repeated for 2 times). Cells were resuspended in 200 µl binding buffer and 10 µl annexin V-fluorescein isothiocyanate (FITC), and 10 µl propidium iodide were added to mix gently for reaction avoiding light for 15 minutes. Finally, 300 µl binding buffer was added for detection within one hour.

Detection of TNF-a level by radioimmunoassay. Serum was taken from -20°C freezer and was added into the test tubes with standard form ^{125}I-tumor necrosis factor (TNF)-α radioimmunoassay kit separately. Labeled markers and antibodies were added into tubes based on the procedure from the kit and mixed thoroughly, and then placed in 4°C refrigerator for 24 hours, and afterwards, separating agents were added and placed for 15 minutes at room temperature after thorough mixing. Two tubes were randomly taken for measuring total radioactivity (T), and all tubes were centrifuged at 1100 g for 20 minutes. The supernatant were discarded and radioactive counting were conducted for each tube. Standard curve was made on logit-Log paper and content of each sample would be identified based on sample B/B_0% from standard curve.

Statistical methods. The Statistical Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software was used for statistical analysis of the results, and measurement data were presented as mean ± standard deviation. Comparison of multiple means was carried out by one-way ANOVA using the least significant difference method, and a $p<0.05$ was set as the criteria for statistically significant difference.

Results.

Synthesis of His-IN for labeling. The synthesized His-IN was light yellow crystal. The mass spectrum (MS) data are mass-to-charge ratio (m/e): 451(compound spectrum molecular weight [M$^+$]). The magnetic resonance hydrogen spectrum (^{1}H-NMR) (ppm) were: ^{s}11.7 (chemical displacement value) (17 H); 8.07 (12 H); 7.60-7.70 (2', 3', 5', 6'[hydrogen ions position); 7.47 (16 H); 7.10 (4 H, J=2.3 Hz); 6.90 (7 H, J=8.9 Hz [coupling constant]); 6.71 (15 H); 6.68 (6 H, J=8.9 Hz, J=2.4 Hz); 3.74 (5-OCH_3); 3.48 (11 H); 3.20 (13 H); 2.60 (14 H); 2.20 (10 H).

The ^{131}I labeled His-IN. The best optimal conditions for labeling of His-IN through chloramine-T method are as follows: 10 µL His-IN in anhydrous ethanol (2 mg/ml), 6 µL Na^{131}I (11 Mbq) and 10 µL chloramine-T (1mg/ml, 50 mmol/L PBS, pH 7.3) were mixed and

placed for reaction for one minute, and then 100 µL sodium thiosulphate (5 mg/ml, 50 mmol/L PBS, pH 7.3) was added to stop the reaction. The labeling rate would decrease if the chloramine-T concentration increases. The prolonged reaction time cannot enhance the labeling rate. The average labeling rate reaches 52% according to the above procedure. The rate of flow (Rf) of ^{131}I-His-IN is 0.6-0.7 and the Rf of free ^{131}I is 0-0.1 under the chromatography method of GF254 silica gel as supporting material and chloroform: methanol 8.5:1.5(V/V) as developing agent (**Figures 2 & 3**). Products were extracted for 3 times by 4 ml ethyl acetate and distilled water (V/V=2/2), and organic phases were collected and dried for final product whose radiochemical purity reaches over 95%. The final crystal products were placed for one day and the radiochemical purities was 93.5%, for 3 days - 90.2%, and 7 days - 89.2%.

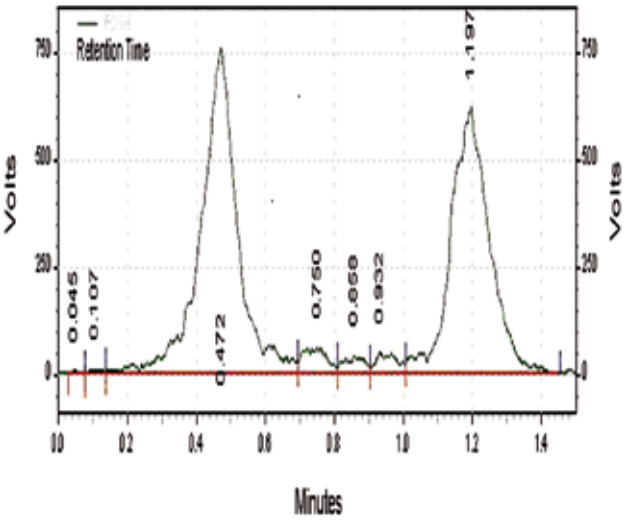

Figure 2 - Thin-layer chromatography (TLC) radioactive thin-layer scanning of labeled marker, the labeled marker peak is regarded as prepeak, the labeled origin marker peak is regarded as post peak.

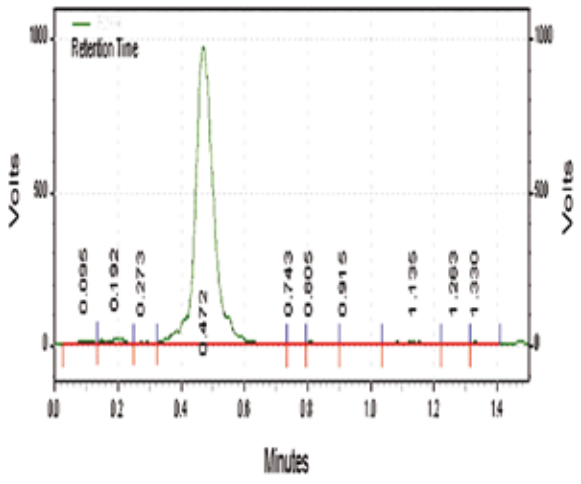

Figure 3 - Thin-layer chromatography (TLC) thin-layer scanning of final products after extraction, the single peak represents final product peak.

Distribution of ^{131}I-His-IN in mice. The in vivo distribution results and tumor/non-tumor (T/NT) ratio in rats bearing Lewis-lung cancer showed that ^{131}I-His-IN was mostly distributed in the blood, heart, liver, kidney, and tumor tissue after 24 hours. The distribution of radioactivity in tumor tissue reached to peak at 2 hours after drug administration, while radioactivity distribution of extra-tumor tissue was rapidly reduced at 4 hours after drug administration. The radioactivity distribution in the kidney had a trend of gradual increase over time and reached to peak at the eighth hour, indicating that this drug is mainly excreted by the kidney. At the fourth hour, the liver had relatively more radioactivity compared with other organs, which may be related to drug detoxification by the liver. The %ID/g tumor tissue reached another peak of 2.31 ± 0.41 at the eighth hour, and the clearance rate of ^{131}I-His-IN after this point was significantly slower than other tissues. After the eighth hour, the ratio of tumor tissue to other organs such as blood, heart, liver, and spleen gradually increased and reached a peak, namely, the ratio of tumor/blood was 1.86 ± 0.28 at eighth hour, 2.67 ± 0.62 at twelfth hour, and 2.45 ± 0.32 at sixteenth hour (**Table 1**), supporting the hypothesis that this drug is detoxifying through the liver and excreted through the kidney.[11]

Tumor suppression rate. **Table 2** shows the variation of tumor volume of the treated group and control group of Lewis-bearing lung cancer mice during the treatment. The tumor growth of all groups is quite slow during treatment and after 5 days of treatment, treated group revealed obvious suppression on tumor growth (p<0.05) in comparison with the control group. All treated groups exhibit suppression of Lewis tumor growth. The difference between the treated group and control group is significant (p<0.05).

Apoptosis rate detected by FCM. Annexin V-FITC and propidium iodide (PI) double standard method were applied to reveal that the mortality and apoptosis rate of treated groups and control group are as follows: 51.69 ± 0.68% for 3.5 mg/kg ^{131}I-His-IN group, 41.24 ± 1.86 % for 3.0 mg/kg ^{131}I-His-IN group, 42.41 ± 1.94% for 3.5 mg/kg IN group, 29.58 ± 2.53% for 3.0 mg/kg IN group, and 10.30 ± 3.45% for physiological saline group. The results suggest that ^{131}I-His-IN and IN has obvious effect on inducing apoptosis and the group of 3.5 mg/kg ^{131}I-His-IN shows highest effect (**Figure 4**).

Table 1 - The ^{131}I-His-IN in C57 mice bearing Lewis lung cancer biology distribution.

Organ	Time in hours (T/NT)						
	2	4	8	12	16	24	48
Blood	5.35 ± 0.36 (0.75 ± 0.05)*	2.02 ± 0.13 (0.86 ± 0.03)*	1.27 ± 0.19 (1.86 ± 0.28)*	0.80 ± 0.16 (2.67 ± 0.62)*	0.70 ± 0.09 (2.45 ± 0.32) *	0.50 ± 0.02 (2.09 ± 0.27) *	0.20 ± 0.01 (1.85 ± 0.32) *
Heart	3.27 ± 0.27 (1.23 ± 0.09)*	1.06 ± 0.20 (1.67 ± 0.25)*	0.67 ± 0.15 (3.53 ± 0.65)*	0.63 ± 0.09 (3.31 ± 0.38)*	0.39 ± 0.08 (4.47 ± 0.82)*	0.40 ± 0.16 (2.94 ± 0.93)*	0.15 ± 0.04 (2.64 ± 0.77)*
Liver	3.12 ± 0.63 (1.29 ± 0.11)*	1.45 ± 0.27 (1.24 ± 0.31)*	0.99 ± 0.14 (2.35 ± 0.32)*	0.59 ± 0.15 (3.76 ± 1.51)*	0.61 ± 0.07 (2.83 ± 0.38)*	0.42 ± 0.03 (2.50 ± 0.34)*	0.19 ± 0.07 (2.23 ± 1.01)*
Spleen	1.80 ± 0.65 (2.40 ± 0.60)*	0.54 ± 0.11 (3.33 ± 0.63)*	0.63 ± 0.07 (3.70 ± 0.43)*	0.61 ± 0.12 (3.47 ± 0.71)*	0.50 ± 0.12 (3.50 ± 0.73)*	0.30 ± 0.05 (3.53 ± 0.62)*	0.14 ± 0.03 (2.65 ± 0.79)*
Lung	2.73 ± 0.24 (1.47 ± 0.13)*	1.13 ± 0.10 (1.54 ± 0.13)*	0.80 ± 0.09 (2.93 ± 0.27)*	0.61 ± 0.04 (3.36 ± 0.17)*	0.52 ± 0.05 (3.30 ± 0.30)*	0.38 ± 0.06 (2.88 ± 0.63)*	0.18 ± 0.10 (1.96 ± 0.34)*
Kidney	2.79 ± 0.47 (1.47 ± 0.98)*	1.91 ± 0.22 (0.92 ± 0.13)	2.00 ± 0.07 (1.16 ± 0.07)*	1.20 ± 0.21 (1.75 ± 0.29)*	0.98 ± 0.06 (1.75 ± 0.29)*	0.61 ± 0.12 (1.75 ± 0.11)*	0.32 ± 0.02 (1.79 ± 0.41)
Stomach	2.10 ± 0.12 (1.91 ± 0.90)*	1.05 ± 0.15 (1.68 ± 0.27)*	0.88 ± 0.10 (2.65 ± 0.28)*	0.61 ± 0.02 (3.35 ± 0.15)*	0.52 ± 0.05 (3.31 ± 0.30)*	0.42 ± 0.04 (2.55 ± 0.44)*	0.17 ± 0.02 (2.21 ± 1.10)*
Thyroid	2.74 ± 0.13 (1.46 ± 0.06)*	1.53 ± 0.26 (1.56 ± 0.19)	0.87 ± 0.13 (2.70 ± 0.41)*	0.64 ± 0.06 (3.19 ± 0.10)*	0.50 ± 0.07 (3.44 ± 0.53)*	0.40 ± 0.03 (2.66 ± 0.36)*	0.21 ± 0.02 (1.73 ± 0.24)*
Tumor	3.99 ± 0.15	1.73 ± 0.70	2.31 ± 0.12	2.06 ± 0.15	1.70 ± 0.11	1.05 ± 0.13	0.37 ± 0.07

^{131}I-His-IN - histamine-indomethacin, T/NT - tumor/non-tumor, *statistically significant (p<0.05)

Table 2 - Comparison of tumor weight and volume in each experimental group (n=12).

Groups	Tumor weight	Tumor volume	Tumor suppression rate
Contrast group	6.38 ± 2.35	6.25 ± 2.11	
3.0 mg/kg IN group	4.36 ± 1.25	4.32 ± 1.13	38.56*
3.5 mg/kg IN group	3.20 ± 1.05	3.05 ± 1.05	52.25*
3.0 mg/kg^{131}I-His-IN group	3.13 ± 0.83	3.25 ± 0.53	57.62*†
3.5 mg/kg^{131}I-His-IN group	1.85 ± 0.62	2.05 ± 0.34	79.21*‡

IN - indomethacin, ^{131}I-His-IN - histamine-indomethacin, tumor inhibition rates of different treatment groups had significant differences than that of the control group (*p<0.05); low-dose ^{131}I-HI-IN had no significant difference compared with the high-dose IN group (†p>0.05), while significant difference was detected between low-dose ^{131}I-His-IN group and low-dose IN group (‡p<0.05); high-dose ^{131}I-His-IN group had significant difference compared to low-dose and high-dose IN groups (p<0.05)

Table 3 - Comparison of the content of tumor necrosis factor (TNF)-α in each experimental group (n=12).

Groups	Content of TNF-α before treatment	Content of TNF-α after treatment
Contrast group	0.58 ± 0.26	0.71 ± 0.24
3.0 mg/kg IN group	0.61 ± 0.29*	1.03 ± 0.36
3.5 mg/kg IN group	0.65 ± 0.31*	1.35 ± 0.42
3.0mg/kg^{131}I-His-IN group	0.62 ± 0.25*	1.39 ± 0.44
3.5 mg/kg ^{131}I-His-IN group	0.59 ± 0.27†	2.11 ± 1.13

IN - indomethacin, ^{131}I-His-IN - histamine-indomethacin, comparison of different treated groups with control group of physiological saline all shows significant difference (*p<0.05), and among them, the 3.5 mg/kg ^{131}I-His-IN group revealed significant difference from other treated group (†p<0.05).

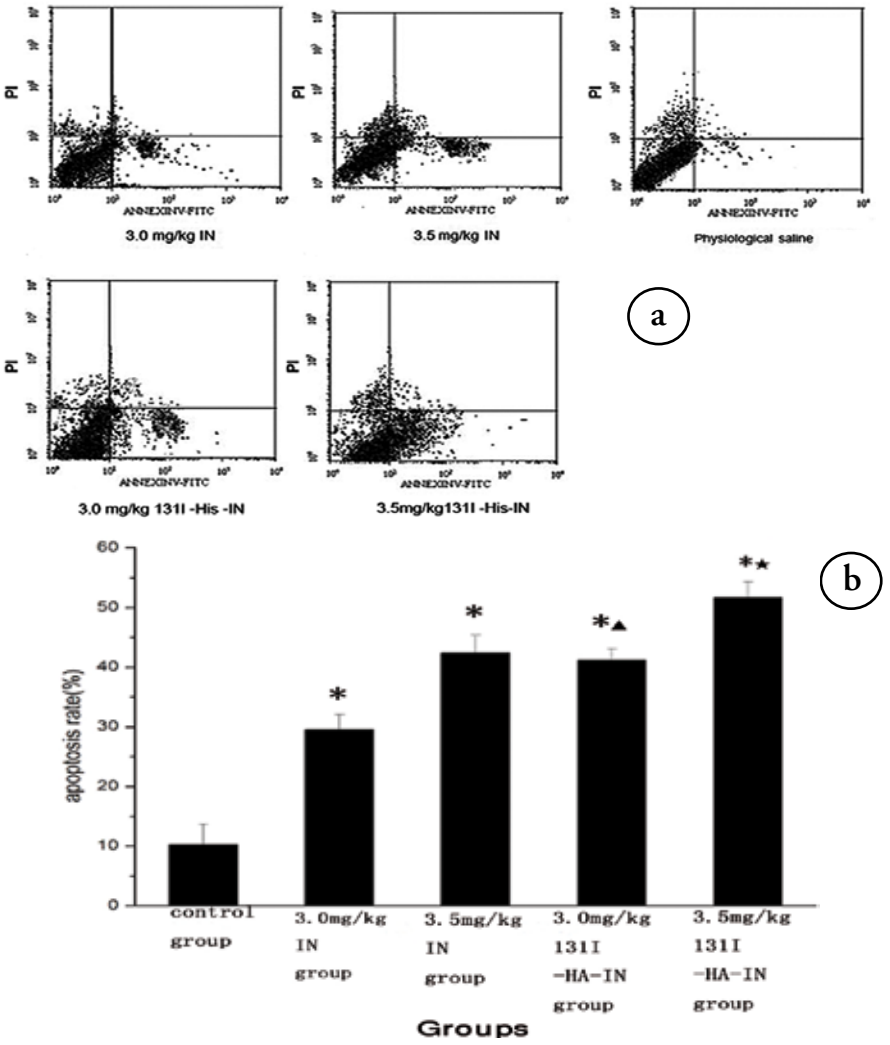

Figure 4 - Apoptosis rate detected by flow cytometry **a)** double labeling propidium iodide (PI) and annexin-V analyze the effect of apoptosis of histamine-indomethacin ([131]I-His-IN) and indomethacin (IN) on Lewis-lung cancer in mice. **b)** Apoptosis rate comparison of [131]I-His-IN and IN on Lewis-lung cancer in mice.*indicate apoptosis rates of different treatment groups had significant differences than that of the control group (p<0.05), ★indicate high-dose [131]I-His-IN group had significant difference as compared to low-dose and high-dose IN groups (p<0.05). [Δ]indicate low-dose [131]I-His-IN had significant difference compared with the low-dose IN group (p<0.05).

Radioimmunoassay test. The TNF-α level in serum of all different groups of Lewis-bearing mice were similar before treatment and all rose after treatment, especially the TNF-α level of the treated groups rose more obviously than the control group (**Table 3**).

Discussion. In a summary of the 2003 Society of Nuclear Medicine Annual Meeting, Wagner[12] pointed out that in the next 20 years, the major direction of tumor therapy study will be the combination of irradiation and chemotherapy by chemotherapeutic drugs labeled with radioactive nuclide to enhance the treatment. As a traditional NSAIDs, IN has been recently proven to be of obvious affinity with tumor. Labeled with appropriate nuclide for treatment such as [131]I or yttrium edotreotid ([90]Y), IN can be transformed to be a complex for tumor therapy with the following advantages theoretically: 1. The IN will act as a vector to carry the nuclide specifically to the surface of the tumor cells or into the tumor cells, and nuclide will bring the ionizing radiation bio-effect on tumor by emitting radiation (ß particle) to injure or kill tumor cells. 2. Meanwhile, IN will exert its chemical toxicity leading to tumor suppressing effect and enhance the ionizing radiation effect of nuclide. 3. Further study will lead to targeted therapeutic complex combining radioactive, chemical, and biological ways to obtain triple effects on the tumor region, and finally to gain obvious anti-tumor treatment.

The IN itself lacks of group for directly labeling radioactive I, while His, tyramine, and other substances which contains histamine ring or tyramine ring that

can be labeled with radioactive iodine easily are usually applied as double-functional chelating agents. The stability testing results reveal that ^{131}I-His-IN stabilizes at least within one week suggesting that it is suitable for the study of biological distribution and further treatment.

The anti-tumor effect of IN works mainly through inhibiting cyclooxygenase (COX)-2 pathway. Khanna et al[13] reported that the change of carboxylic acid structure does not reduce the inhibitory effect of IN to COX-2 activity and the replacement of some group will enhance its inhibitory effect.

Our study is designed to combine acetic acid at the third position of IN with primary amine at the side chain of His to form amido bond leading to a stable chelate. This derivative providing a His ring for radioactive iodine labeling does not reduce the inhibitory effect of IN to COX-2 activity, and does not alter the anti-tumor effect of IN theoretically. Our data suggest that this derivative labeling method is easy-to-operate with high labeling productivity, and in vitro stability.

The data of in vivo biological distribution of Lewis-bearing lung cancer C57 mice revealed that after 2 hours of administration, the radio distribution reaches a peak in tumor tissue and reduces as time extends. Also, other tissues with rich blood supply exhibited relatively high radio distribution at this time point. These phenomena suggest that the intake peak of tumor tissue at this time point is due to better blood supply than other organs instead of a selective intake by non-tumor cells. The %ID/g value reaches the second peak at the time point of 8 hours after administration in tumor tissues, and the clearance in tumor tissues is obviously slower than that in other tissues. The ratios of tumor/blood and tumor/liver are increasing after 4 hours of administration as time extends and decreases gradually after 16 hours of administration with statistical significance. After 48 hours of administration, most of ratios of tumor/non-tumor tissues are still more than one with statistical significance suggesting the radioactive drug takes effect at least within 48 hours after administration in tumor tissue. The ^{131}I-His-IN keeps the affinity of IN for tumor and specific accumulation in tumor tissue with irradiation by ^{131}I at the tumor region, meanwhile, IN provides enhancing effect to chemotherapy, sensitivity, and chemical toxicity realizing the possibility of combination of chemical toxicity by IN and irradiation by nuclide.

As an effective tumor cell killing factor, TNF-α is also an important regulatory factor[14] in inflammatory response and immune response leading to anti-tumor and assisting tumor-suppression through adjusting immuno-status and stimulating factors including TNF, IL-2, and INF, and so on. Radiotherapy can induce tumor cell apoptosis and stimulate the anti-tumor factors.[15] Our study on detection of TNF-α level by radioimmunoassay shows the TNF-α levels in serum of different treated Lewis-bearing mice groups all increase significantly and the 3.5 mg/kg ^{131}I-His-IN group shows significant difference from other treated groups in terms of TNF-α level (p<0.05). Those results suggest that 3.5 mg/kg ^{131}I-His-IN group takes significant effect on enhancing TNF-α level confirming that ^{131}I can synergize with IN to enhance the release of TNF-α leading to anti-tumor effect.

In addition, the daily measurement data of tumor volume and weight of Lewis-bearing mice revealed the tumor volume and weight of different treated groups all reduced in comparison with those before treatment and the tumor suppression rates of all treated groups have been increased obviously, among which the 3.5 mg/kg ^{131}I-His-N group exhibits the highest tumor suppression rate. The chemotherapy and radiotherapy to cancer all take effects through inducing tumor cell apoptosis,[16] Our study on detecting tumor cell apoptosis in Lewis-bearing lung cancer mice by combination of Annexin V-FITC and PI double standard method FCM suggests shows that the significant difference between 3.0 mg/kg ^{131}I-His-IN group and 3.5 mg/kg ^{131}I-His-IN group demonstrates the treatment of lung cancer by ^{131}I-His-IN is in dose-dependent manner, and optimal dosage should be determined by further study. However, further studies are needed to label ^{125}I with His-IN in order to extend retention time in the tumor. Therefore, it can preferably exert radiotherapy to tumor.

In conclusion, we synthesized His-IN and applied ^{131}I labeled His-IN to tumor therapy. In this way, the selective tumor-affinity, and tumor-therapeutic effect of IN were kept and ^{131}I can exert its irradiative effect. Meanwhile, IN enhances sensitivity of radiotherapy and chemical toxicity with improved tumor imaging.

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