Biliary and duodenal drainage for reducing the radiotoxic risk of antineoplastic ^{131}I -hypericin in rat models

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Abstract

Necrosis targeting radiopharmaceutical ^{131}I -hypericin (^{131}I -Hyp) has been studied for the therapy of solid malignancies. However, serious side effects may be caused by its unwanted radioactivity after being metabolized by the liver and excreted via bile in the digestive tract. Thus the aim of this study was to investigate two kinds of bile draining for reducing them. Thirty-eight normal rats were intravenously injected with ^{131}I -Hyp, 24 of which were subjected to the common bile duct (CBD) drainage for gamma counting of collected bile and tissues during 1–6, 7–12, 13–18, and 19–24 h ($n=6$ each group), 12 of which were divided into two groups ($n=6$ each group) for comparison of the drainage efficiency between CBD catheterization and duodenum intubation by collecting their bile at the first 4 h. Afterwards the 12 rats together with the last two rats which were not drained were scanned via single-photon emission computerized tomography/computed tomography (SPECT/CT) to check the differences. The images showed that almost no intestinal radioactivity can be found in those 12 drained rats while discernible radioactivity in the two undrained rats. The results also indicated that the most of the radioactivity was excreted from the bile within the first 12 h, accounting to 92% within 24 h. The radioactive metabolites in the small and large intestines peaked at 12 h and 18 h, respectively. No differences were found in those two ways of drainages. Thus bile drainage is highly recommended for the patients who were treated by ^{131}I -Hyp if human being and rats have a similar excretion pattern. This strategy can be clinically achieved by using a nasobiliary or nasoduodenal drainage catheter.

Keywords: ^{131}I -hypericin, solid tumor therapy, radioactivity, distribution, common bile duct drainage, duodenal drainage

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Introduction

In addition to showing anti-depressant, anti-bacterial, and anti-tumor effects,¹ hypericin (Hyp) has been demonstrated to exhibit a superior affinity for irreversibly damaged or necrotic tissues.² Radioiodinated Hyp, particularly ^{131}I -Hyp, is a necrosis avid tracer that can be used for simultaneous cancer imaging diagnosis and targeted radiotherapy to effectively inhibit tumor growth.³ Because 30–80% of the solid tumor mass is accompanied by necrosis, which is rarely observed in normal subjects, Hyp is now under active development for use in the diagnosis and treatment of malignant tumors;^{2–8} accordingly, a dual-targeting anticancer approach has been introduced.^{3,8} Briefly, vascular disrupting agents (VDAs) can block the blood supply to solid tumors, causing a large area of tumor necrosis,^{9–11} thereby creating an ideal necrotic target for Hyp accumulation. As

^{131}I -Hyp preferentially accumulates in necrotic tissues, it delivers its radioactive energy to the adjacent living tumor remnants.^{1,6,12} The β -particles emitted by ^{131}I -Hyp can kill living cells within 2.0 mm, and the γ -rays produced by ^{131}I -Hyp aid in the detection of necrotic primary and metastatic tumors by means of nuclear imaging. Additionally, each treatment cycle may achieve long-term therapeutic efficacy and allow monitoring for weeks to months due to the eight-day decay half-life of ^{131}I . Because of these highly complementary properties, the combination of this necrosis avid tracer with a VDA makes this new theragnostic strategy potentially attractive for clinical oncology.³

Although this dual-targeting anticancer approach looks promising, its possible side effects are a matter of concern. Hyp and ^{131}I -Hyp are mainly metabolized by the liver and excreted into the bile.^{13–15} The metabolites, including free

^{131}I and the bound derivatives of ^{131}I -Hyp, are still radioactive. Some are reabsorbed by the intestines entering the enterohepatic circulation (EHC), while others are mixed with the chyme in the intestines for a substantially long period before they are discharged with the feces.¹⁶ The metabolites absorbed by the intestines begin to re-circulate with the blood. Free ^{131}I may accumulate in and persistently irradiate the thyroid gland if it is not protected, while the ^{131}I -Hyp derivatives may no longer show a superior affinity for necrotic tissue and are viewed as the radioactive waste. When trapped in the intestines, these radioactive metabolites will evoke persistent β -radiation, causing radiation enteritis.^{17–19}

To avoid such side effects caused by radioactive wastes from the bile, a duodenal drainage catheter has been designed and constructed for bile drainage in rats.¹⁵ Thus, the bile together with the intestinal juice containing radioactive substances can be drained outside of the body. A preliminary study indicated that rats with this duodenal drainage catheter showed lower radioactivity in tissues and required less time for radioactivity elimination.¹⁵ Nevertheless, the efficiency of this catheter for bile drainage has not yet been tested. Moreover, the optimal time for bile drainage needs to be investigated. According to several reports, Hyp is efficiently discharged during the first 24 h after injection,^{20–23} however, its profile in the bile, especially within the first 24 h, has not been established. Because intubation and draining may cause discomfort to patients, we sought to first determine the optimal period for the maximum drainage of radioactive metabolites in rats for extrapolation to human scenarios.

Most of the previous studies addressing the use of Hyp have focused on oral^{20,24,25} or intraperitoneal²² administration, and only a few references are available concerning the distribution and discharge of Hyp following intravenous (i.v.) administration.^{15,26} In this study, rats were subjected to i.v. injection of ^{131}I -Hyp, after which the distribution of radioactivity in the bile and tissues was detected within 24 h, and the efficiencies of bile drainage were compared between the methods employing a biliary and duodenal catheter. Our findings may provide evidence regarding the necessity and feasibility of bile drainage and the proper time to implement this approach.

Materials and methods

Preparations of ^{131}I -Hyp

Hyp with a purity >98.5% was purchased from Purui Technology (Chengdu, Sichuan, China). The Na^{131}I solution (10 mCi/mL) was supplied by HTA Co., Ltd (Beijing, China). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO), unless specifically mentioned.

The preparation and testing of the radiochemical purity of ^{131}I -Hyp have been described previously.²⁶ Hyp was dissolved in dimethylsulfoxide (DMSO), radioiodinated by Na^{131}I solution in the presence of Iodogen as an oxidant (Figure 1).²⁷ The radiochemical purity of ^{131}I -Hyp was determined via thin-layer chromatography (TLC) using Waterman No 1 filter paper as a stationary phase and 0.1 N hydrochloric acid as a developing solvent system, and the results were analyzed by a radioactive TLC scanner. The stability of ^{131}I -Hyp was tested by incubating ^{131}I -Hyp in solution at room temperature and in rat plasma (1:20 v/v) at 37°C and was determined via TLC at the time points of 0.5 h, 12 h, 1 d, 2 d, 4 d, and 8 d.

The ^{131}I -Hyp solution was formulated in DMSO/polyethylene glycol (PEG400)/propylene glycol (PG) (1:1:1 v/v/v) for i.v. injection at 3.96 MBq/kg of ^{131}I and 0.1 mg/kg of Hyp in rats.

Animal modeling

Adult male Wistar rats, weighing 250–300 g, were provided by the Experimental Animal Center of the Jiangsu Academy of Traditional Chinese Medicine, Nanjing, Jiangsu, China. The protocols for animal care, use, and experiment were approved by the institutional Animal Affairs Committee. To prevent the thyroid from taking up free ^{131}I , the rats were fed with a 0.5% potassium iodide solution 24 h before ^{131}I -Hyp injection. The rats were anesthetized intraperitoneally with pentobarbital (40 mg/kg body weight; Nembutal Veterinary, Sanofi Sante Animale, Paris, France), followed by surgery.

The catheterization of the common bile duct (CBD) for bile drainage under open surgery is shown in Figure 2a, b. Briefly, a 24 G venous indwelling needle was used as the drainage catheter. After laparotomy, the CBD between the liver and duodenum was exposed. The CBD was then

Figure 1 Iodogen method for preparation of ^{131}I -Hyp

pierced with the needle near the duodenum, directed towards the hepatic hilum, and the needle was then fixed via suturing for continuing bile drainage.

The surgery for duodenal drainage (Figure 2d) was performed using a duodenal drainage catheter modified from a pediatric urinary Foley catheter (Figure 2c). The catheter contains two channels; one is used for balloon extension, and the other is for juice drainage. The rats were subjected to laparotomy. The catheter (B,C end) was orally implemented through the esophagus, stomach, and duodenum until reaching 1 cm below Oddi's sphincter at the duodenum. Air was pumped into the catheter from the A end to dilate the balloon at the B end, as shown in B', to block the duodenum. Thus the bile mixed with duodenal juice could be drained outside through the catheter from the C end to the D end.

Study designs

Experiment 1: Radiobiodistribution in the bile and tissues after CBD drainage. As shown in Figure 3a, 24 rats were

divided into four groups ($n=6$ each), and ^{131}I -Hyp was i.v. injected at 0 h. For bile drainage at intervals of 1–6 h (group 1), 7–12 h (group 2), 13–18 h (group 3), and 19–24 h (group 4), under anesthesia, these rats were subjected to abdominal surgery for CBD catheterization. The bile was drained and collected, with one sample being obtained per hour, for the subsequent 6 h in each group. Then, the rats were euthanized through an overdose of anesthetic. To compare the radiobiodistribution in function of time in the same organs, tissues including the heart, lung, liver, spleen, kidney, small intestines, large intestines, and blood were sampled to perform radioactivity measurements through gamma counting. The urine and feces were also collected for measurement.

Experiment 2: Drainage efficiency of duodenal and CBD catheters. As outlined in Figure 3b, 14 rats were fasted for 8 h before surgery and were divided into three groups. One group received surgery to achieve duodenal drainage ($n=6$); one group received surgery to achieve CBD

Figure 2 The surgeries for animal modeling. The catheterization of CBD is shown in the photograph (a) and diagram (b). The yellow arrow indicates the site for cannulation. The surgery for duodenal drainage was performed using a duodenal drainage catheter modified from a pediatric urinary Foley catheter (c). The catheter (B,C end) was implemented from the esophagus, stopped until reaching 1 cm below Oddi's sphincter at the duodenum. A balloon was blown up at the B end by pumping air into the A end, as shown in B'. The duodenum was blocked and the bile mixed with duodenal juice could be drained outside through the catheter from the C to D end. The image d is the diagram of the duodenal drainage. (A color version of this figure is available in the online journal.)

Figure 3 Flow diagram of protocol. Experiment 1: radiobiodistribution in the bile and tissues after CBD drainage (a). Experiment 2: Comparing the bile drainage efficiency with CBD or duodenal cannulation (b)

drainage ($n=6$); and another group ($n=2$) was maintained as controls, without drainage. Following i.v. injection of ^{131}I -Hyp, the duodenal juice and bile were collected at a rate of one sample per hour for the subsequent 4 h, when the peak of radioactivity excretion had been passed according to experiment 1 and a previous study.¹⁵ Then, all the rats were euthanized and scanned via single-photon emission computed tomography/computed tomography (SPECT/CT) for *ex vivo* imaging of the distribution of ^{131}I . The radioactivity of the drained samples from the two drainage experiments was measured and compared. The ^{131}I binding rate in the aspirates was tested via TLC.

^{131}I radioactivity detection

The collected samples were weighed, and their radioactivity was measured using an automatic gamma counter (SN-695; Hesuo Rihuan photoelectric instrument, Shanghai, China). Backups of the ^{131}I -Hyp solutions were used as decay correction standards. The results were corrected for background radiation and physical decay. The radioactivity of these samples was calculated and expressed as the percentage of the injected dose per gram of tissue or bile (%ID/g) and the percentage of the injected dose per organ or per hour in bile (%ID).

The radioactive substances present in the drained bile and juice included free ^{131}I and bound ^{131}I -Hyp and its derivatives, which were determined via TLC. The ^{131}I binding rate (%) was calculated as follows: ^{131}I binding rate (%) = $\text{count of bound } ^{131}\text{I} / (\text{count of free } ^{131}\text{I} + \text{count of bound } ^{131}\text{I}) \times 100\%$.

SPECT/CT imaging

A variable-angle dual detector SPECT coupled to a 16-slice CT (Symbia T; Siemens Medical Systems, Chicago, IL) was used to perform ^{131}I imaging of the rats in experiment 2. The dead rats were positioned in the center of the field-of-view in a supine position. Anterior and tomographic images were collected using the following acquisition parameters: static image matrix size, 128×128 ; acquisition count limit, 50,000; SPECT tomographic image matrix, 64×64 ; and continuous acquisition, 15 s/frame $\times$ 24 frames.

Statistical analysis

All statistical analyses were performed with SPSS11.0 (StatSoft, USA). Numerical data were expressed as the mean $\pm$ standard error and were compared through one-way analysis of variance (ANOVA). When significant, the differences between the means were assessed using the least significant difference test. $P < 0.05$ was considered significant.

Results

Radiolabeling and stability of ^{131}I -Hyp

The radiochemical purity of ^{131}I -Hyp was greater than 98% and the specific activity was typically 19.0 MBq/ μmol . The TLC scanning results were consistent to that previously published with a retention factor (Rf) of 0.15 for ^{131}I -Hyp.^{26,28} The stability of ^{131}I -Hyp stored in solution at room temperature and in rat plasma at 37°C was excellent, with a radiochemical purity over 95% being observed for up to eight days (Table 1).

By electrophilic substitution reaction, Hyp was mono-iodinated at position 2 of its polycyclic aromatic structure as shown in Figure 1, which was suggested by a previous study using stable iodide with high-performance liquid

chromatography (HPLC) and layer chromatography-mass spectroscopy (LC-MS) for characterization.²⁹

The radioactivity excreted in the bile

For experiment 1, radioactivity in the bile presented as %ID/g is graphed as a function of time in Figure 4a. The highest levels of radioactivity were observed within 12 h after injection, especially in the first 6 h. Radioactivity reached a peak at approximately 2 h, followed by a decline from 3 to 12 h, and remained unchanged after 18 h. In addition to the highest concentration observed at 2 h ($14.75 \pm 2.13\%$), two smaller peaks appeared during the initiation of draining in the 7–12 h group and 13–18 h group, at $7.06 \pm 0.49\%$ and $2.57 \pm 0.52\%$, respectively.

The radioactivity in the bile presented as %ID is plotted for each hour in Figure 4b, which followed the same pattern as the results for %ID/g shown in Figure 4a. Approximately 58% of the injected radionuclide was eliminated within the

Table 1 In vitro stability of ¹³¹I-Hyp in solution at room temperature and in rat plasma at 37°C.

Incubation time (h)	In solution at room temperature (%)	In rat plasma at 37°C (%)
0.5	98.63	98.21
12	98.37	98.20
24	98.35	97.63
48	98.21	96.82
96	98.11	96.35
192	98.02	95.11

Figure 4 The radioactivity in the bile within 24 h after ¹³¹I-Hyp i.v. injection. The radioactivity in the bile presented as the percentage of injected dose per gram (%ID/g) (a) and the percentage of the injected dose per hour (%ID) (b). The bile was collected at intervals of 1–6, 7–12, 13–18, or 19–24 h (n = 6 each), weighed and their radioactivity was measured using an automatic gamma counter. The result is expressed as the mean ± standard error

Figure 5 The radiobiodistribution in tissues, blood, and excrements within 24 h after ^{131}I -Hyp i.v. injection. The radioactivity presented as the percentage of injected dose per gram of tissues (%ID/g) (a) and the percentage of the injected dose per organ (%ID) (b). Test samples were collected at 6, 12, 18, 24 h ($n = 6$ each), weighed and their radioactivity was measured using an automatic gamma counter. The results are expressed as the mean $\pm$ standard error. Within the same tissue, data sharing no common letter are different ($P < 0.05$). S.I.: the small intestines. L.I.: the large intestines. Data indicating radioactivity of large intestines (including feces) at 6 h were not collected

first 6 h, 23.6% between 7 and 12 h, and 7% during 13–24 h. The radioactivity (%ID) recorded within the first 12 h was much higher than that from 13 to 24 h, accounted to 82% of the injected dose or 92% of the total bile radioactivity within the first 24 h.

The radiobiodistribution in tissues, blood, and excrements

The radioactivities (%ID/g) of samples collected at different time points are indicated in Figure 5a. The radiobiodistribution evolved in various tissues within the first 24 h after the injection of ^{131}I -Hyp. The radioactivity reached a peak in the small intestines (including the chyme) at 12 h; this peak was significantly higher than that recorded at 6 h, 18 h, or 24 h ($P < 0.001$ for all) and decreased over

time thereafter. The radioactivity in the large intestines (including feces) reached a maximum at 18 h, and the radioactive concentrations measured at 12 h and 18 h were significantly higher than that at 24 h ($P < 0.05$, $P < 0.01$, respectively). However, when the feces were removed from the large intestines, there were no longer significant differences in radioactivity between all time points. In addition, radioactivity in the lung remained almost constant over the entire 24 h period, at a relatively high level. The radioactivity in other tissues showed a similar profile, reaching the highest values at 6 h and decreasing over time thereafter. The radioactive concentration in the blood, liver, spleen, kidney, and stomach at 6 h was significantly higher than at the other time points ($P < 0.001$ for all, except $P < 0.05$ in the stomach between 6 and 12 h).

Figure 6 SPECT/CT images in rats with CBD, duodenal drainage, and without drainage. Image values are expressed as SUV be used in the same scaling. Sagittal views were performed 4 h after i.v. injection of ^{131}I -Hyp in rats with CBD drainage (a), duodenal drainage (b), and without drainage (c). The liver, intestines, and penile are indicated by the letter L, I, and P, respectively. Intestinal uptake of radioactivity (dashed box) was shown only in rats without drainage (c) (A color version of this figure is available in the online journal.)

We next calculated the radioactivity for total organs at indicated time points (Figure 5b). The contents of radioactive substances appeared to be highest in feces and negligible in urine. The feces discharged during the first 6 h of bile drainage contained no radioactivity. The contents of radioactivity in the feces increased greatly from 12 h after injection, and up to 35% of the injected dose was recovered after 24 h. The radioactive contents of the feces after 24 h were significantly greater than after 18 h ($P < 0.01$), and those after 18 h were higher than at 12 h ($P = 0.01$). In line with the findings regarding the radioactivity per gram of tissue (Figure 5a), the level of radioactivity in the whole lung was relatively high. In the blood, liver, spleen, and kidney, the recorded radioactivities were all significantly higher at 6 h after injection than at the other time points ($P < 0.001$ for all time points, except in the kidney between 6 and 12 h, where $P < 0.01$), subsequently exhibiting a decreasing trend and reaching relatively constant values after 12 h.

Drainage efficiency of the CBD and duodenal cannulation

In experiment 2, SPECT/CT showed less radiouptake in the liver and almost no uptake in the intestines in the rats with CBD and duodenal drainage at 4 h after injection, compared with the rats without drainage (Figure 6). The penile vein used for injection was also observed as a hot spot in all treatments. The radioactivity in the drained duodenal juice was compared with that in the bile collected via

CBD cannulation at 4 h. The results showed that there was no significant difference in radioactivity between these two sources of bile at each hour examined (Figure 7a). Using both of these drainage methods, the radioactivity during the first hour was found to be lower compared with that at 2 and 3 h; i.e. in the CBD cannulation group ($P < 0.05$, $P < 0.01$) and in the duodenal intubation group ($P < 0.001$, $P < 0.01$).

The radioactive substances in the drained fluids included free ^{131}I and bound ^{131}I -Hyp derivatives. The results from TLC testing showed that the binding rate of radioactive ^{131}I in the fluids using both methods was approximately 80% (Figure 7b), which was lower than the binding rate of the original injectants ($>98\%$). Further analysis indicated that the rate of ^{131}I binding in the bile from the CBD remained relatively constant during the draining period, whereas there was a peak in the binding rate during the first hour in the duodenal juice, which was lower than the rates recorded in other draining periods (1 h vs. 2 h, $P < 0.05$). During the first draining hour, the rate of ^{131}I binding in the duodenal juice was significantly lower than in the bile from the CBD ($P < 0.01$).

Discussion

Animal models

Most of the studies conducted to date on the distribution and metabolism of Hyp or its derivatives have been carried out in tumor-bearing animal models with the aim of verifying the affinity of ^{131}I -Hyp for tumors or tumor

Figure 7 The comparison between the CBD and duodenal drainage. The radioactivity of the drained samples (a) and the binding rate of radioactive ^{131}I (b) were compared between the two drainages ($n=6$ each). The drained juice and the bile during each hour within 1–4 h were collected, weighed, and their radioactivity was measured using an automatic gamma counter. Free ^{131}I and bound ^{131}I -Hyp derivatives in samples were determined via TLC, and the ^{131}I binding rate (%) was calculated (b). The result is expressed as the mean $\pm$ standard error. Within the same group, data sharing no common letter are different ($P < 0.05$). Mean value was significantly different between two groups: $*P < 0.05$.

necrosis.^{20–23,30} Normal rats were used in the present study to address the radioactivity biodistribution in normal tissues and radioactivity excretion in the bile after the i.v. administration of a dose of ^{131}I -Hyp, especially considering the feasibility of the two applied draining methods.

The necessity for bile drainage

It has been noted that the radioactive metabolites of ^{131}I -Hyp are mainly processed by the liver and discharged via the bile,^{13,15} which was verified in the present study via the observation of nearly exclusive liver uptake, as showed on SEPCT/CT. This phenomenon is consistent with the high dose of radiation in feces and the negligible radioactivity in urine recorded in this study. However, there was previously no direct evidence revealing the time distribution of such

radioactive metabolites in the bile. Hence, for the first time, we sequentially measured the radioactivity in the bile for 24 h after the i.v. injection of ^{131}I -Hyp. Approximately 90% of the injected radiation was eliminated during the first day when no necrosis existed to allow ^{131}I -Hyp accumulation to occur. If such highly radioactive metabolized substances are not drained directly out of the body, they will be discharged gradually through the intestines, which could cause serious side effects or radiotoxicity.

Re-absorption of radioactive metabolites in the intestines is deemed harmful to the body. The radioactive metabolites in the bile include free ^{131}I and bound ^{131}I -Hyp derivatives. The free ^{131}I is mainly generated through the deiodination of ^{131}I -Hyp and may accumulate in the thyroid gland (if not pre-saturated with non-radioactive iodine) after re-absorption, resulting in hypothyroidism due to long-term

irradiation. According to our results, free ^{131}I accounted for approximately 10–30% of the activity in the drained duodenal juice, or approximately 1.5% of the originally injected activity per hour, which may pose a serious threat to the thyroid gland or other vulnerable organs if the dose of ^{131}I -Hyp applied for cancer treatment varies from 100 to 300 mCi. Moreover, ^{131}I -Hyp derivatives in the bile likely no longer display necrosis avidity due to structural changes, and the risk of bone marrow suppression might be increased after their re-absorption.³¹ Therefore, re-absorption and re-distribution of such radioactive metabolites should be avoided through proper bile drainage.

The remaining unreabsorbed radioactive metabolites are mixed with the chyme. Our data regarding the radioactivities obtained with and without chyme in the intestines suggested that most of the radioactivity came from the contents of the intestines, rather than the intestines themselves. Although all of the radioactive contents in the intestines would be finally discharged as the feces, these radioactive wastes remain in the intestines for more than 18 h in rats. In humans, the intestinal transit time could be even longer.³² The β -particles emitted from the radioactive metabolites could injure or kill living cells within a 2.0 mm distance,^{3,6} leading to possible radiation enteritis.^{17–19} Therefore, bile drainage is necessary to avoid the side effects of unwanted irradiation in normal tissues.

Comparing the bile drainage efficiency with CBD or duodenal cannulation

A duodenal balloon catheter was shown to enable faster elimination of radioiodinated Hyp and decreased its radiobiodistribution in normal organs and tissues in a preliminary study in rats.¹⁵ This study further demonstrated that the radioactive waste in the gastrointestinal (GI) tract could be drained equally efficiently through duodenal and CBD drainage, and there was no significant difference in the elimination of radioactivity per hour between these two treatments. Nevertheless, a greater amount of free ^{131}I was associated with duodenal drainage, likely as a result of the deiodination of ^{131}I -Hyp in the active enzymatic environment of the duodenum, which shows a different pH.

Regarding whether the drainages via both ways may impair the uptake of ^{131}I -Hyp in necrotic tissue, in general, this influence is regarded negligible. This is due to 1) the proportion of the ^{131}I -Hyp after re-uptake via the EHC, if any, is minimum because no significant difference in serum concentration of ^{131}I -Hyp was found between animals with and without bile drainage;¹⁵ and 2) after biliary excretion into the GI tract, ^{131}I -Hyp is likely dissociated into ^{131}I and Hyp, both of which will not contribute anymore to the necrosis-specific uptake.¹⁵

Clinical implications

Biodistribution of radioiodinated Hyp in both %ID and %ID/g among organs and tissues has been extensively studied, compared and published in many papers.^{3,15,16,26,28,30} In line with the previous research on Hyp metabolism,^{20–23} we found that the majority of radioactivity was cleared from most tissues and organs in rodents within 24 h after ^{131}I -Hyp

injection. Hyp and its derivatives are solely eliminated via the hepatobiliary route, and the radioactive waste in the bile should be removed from the body as early and completely as possible to reduce possible radiotoxicity. Under open abdominal surgery, this rodent study demonstrated that drainage via either the CBD or duodenum was equally efficient, with over 82% of the injected activity being discharged within the first 12 h. Considering the roughly similar metabolism and GI transit times of rodents and humans,³² our findings have clinical implications. With the assistance of imaging and fiber optic endoscopic technologies, bile drainage can be achieved clinically using a nasobiliary or nasoduodenal catheter.^{33,34} Both types of catheter can be inserted and removed easily, quickly, and safely, though both also cause a certain degree of discomfort. Therefore, efficient drainage during the first 12–24 h would be recommended to significantly reduce the bodily retention of radioactivity and possible radiotoxicity. The amount of radioactive waste can be quantitatively monitored using a gamma counter to measure the cumulative activities in the collected fluids, based on which a decision can be made regarding when to discontinue the drainage. Alternatively, medications that stimulate bile excretion and/or intestinal movement could also be applied to help speed up the clearance of radioactive waste from the body. Furthermore, from a radio-safety point of view, this strategy can be extended to other radionuclide therapies as long as the tracers are mainly eliminated through the hepatobiliary pathway.

In conclusion, to reduce the potential side effects of ^{131}I -Hyp administered for anticancer treatment, bile drainage is highly recommended, and should ideally be performed within 12–24 h after radionuclide injection. Biliary and duodenal drainage catheters are equally effective for achieving such a drainage, which can be implemented clinically by applying a nasobiliary or a nasoduodenal catheter.

Author contributions: YN and MMC designed and YL, CHJ and JZ conducted the experiments and animals. YL wrote the manuscript and analysis of the data. XJ and ZPS prepared radiopharmaceutical and test the radioactivity of the samples. SPECT/CT was performed by WL.

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