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The Study on Local Thermo-therapy with CT Measure Temperature: the Pharmacokinetics Study of Iohexolthermosensitive Liposomes During Local Thermotherapy

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To study on using CT measure temperature during local thermo-therapy, we study the pharmacokinetics of intravenous injecting iohexol thermosensitive in rats. Heating group and non-heating group use C_6 Glioma model rats, the heated to 43! and normal temperatureÿNormal rats are intravenous injected with iohexolstandard product iohexol thermosensitive liposome,the $t_{1/2}$ of heating group prolongs significantly compared with the non-heating group,AUC increases significantlyÿThe AUC of heating group and non-heating group increases significantly compared with the standard product group, demonstrating that under the same dose condition, compared with iv. standard product, iohexol thermosensitive liposome can prolongs the time of iohexol in blood circulation;The thermosensitive liposome encapsulated with iohexol in C_6 Glioma model are release theirs content sharply in heat group when the local tumor is heated.

Key words: Iohexol; Thermosensitive liposome; Pharmacokinetics, CT, Measure temperature.

Intravenous injects freeze-dried needles including iohexol after heating the tumor local to above 43°C (the convert temperature of thermosensitive liposome),observe the release and its effects to the pharmacokinetics of iohexol encapsuled in thermosensitive liposome above covert temperature(heat) and body temperature, provides the theoretical basis for the CT measure and control temperatured during local thermotherapy .

MATERIALS AND METHODS

Drugs

Thermosensitive liposome freeze-dried needles compounded with CT contrast agents (iohexol),thermosensitive liposome. Specifications: 2ml per bottle,including 372 mg/ml, Batch number: 1st batch, provided by tianjin institute pharmaceuticals research.Iohexol(Zhejiang starry people Co., LTD, Batch number 070819),Hydroxyl styrene-acrylic ester(PP, Sigma company products,purity>99%),Tofu helicid(Batch number 050301,content 97.0%, Kunming YunKe pharmaceutical Co., LTD) (ethylene glycol) – 400, Ethyl acetate is analysis alcohol, reagent for HPLC is chromatographic pure.

Animals

C_6glioma model(SD strains),provided by Tianjin First Central Hospital HealDepartment Acute and Serious illness Key Laboratories.Normal SD strains rats, bought in the Chinese Academy of Medical Science. All animals usage of male and female, body weight 200~250g(Experimental animals qualified number:0001662).

Instrument

Thermostatic circulation water bath (Shanghai medical equipment factory)Peristaltic

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pump(Shanghai faith instrument Co., LTD) Homemade u-shaped capillary.HPLCtesting instruments(Series b! pump, SHIMADZU SPD-10A ultraviolet detector, Lab Alliance AS-3000 automatic sampler,ANASTAR chromatographic data stations; LD - 5 type low speed centrifuge (Beijing medical centrifuge plant production); Sartorius BS series electronic balance; TGL - 16C centrifuge table high speed).

EXPERIMENTAL

Design

C_6 glioma model rats are divided into 2 groups randomly,the first group is treated with opening craniotomy window,Expose tumorsÿconnect the homemade u-shaped capillary with the thermostatic circulation baths and peristaltic pump, heat the tumor local,let the temperature arrive at above 43!,intravenous inject freeze-dried needles after 20min heat, continue to heat for 10min; the second group is treated with opening craniotomy window but no heating,intravenus inject freeze-dried needles;And set another normal rats intravenus inject standard product solution including iohexol(3ml (ethylene glycol) – 400,join iohexol,dilute it to 10ml with injection water).Three groups of rats are all intravenous injected 2ml/200g weight, equivalent toiohexol 3720 mg/kg. Picks before giving drugs(0h),after giving drugs 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 and 8h respectively from venous blood,heparin anticoagulation, measure blood medicine density after separating plasma regularly.

HPLC assay blood samples determination

Blood sample pretreatment Iohexol; Take plasma 10μl,10 times diluted with blank plasma,join Tofu helicid internal standard solution(10mg/ml)10μl, blending, join 300μl methanol,vortex oscillation 30 seconds, Room-temperature placed 30min, 12000r/min centrifugal 10min,take supernatant fluid 200μl,join 800μl no ion water,centrifugal again 2min, into sample analysis. Standard curve preparation: Weigh out iohexol standard product 100mg precisely, made into 10mg/ml reserve liquid by water.Dilute reserve liquid and prepare plasma sample including iohexol with mass concentration is 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000ug/ml.Following”2.2.1" start blood sample processing and analysis, use the ratio of iohexol and internal standard chromatographic peak area to calculate and work out the linear regression equation and its correlated coefficient.

Methods recovery

Prepare the plasma sample including iohexol which mass concentration is 10050 and 500 ug/ml, determine after pretreatment,put it into standard curve regression equation,calculate the titer, recovery (%) = (determined concentration/join concentration) × 100%.

Rats blood drug concentration measurement and Drug metabolic parameters calculation

The three group’s rats blood sample are processed following the”2.2.1" and then into sample analysis, put the measured peak area ratio into the regression equation to caculate the concentration.Fitting by the 3p87 pharmacokinetic programs made by China Drugs Institue,and caculate the correlvent drug metabolism kinetic parameters,among them the Cmax, Tmax are calculated by actual measured value, AUC is calculated by the trapezoidal area of law.

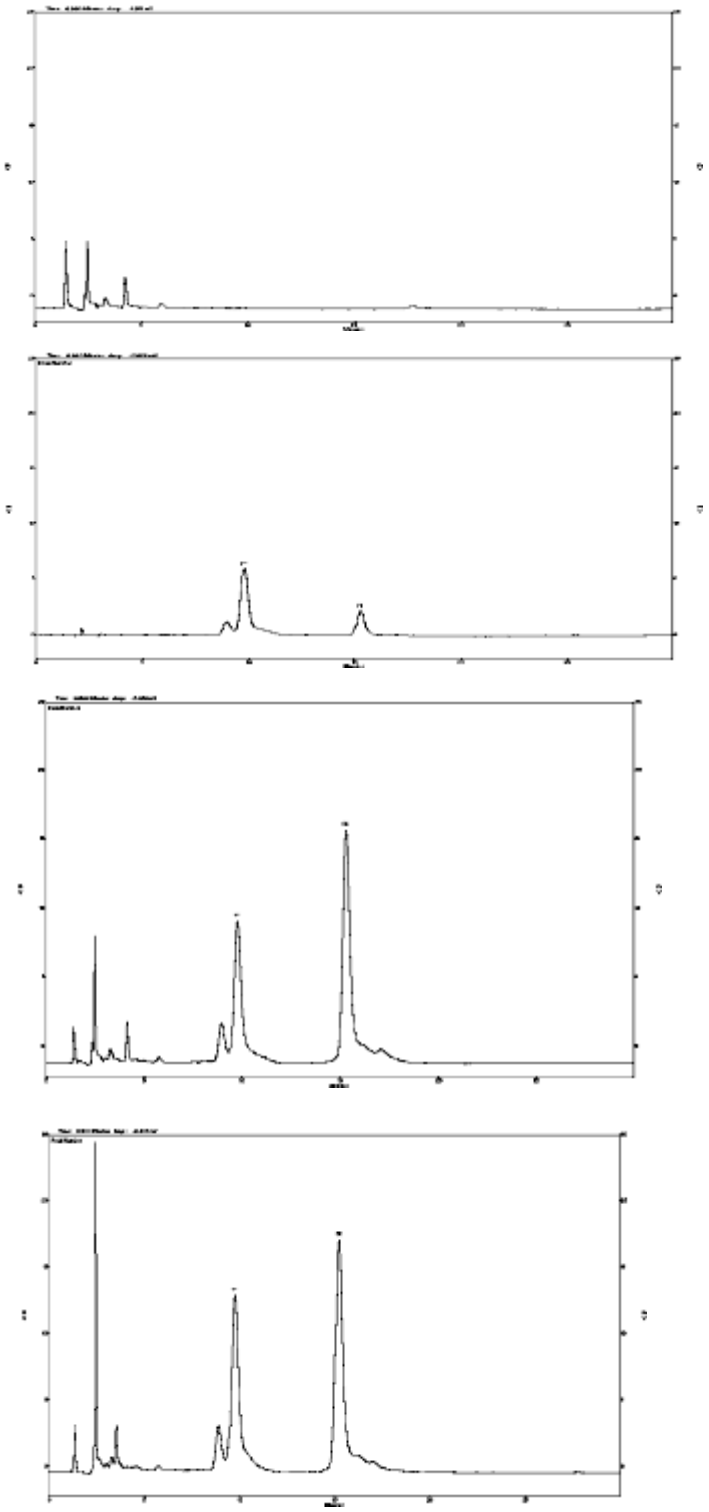
RESULTS

Specificity of chromatographic method

Under the above chromatographic conditions, iohexol, internal standard and plasma impurity peak get good separation,Iohexol’s retention time (t_R) is 9.8min, internal standard’s retention time (t_R)is 15.3min(figure1).Use the ratio of chromatographic peak A as the abscissa denotes, mass concentration of iohexol(ug/ml)as the y-coordinate, Iohexol’s standard curve:Y=0.0026X+0.0095,r=0.9992.demonstrates that iohexol in 1^2000ug/ml range linear good, the method recovery of ioexol 10, 50 and 500 ug/ml is(78.73±10.05, 102.10±6.34 and 107.33±7.20)%.

Drug metabolism kinetic parameters The blood drug concentration of iohexol in rats bodies see table 1, the blood drug concentration - time curves of iohexol see Figure2.Pharmacokinetic computer program 3p87 is used to do data processing,after model fitting,the A I C minimum principle is used todetermine the blood drug concentration of iohexol liposome after intravenous injected into rats bodies are both match the one room model, the pharmacokinetic parameters of iohexol see table 2.

Experimental results of pharmacokinetics



(1 - iohexol, 2 - internal standard, t_{R1}=9.8min, t_{R2}=15.3min)
A: blank plasma b: iohexol standard solution c: blood sample added iohexol d: rats blood sample after iv.

Fig. 1. Iohexol HPLC chromatograms

Table 1. Rats plasma iohexol concentration after i.v. liposome freeze-dried needle ($\bar{\chi}$±s.d.,n=3)

Time	Plasma iohexol concentration (mg/ml)		
(h)	Heating group	Non-heating group	Standard product group
0.08	13.67±0.58	15.33±0.31	12.85±0.10
0.25	11.80±0.46	11.93±0.17	7.67±0.04
0.5	10.16±0.39	6.11±0.06	4.32±0.04
1.5	7.62±0.15	2.25±0.03	1.79±0.04
2	6.71±0.68	0.26±0.12	0.42±0.03
4	3.97±0.07	0.08±0.03	0.09±0.00
6	2.50±0.08	0.04±0.01	0.02±0.00

Table 2.iohexol drug metabolism kinetic parameters after i.v. liposome freeze-dried needles ($\bar{\chi}$±s.d., n=3)

Parameters	Units	Iohexolmetabolism kinetic parameters		
		Heating group	Non-heating group	Standard product group
Ke	1/h	0.29±0.01	1.89±0.18	1.70±0.04
t1/2(Ke)	h	2.38±0.03	0.37±0.03	0.41±0.01
AUC	(mg/ml)*h	35.45±1.40	11.09±0.25	8.50±0.12
V(c)	(mg/kg)/(mg/ml)	296.20±12.03	208.26±7.04	294.37±5.20
CL(s)	mg/kg/h/(mg/ml)	86.16±3.49	393.13±25.66	499.23±4.29

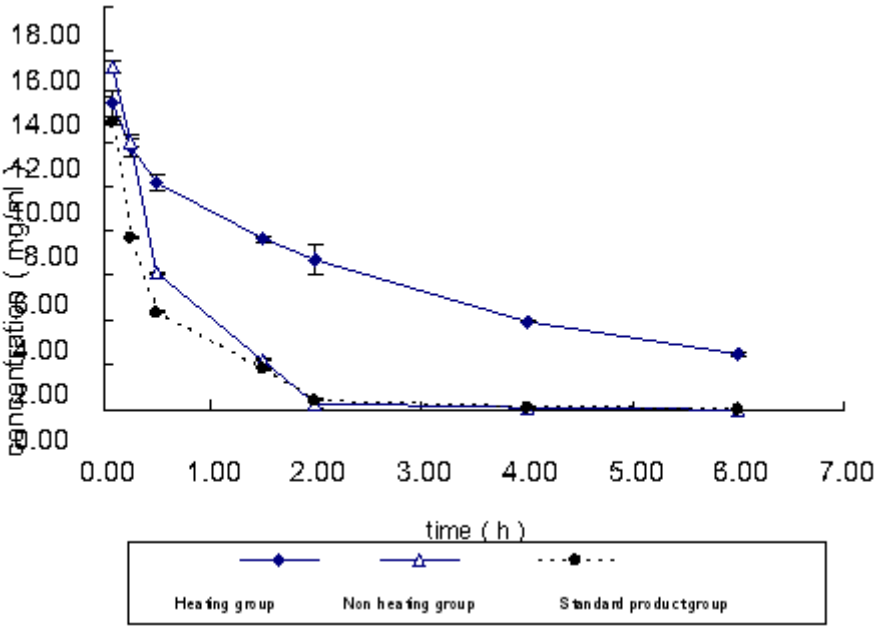


Fig. 2. Time - blood drug concentration curve after i.v. liposome freeze-dried needles (iohexol 3720mg/kg)

in rats bodies show that, freeze-dried needles including iohexol encapsulate with thermosensitive liposome. The t1/2 of heating group prolongs significantly compared with the non-heating group. AUC increases significantly; the AUC of heating group and non-heating group increases significantly compared with the standard product group, demonstrates that under the same dose condition, compared with intravenous inject standard product, freeze-dried needles including iohexol encapsulate with thermosensitive liposome can prolongs the time of iohexol in blood circulation, increases the bioavailability of iohexol; The thermosensitive liposome encapsulated with iohexol in C6 Glioma model are release theirs content sharply in heat group when the local tumor is heated.

DISCUSSION

Over half the century, oncology has made great progress around the world, integrated application of all existing possible ways to treat tumor has enjoyed popular support and has been accepted by clinical doctors, it has become the best and most popular model to treat malignant tumor. The cooperation of surgery, radiotherapy, chemotherapy, thermal therapy and biotherapy has made remarkable therapeutic effect in the treatment of many different kinds of malignant tumors(Chen *et al*,2003; Mane *et al*, 1999; Li *et al*, 1999).

As an auxiliary method to treat malignant tumor, thermal therapy has its distinguishing feature compared with other treatment: low cost, less side-effect, tumor immunity stimulation, curative effect affirmation(Seegenschmiedt *et al*, 1995). Using thermosensitive liposome as carrier, thermal therapy works with targeted chemotherapy are given increasing attention(Dresen *et al*, 2005; Takahashi *et al*, 2002; Aoki *et al*, 2004). Thermotherapy using laser, radio frequency, microwave or ultrasound focus, and triggers the thermosensitive liposome release the chemotherapeutics locally. Thermosensitive liposome has the character of temperature phase transition and encapsulation, and indiscriminate package of water soluble drug and fat soluble drug, when heated to phase-transition temperature, its content will release. However, the promotion and application of thermotherapy was restricted to a great extent because of the difficulty of temperature measure during treatment. The current thermometric method is using impaired thermometer or using body model to estimate the internal possible temperature distribution. The thermometric way is impaired punctuation way, it is hard to reflect the full view of temperature distribution and it always leads to complication such as infection, bleeding, etc. The body model estimation method could only give a rough estimation of temperature distribution, which is very different from the real temperature. The ideal temperature measure method should be non-impair, time-real and three-dimensional, only in this way, can the cells around the tumor be heated to death, avoiding the existing of "cold area" which leads to recur; meanwhile, only when the temperature is observe and control precisely can the normal tissue be avoid being damaged too much, and reduce the occur of complication. As a result, looking for an ideal method to measure the temperature is an important and difficult problem that is exigent to be solved. With CT is widespread gradually. CT scan could reveal the tumor image without damage, real-time and three dimensional, compared with MRI, quick imaging and low cost is its advantage. CT scan combine with temperature sensitive contrast agent is a promising ideal thermometric method.

Take advantage of temperature phase transition character and packaging character of thermosensitive liposome, its thermal treatment temperature (43°C) as the phase transition temperature, pack the CT contrast agent and chemotherapeutics together, make it a compound thermal sensitive liposome that packing two drugs at the same time; during the local mesenchyme thermal treatment, iv the drug, when the target area reach above the thermal treatment temperature (43°C), the thermosensitive liposome changes its structure, and release its extent, CT would detect the dose changing of local contrast agent, thus it could reveal the heating area effectively, avoid the failure possibility caused by uneven temperature of thermal treatment, and reduce the thermal damage to normal tissue greatly, remove the complication brought by the traditional temperature measurement. While use CT to measure the temperature range, thermosensitive liposome release the chemotherapeutics in the tumor,

express the combined effect of local thermotherapy and chemotherapy targeted heat, enhanced theirs curatives effect, avoid the side-effect of systemic chemotherapy. There isn't any study that treating tumor under CT observed and controlled temperature, Fossheim 2000 studied the temperature control feasibility of thermo-sensitive paramagnetic liposome in the MRI oriented thermal treatment(Fossheim *ET AL*,2000; Fossheim *ET AL*, 1997; Fossheim *et al*,1998).

This test aims at freeze-dried needles including iohexol encapsulate with thermosensitive liposome, intravenous injects to C_6Glioma model ratsÿunder the tumor local heating, non-heating condition, observe the pharmacokinetics in rats bodies,and set another normal group rats get intravenus injection with iohexol standard product solution as the comparation.The test of pharmacokinetics in rats bodies shows,iohexol encapsulate with thermosensitive liposome made into freeze-dried needles, can prolong the time of iohexol in blood circulation significantly, increase the absorb of iohexol. It is more favorable for the release of iohexol in rats bodies when the C_6Glioma model ratstumor local is heated.We can guess that the structure of thermosensitive liposome have changed in heat point the drug concentration may inrease sharply. theirs metabolism form are seem to freeiohexol. However, if the drug concentration in the heated local is increased or not,and if the toxicity it brings to each organizations while its bioavailability increased or not, further research in the future is needed.

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