Preparation and distribution of 5-fluorouracil $^{125}$I sodium alginate-bovine serum albumin nanoparticles

YI Yi-Mu¹, YANG Tang-Yu² and PAN Wei-Min³

**Subject headings** 5-fluorouracil (5-FU); sodium alginate-albumin; preparation of nanoparticle (NP); distribution

**Abstract**

**AIM** To prepare 5-FU sodium alginate $^{125}$I bovine serum albumin nanoparticles (BSA NP), to determine the radioactive count in different organs of rats at different time points after oral administration of 5-FU $^{125}$I sodium alginate-BSA NP and to calculate the kinetic parameters of its metabolism.

**METHODS** Emulsion solidification method was used to prepare 5-FU $^{125}$I sodium alginate-BSA NP, and to determine its diameter under transmission electronic microscope (TEM). Then the rate of NP and external drug releasing velocity were measured. Radioactive counting in different organs of rats was made after oral administration of the NP by GAMA Counter, and the kinetic parameters of drug metabolism were calculated by handling the data with the two-department model.

**RESULTS** The average arithmetic diameter of the NP was 166nm±34nm, the rate of 5-FU was 32.8% and the cumulative external releasing ratio amounted to 84.0% within 72 hours. The NP was mainly distributed in the liver, spleen, lungs and kidneys after NP oral administration to rats. The microscopic experiment showed that NP was distributed in the Kupffers cells of liver, liver parenchymal cells and the phagocytes of spleen and lungs. The kinetic parameters of metabolism were: $T_{1/2} = 9.42$ h, $C_{max} = 2.45 \times 10^7$ Bq, $T_{max} = 2.18$ h, AUC=$148 \times 10^9$ Bq. CONCLUSION NP is difficult to pass through the blood-cerebral barrier, and $^{125}$I sodium alginate-BSA NP enters the body circulation by gastrointestinal passage.

**INTRODUCTION**

Nanoparticle (NP) is a colloidal dispersion system, with diameters ranging from 10nm to 1000nm. The particles exist mainly in the organs rich in phagocytes after absorption, such as liver, spleen and lymph system. Since its introduction in the 1980s, scientists have done a lot of researches on its preparation, stability and targeting[1,2]. Now most materials used to prepare NP are synthetic substances, e.g. cyanoacrylate, methylacylate and polylactic acid. However, we selected natural substances—sodium alginate and bovine serum albumin as carriers, and 5-fluorouracil (5-FU) as model drug to prepare NP by emulsion solidification, and studied its distribution after oral administration.

**MATERIALS AND METHODS**

**Animal**

The Kunming rats weighing 20 g±2 g, were provided by the Experimental Animal Centre of Tongji Medical University.

**Reagent**

Sodium alginate (chemical reagent), bovine serum albumin (Sigma), pentane dialdehyde (biochemical reagent, Merck), Na $^{125}$I (specific activity 7.78 TBq/L, Chinese Atomic Energy Institution), IV liquid nuclear emulsion (Physics Institute of Chinese Atomic Energy Research Institution).

**Preparation of 125I bovine serum albumin (BSA)**

The BAS (10mg) was dissolved in 10mL distilled water and marked with $^{125}$I according to the chloromercuric-T method. After the reaction was completed, the mixture was separated by column chromatography (10 mm×340 mm), using Sephadex G-50 as column material and Na$_2$S$_2$O$_3$ as eluant. Then the
radio-chemical purity of eluate was determined by dichloroacetic acid method.

**Preparation of 125I sodium alginate-BAS NP**

According to the literature, 1 mL-above I\(^{125}\)BSA (1g/L, SpA 107 Bq) was added into sodium alginate solution to prepare sodium alginate-BSA NP.

**Determination of the diameter of nanoparticles**

The NP size in the suspension was detected on TEM.

**Determination of the encapsulation efficiency**

The standard curve of 5-FU was drawn first. One hundred and five mg of 5-FU was weighed precisely, dissolved in thin HCl solution (9-1000) and adjusted to given volume. This solution was diluted to its half concentration. After 3.0, 6.0, 9.0, 12.0 and 15.0 mL-diluted solution were taken into 100mL-volumetric flasks and adjusted to given volume respectively, their absorbance (\(A\)) values were detected on ultraviolet-visible spectrophotometer at 265 nm. The following regression equation was obtained:

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C = 16.72A \pm 0.0416 \quad (r = 0.9998).
\]

A total of 1.0 mL 5-FU NP suspension was added accurately to a test tube with 9 mL 6mol/HCl. The mixture was hydrolyzed for 20h at 100°C water bath. The hydrolyzate was adjusted to given volume with distilled water to detect its A value as standard curve item, the hydrolyzate of bland NP suspension served as control. The result showed that the control group had no obvious absorption at 265nm (A=0.01). By putting the A value into the regression equation we worked out the concentration of 5-FU in the hydrolyzate and got the encapsulation efficiency consequently.

**Determination of recovery rate**

The same quantities of albumin, sodium alginate and pentane dialdehyde as “Preparation of NP” items were mixed with 5-FU weighed precisely, the mixture was dissolved in distilled water. Its A value was detected according to the standard curve and the content of 5-FU was calculated.

**Determination of drug release**

Appropriate quantity of 5-FU NP suspension was taken precisely, washed for three times with distilled water, and suspended in 200mL-thin HCl solution (9-1000). The drug release state of the suspension was measured at 37°C by paddle method (100r/min). The samples were taken at the beginning, the 2nd, 4th, 8th, 12th, 24th, 48th and 72nd hour, and filtered through 0.2\(\mu\)m microporous membrane. The filter liquor was detected at 265nm to get its A value and cumulative rate of drug release.

**The distribution of 125I sodium alginate-BSA in rats**

Fourty-five Kunming rats were divided into 15 groups (3 rats per group). After starvation for 16h, the rats were given the 5-FU NP orally 148×10\(^{8}\)Bq per rat. Right after its blood was taken from the eye at different time points (heparin was added to prevent coagulation), the rats were sacrificed by breaking its spine. The relevant tissues and organs were taken. When the water had been absorbed with filter paper after washing, they were weighed. The amounts of the NP in unit weight in different tissues were measured by GAMA Counter. In accordance with the counting result, we calculated the tissue blood ratio at different time points and used two-department model to process the data in order to get pertinent kinetic parameters.

**Radioautography**

While carrying out the histological experiment, we chose the tissues of liver, spleen, lungs, and kidneys at some time points (the 4th, 8th and 12th hour), and frozen sections and paraffin wax sections about 6\(\mu\)m-thick were made. IV liquid emulsion was used for microradioautography.

**RESULTS**

**Diameter of NP**

On the 5-time enlarged photograph (diagram 1) of TEM, the average arithmetic mean diameter of 300 detected NPs was 166 nm±33 nm, 8% < 130 nm, 36% 130 nm - 150 nm, 42% 150 nm - 170 nm and 14% >180nm.

**Encapsulation efficiency**

After two batches of 5-FU NPs were hydrolyzed in acid medium, their encapsulation efficiency was calculated (Table 1).

| Weight of drug added (g) | A value | Weight of drug encapsulated (g) | Encapsulation efficiency (%) |
|-------------------------|---------|--------------------------------|-----------------------------|
| 0.6162                  | 0.679   | 0.208                          | 33.8                        |
| 0.6113                  | 0.625   | 0.193                          | 31.6                        |
| 0.5917                  | 0.676   | 0.195                          | 32.9                        |

**Rate of recovery**

The rate of recovery of three batches of samples is shown in Table 2.
Table 2  Rate of recovery of 5-FU

| Weight of 5-FU added (g) | Weight of 5-FU determined (g) | Rate of recovery (%) |
|-------------------------|-------------------------------|---------------------|
| 0.1348                  | 0.1326                        | 98.4                |
| 0.1463                  | 0.1449                        | 99.1                |
| 0.1429                  | 0.1395                        | 97.6                |

Variation of drug releasing ratio of 5-FU NP before and after storage
The cumulative ratio of drug release within 72 h decreased from 84.8% before storage to 80.6% after storage under 40°C±1°C for three months.

The pharmacokinetics of 125I sodium alginate-BSA NP in rats
Thirty min after the rats administrated 125I sodium alginate-BSA NP orally, obvious radioactivity was shown in its blood. Eight hours after the administration, the radioactivity of their liver, spleen and lungs was 2.08 times, 2.32 times and 1.60 times, as much as that of blood, while 24 hours after the administration, they decreased to 1.18, 1.22 and 0.87 times respectively (Table 3).

The data in the parentheses show the ratio of radioactivity of the relative organ to that of blood. The data in Table 3 were put into computer, and processed according to the two-department model to calculate the relevant pharmacokinetic parameters. The results are shown in Table 4.

Radioautography
The microradioautographical experiment indicated that the 125I sodium alginate-BSA NP were mainly distributed in the Kupffer cells of liver and liver parenchymal cells (Figure 2) after oral administration to rats. And there were Ag particles in the phagocytes of spleen (Figure 3) and also in the pulmonary cells (Figure 4).

Table 3  Distribution of 125I sodium alginate-BSA NP in rats  (n = 3, dosage 148×108Bq per rat, po.)

| Organ         | Value of radioactive counting of 100mg tissue in 30s at different time points |
|---------------|--------------------------------------------------------------------------------|
|               | 1h          | 4h          | 8h          | 16h         | 24h         | 36h         |
| Blood         | 502±100     | 601±135     | 373±22      | 298±19      | 191±17      | 129±23      |
| Heart         | 436±110(0.86)| 801±66(0.33)| 265±23(0.71)| 230±23(0.77)| 190±10(0.99)| 87±35(0.67) |
| Liver         | 436±110(0.86)| 908±100(1.51)| 777±69(2.08)| 405±97(1.36)| 226±16(1.18)| 153±21(1.19)|
| Spleen        | 663±140(1.32)| 1029±168(1.71)| 834±272(2.32)| 436±195(1.46)| 233±25(1.22)| 169±27(0.95)|
| Lungs         | 717±314(1.42)| 818±172(1.36)| 597±121(1.60)| 443±31(1.49)| 166±40(0.87)| 119±27(0.92)|
| Kidneys       | 526±139(1.05)| 185±39(0.14) | 401±71(1.08) | 387±105(1.30)| 133±13(0.69)| 123±14(0.95)|
| Brain         | 105±31(0.21)| 1124±99(0.19)| 111±47(0.30)| 96±27(0.32) | 50±26(0.26) | 39±11(0.30) |
| Stomach       | 89654±7143  | 28674±3647  | 8362±1902   | 4641±1400   | 698±34     | 527±114     |
| Intestine     | 765±137     | 5573±891    | 30±599      | 403±118     | 115±64     | 204±46      |
| Bowel         | 1256±664    | 639±1023    | 30±599      | 3221±863    | 125±60     | 256±102     |

Table 4  Pharmacolinetical parameters of 125I sodium alginate-BSA NP in rats

| Parameter | Unit | Value   |
|-----------|------|---------|
| A         | Bq   | 3022×10² |
| α         | 1/h  | 0.07    |
| B         | Bq   | 3.03×10⁷ |
| β         | 1/h  | -0.11   |
| Ka        | 1/h  | 1.46    |
| V/F       | Bq   | 1428×10³ |
| T1/2α     | h    | 9.42    |
| T1/2β     | h    | -6.07   |
| T1/2Ka    | h    | 0.47    |
| K0        | 1/h  | -0.11   |
| K1        | 1/h  | 0.07    |
| K2        | 1/h  | -0.00035|
| AUC       | Bq/h*| 3.9×10⁹ |
| CL(s)     | Bq/h| 105×10⁷ |
| T(peak)   | h    | 2.18    |
| Cmax      | Bq   | 2.45×10⁷ |
DISCUSSION

Since the introduction of NP in the early 80s, scientists have done a lot of researches in its preparation, stability, distribution and targeting. It is suggested that the stability of NP is better than that of liposome and it can carry more drug than liposome.

At present, the materials, which are reported to prepare NP in literature abroad, are all man-made synthetic materials. While NP is mostly administrated in travenously, we chose natural polysaccharide and protein to compose the carrier, and observed its distribution and metabolism after oral administration to rats.

Thirty minutes after the rats administrated $^{125}$I sodium alginate-BSA NP orally, the radioactive substances in their blood were detected.

The results indicate that NP is mainly distributed in the liver, spleen and lungs, quantitatively in heart and slightly in brain. This shows NP is difficult in passing through the bloock-cerebral barrier. The experiments also display that the $^{125}$I sodium alginate-BSA NP enters the body-circulation by gastrointestinal passage and is chiefly distributed in the tissues rich in phagocytes, such as in the liver and spleen.

In the study of its distribution, we discovered that there is still a large amount of radioactivity in the gastrointestinal passage 12 hours after the administration. This illustrates that the absorption of NP is not complete because NP exists in the suspension as cloudy polymer but not mono-dispersion, and the large diameter of the polymer makes it difficult for the NP to be absorbed by the gastrointestinal passage.

REFERENCES

1. Keyser JLDE, Poupaert JH, Dumont P. Poly (diethyl methylidenemalonate) Nanoparticles as a potential drug carrier: preparation, distribution and elimination after intravenous and peroral administration to mice. *J Pharm Sci*, 1991;80:67-70
2. Alain R, Brigitte C, Roger LV, Louis T. Blood clearance and organ distribution of intravenously administered polymethacrylic nanoparticles in mice. *J Pharm Sci*, 1989;78:481-484
3. Dieter Scherer, J.R. Robinso N, Jorg Kreuter. Influence of enzymes on the stability of polybutylcyanoacrylate nanoparticles. *In J Pharm*, 1994;101:165-168
4. Nazin Ammoury, Hatem Fessi, Devissaguet JP, Puisieux F, Benita S. In vitro release kinetic pattern of indomethacin from poly (D, L-Lactide) Nanocapsules. *J Pharm Sci*, 1990;79:763-767

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