Acta Medica Okayama

Volume 40, *Issue* 1 1986 *Article* 9

FEBRUARY 1986

Organ distribution of asialo-red blood cell ghosts: an attempt at targeting to the liver.

Shu Nakamoto* Yoshito Sadahira†

Masaharu Mori‡ Michiyasu Awai**

*Okayama University,

†Okayama University,

‡Okayama University,

**Okayama University,

Organ distribution of asialo-red blood cell ghosts: an attempt at targeting to the liver.*

Shu Nakamoto, Yoshito Sadahira, Masaharu Mori, and Michiyasu Awai

Abstract

We investigated the organ distribution of four types of red blood cells (RBC) preparations: native RBC, asialo-RBC, native ghosts and asialo-ghosts. Intravenously injected asialo-ghosts were rapidly removed from the blood stream and accumulated mainly in the liver 120 min after the injection. Our results suggest that asialo-ghosts are a simple and effective carrier for targeting of drugs to the liver.

KEYWORDS: red blood cell ghosts, sialidase-treatment, orgam distribution, targeting to liver.

---

*PMID: 3008506 [PubMed - indexed for MEDLINE]

Acta Med Okayama 40 (1) 61-64 (1986)

— Brief Note —

Organ Distribution of Asialo-Red Blood Cell Ghosts: an Attempt at Targeting to the Liver

Shu Nakamoto, Yoshito Sadahira, Masaharu Mori and Michiyasu Awai

First Department of Pathology, Okayama University Medical School, Okayama 700, Japan

We investigated the organ distribution of four types of red blood cells (RBC) preparations: native RBC, asialo-RBC, native ghosts and asialo-ghosts. Intravenously injected asialo-ghosts were rapidly removed from the blood stream and accumulated mainly in the liver 120 min after the injection. Our results suggest that asialo-ghosts are a simple and effective carrier for targeting of drugs to the liver.

Key words: red blood cell ghosts, sialidase-treatment, organ distribution, targeting to liver.

Recently, the administration of liposome-entrapped drugs to animals has been widely studied. This method can reduce the side effects of drugs and can "target" them to a certain organ or to a certain cell type (1). Modification of the liposomal surface with antibodies or carbohydrates (1) alters their destination. Since liver cells have galactose-receptors on their surface (2), the introduction of galactose residues to the liposomal surface by asialo-glycolipids or asialo-proteins gives the liposomes an affinity for liver cells (1). Red blood cells (RBC) also have been used as a carrier of drugs by converting them into so-called resealed ghosts; this apporoach has been attempted clinically (3) and markedly prolonges the blood concentration of some drugs (4). Sialidase-treated RBC show a shorter life span in the blood stream and accumulate in the liver (5). In the present study, we examined the usefulness of asialo-ghosts for targeting to the liver. Four types of RBC preparations (native RBC, asialo-RBC, native ghost and asialo-ghost) were prepared, and their blood clearance and organ accumulation were investigated.

Fresh RBC were obtained from the jugular vein of Swiss mice (Animal Center of Okayama University Medical School) with heparin and washed four times with cold Dulbecco's phosphate-buffered saline (PBS). Asialo-RBC were prepared as follows: Packed RBC (1.0 ml) were incubated with 0.5 ml of PBS and 0.5 ml of sialidase type IV (0.4 unit/ml, Sigma) at 37℃ for 60 min and then washed with cold PBS three times. By this treatment, 56 μg sialic acid was released from 1 ml of packed RBC. Resealed RBC ghosts were prepared according to the method described by Furusawa (6). Packed native RBC and asialo-RBC (0.5 ml) were diluted with 4.5 ml of reverse PBS (137 mM KCl, 2.7 mM NaCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4 and 4 mM $MgCl_2$) and dialyzed against 2400 ml of one-sixth-diluted reverse PBS for 30 min at room temperature. After being re-dialized against 2400 ml of reverse

Table 1 Organ concentrations[a] of native RBC, asialo-RBC, native ghosts and asialo-ghosts 120 min after the injection.

	Blood	Liver	Spleen	Kidney
Native RBC	52.5±13.3	4.1±0.5	11.8±5.2	5.1±2.9
Asialo-RBC	26.4±2.9	18.7±2.7	7.4±1.3	3.0±1.2
Native ghosts	12.7±1.3	18.3±1.8	64.9±13.1	7.5±2.8
Asialo-ghosts	2.3±0.7	39.3±4.4	28.8±3.6	10.4±0.4

a: The values were calculated as (% of injected dose)/(wet weight, g, of the organ) and expressed as the mean ± SD.

PBS for 30 min at room temperature, they were washed with cold PBS three times. The four types of RBC preparations thus prepared were labelled with $Na_2{}^{51}CrO_4$ (Amersham Corp.) by the method described by Berlin (7). After washing, they were resuspended in PBS and the hematocrit value was adjusted to about 5%.

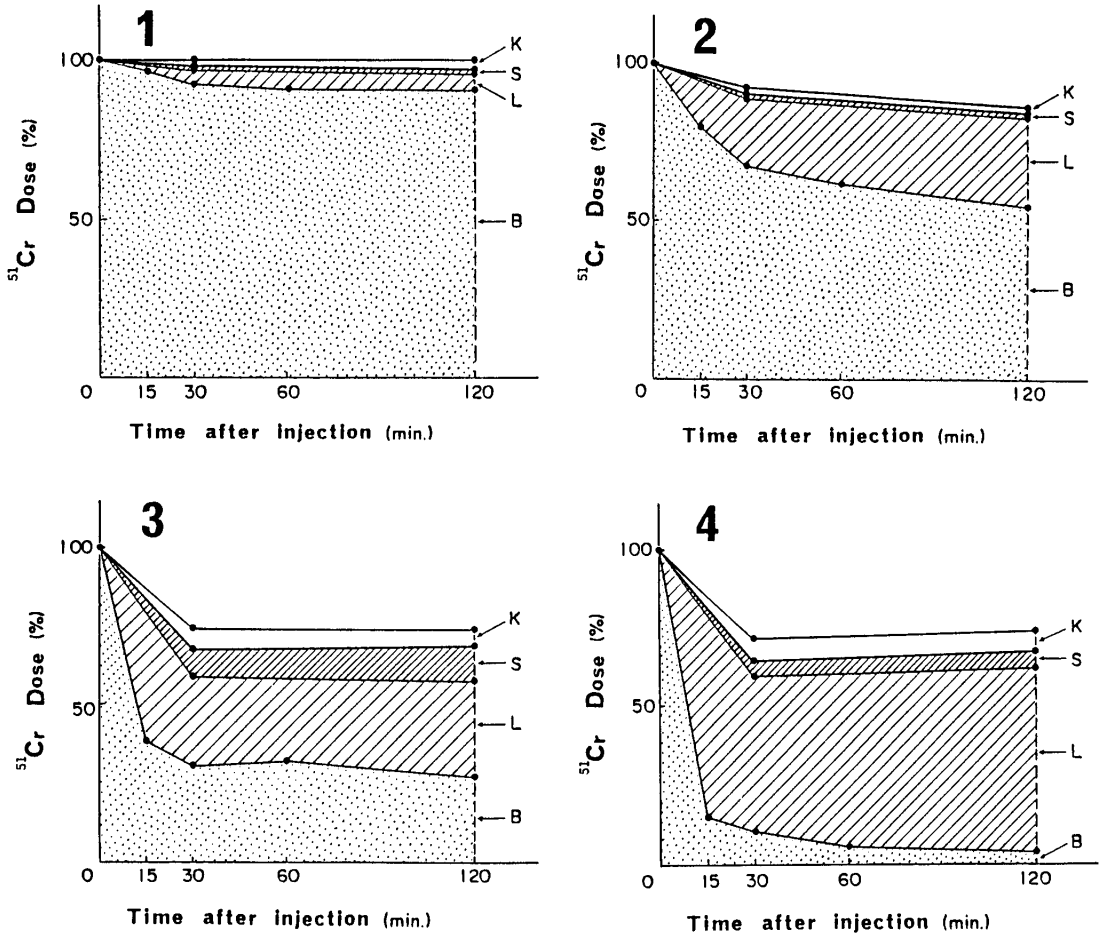
Thirty-two Swiss mice were divided into four groups and were administered 0.1 ml of the four respective suspensions via the tail vein. A half of the mice in each group were bled from the orbital venous plexus (about 0.1 ml) 15 min after the injection and sacrificed by cutting the abdominal aorta 30 min after the injection. The other half were bled 60 min after the injection and sacrificed 120 min after the injection. The removed organs (liver, spleen and kidney) and blood were put into test tubes and their radioactivities of Cr^{51} were estimated using an Aloca ARC-500 auto well gamma system. Total circulating blood volume was calculated by multiplying the body weight by 0.07 (8).

Fig. 1 shows the results of the experiments in which the native RBC were administered to mice. Almost all of the radioactivity (90.0 ± 7.3%) was found in the blood stream 120 min after the injection, compared to the liver (6.4±0.7%), spleen (1.4±0.2%) and kidney (2.3±0.9%). The RBC in these organs were thought to be derived from the blood remaining in the blood vessels of the organs.

Treatment with sialidase increased RBC affinity to the liver (Fig. 2, Table 1). However, the radioactivities of the spleen and kidney were almost the same as those in the experiments with native RBC. The hepatic concentration of asialo-RBC was five times as high as that of native RBC 120 min after the injection (Table 1). These results are consistent with those reported by Durocher *et al.* (5).

Native ghosts were removed more rapidly from the blood stream. Blood, liver, spleen and kidney showed 27.9±1.0, 39.3±3.5, 11.1±2.5 and 4.9±1.7% of the injected dose, repectively, 120 min after the injection (Fig. 3). Accumulation of this preparation in the liver and spleen was prominent. The splenic concentration of native ghosts was four times as high as the hepatic concentration after 120 min (Table 1). Alterations in RBC shape and rigidity induced by hypotonic treatment (9) facilitate entrapment of the ghosts by the reticulo-endothelial system. The liver and spleen are the major components of this system. The recovery rate of the radioactivity was about 83%, which was the lowest among the four preparations. This suggests the uptake of the native ghosts by the reticulo-endothelial system in other organs.

Many other techniques for introducing drugs into RBC have been reported: modifying the hypotonic process (3, 4, 9), applying an electric fields (10) or administering amphotericin B (11). However, it is neces-



Figs. 1-4 Serial changes in the organ distribution of the four RBC preparations. Swiss mice were injected with 0.1 ml of native RBC (Fig. 1), asialo-RBC (Fig. 2), native ghosts (Fig. 3) and asialo-ghosts (Fig. 4), which were already labelled with ^{51}Cr. The radioactivity in the blood (B) was measured 15, 30, 60 and 120 min after the injection. The radioactivity in liver (L), spleen (S) and kidney (K) was measured 30 and 120 min after the injection, and calculated as the percent of the injected dose.

sary to prevent the removal of the ghosts from the blood stream by the reticulo-endothelial system. Zimmerman *et al.* (10) reported that only 8.3% of the injected electric field-prepared ghosts were found in the blood stream, but that 55.2% were found in the liver and 4.5% in the spleen 30 min after the injection. Interestingly, asparaginase entrapped in hypotonically prepared ghosts had a somewhat longer life span in the blood stream than free asparaginase (4).

Asialo-ghosts disappeared from the blood stream most rapidly and accumulated mainly in the liver, where $75.0 \pm 2.5\%$ of the injected dose was found 120 min after the injection (Fig. 4). The radioactivity in the blood, spleen and kidney was 4.6 ± 1.3, 4.3 ± 0.4 and $6.6 \pm 0.1\%$, respectively. The hepatic concentration of asialo-ghosts was ten times as high as that of native RBC and twice as high as that of native-ghosts 120 min after the injection, which the splenic

concentration of asialo-ghosts was twice as low as that of native-ghosts (Table 1). The hepatic concentration of asialo-ghosts was higher than the splenic concentration.

Treatment of RBC with sialidase removes sialic acid from their surface, which resultes in the reduction of the surface charge and exposure of penultimate galactose residues. Galactose-receptors on liver cells (2) are thought to play an important role in the capturing of both asialo-ghosts and asialo-RBC by the liver (12), though a decrease in the surface charge of RBC may make their role less important as seen in aged RBC (13). It is reported that both liver parenchymal cells and Kupffer cells have galactose-receptors, and form aggregates with asialo-RBC (12). Other organs such as the spleen and kidney are thought to not have this receptor.

It is likely that administration of drugs entrapped in asialo-ghosts targets the drugs directly not only to Kupffer cells but also to liver parenchymal cells and can raise their therapeutic effects by increasing their intra- and extra-cellular concentration. Therefore, greater effectiveness of anti-neoplastic drugs may be expected by applying this method against liver neoplasms, especially against hepatomas having the galactose-receptor. This method may improve the treatment of hemochromatosis, a disease of excess iron deposition in liver parenchymal cells as well as in Kupffer cells.

References

1. Ghosh P, Das P K and Bachhawat B K : Targeting of liposomes towards different cell types of rat liver through the involvement of liposomal suface glycosides. Arch Biochem Biophys (1982) **213**, 266-270.
2. Matsuura S, Nakada T, Sawamura T and Tashiro Y : Distribution of an asialoglycoprotein receptor on rat hepatocyte cell surface. J Cell Biol (1982) **95**, 864-875.
3. Beutler E, Dale G L, Guinto E and Kuhl W : Enzyme replacement therapy in Gaucher's disease : Preliminary clinical trial of a new enzyme preparation. Proc Natl Acad Sci USA (1977) **74**, 4620-4623.
4. Updike S J, Wakamiya R T and Lightfoot E N : Asparaginase entrapped in red blood cells : Action and survival. Science (1976) **193**, 681-683.
5. Durocher J R, Payne R C and Conrad M E : Role of sialic acid in erythrocyte survival. Blood (1975) **45**, 11-20.
6. Furusawa M, Yamaizumi M, Nishimura T, Uchida T and Okada Y : Use of erythrocyte ghosts for injection of substances into animal cells by cell fusion. Methods Cell Biol (1976) **14**, 73-80.
7. Berlin N I : Detemination of red blood cell life span. J Am Med Assoc (1964) **188**, 375-378.
8. Keighley G, Russell E S and Lowy P H : Response of normal and genetically anemic mice to erythropoietic stimuli. Br J Haematol (1962) **8**, 429-441.
9. Ting-Beall H P, Costello M J, Shoemaker D and Holland V F : Ultrastructure of hemoglobin-depleted human erythrocyte resealed ghosts. Biochim Biophys Acta (1981) **640**, 807-811.
10. Zimmermann U, Pilwat G and Esser B : The effect of methotrexate in mice. J Clin Chem Clin Biochem (1978) **16**, 135-144.
11. Kitao T and Hattori K : Erythrocyte entrapment of daunomycin by amphoterincin B without hemolysis. Cancer Res (1980) **40**, 1351-1353.
12. Schlepper-Schäfer J, Kolb-Bachofen V and Kolb H : Analysis of lectin-dependent recognition of desialated erythrocytes by Kupffer cells. Biochem J (1980) **186**, 827-831.
13. Yaari A : Mobility of human red blood cells of different age groups in an electric field. Blood (1969) **33**, 159-163.

Received: June 22, 1985
Accepted: September 24, 1985

Reprint requests to:
Mesaharu Mori
First Department of Pathology
Okayama University Medical School
2—5—1 Shikata-cho
Okayama 700, Japan