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Research Paper

Pharmacokinetic and Tissue Distribution Mechanism of Mouse Recombinant Heat Shock Protein 70 in Mice

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Purpose. To investigate the *in vivo* pharmacokinetics and uptake mechanisms of recombinant mouse heat shock protein 70 (Hsp70) by hepatocytes in mice.
Methods. The tissue distribution and intrahepatic localization of Hsp70 were determined after an intravenous injection of ^{111}In-Hsp70 (^{111}In-Hsp70) into mice. Ligands of CD91 or scavenger receptors were injected prior to Hsp70 to examine the involvement of these molecules on the distribution of ^{111}In-Hsp70. The uptake of ^{111}In-Hsp70 by primary mouse hepatocytes was also examined.
Results. After intravenous injection, ^{111}In-Hsp70 was rapidly eliminated from the circulation and taken up mainly by the liver. The hepatic uptake was significantly inhibited by preinjection of ligands for CD91 or scavenger receptors. The separation of liver-constituting cells revealed a major contribution of hepatocytes to the overall hepatic uptake of ^{111}In-Hsp70. The uptake of ^{111}In-Hsp70 by cultured hepatocytes was inhibited by a CD91 ligand or anti-CD91 anibody. In addition, after subcutaneous injection, ^{111}In-Hsp70 gradually disappeared from the injection site and accumulated in primary lymph nodes.
Conclusions. These results indicate for the first time that intravenous Hsp70 is, at least partially, recognized by CD91 and eliminated by hepatocytes, whereas subcutaneous Hsp70 is efficiently delivered to regional lymph nodes.

KEY WORDS: delivery; heat shock protein 70; hepatocytes; pharmacokinetics; uptake; vaccine.

INTRODUCTION

Heat shock proteins (Hsps) are a family of highly conserved molecules that are induced under stress conditions and exhibit ATPase activity and peptide binding activity (1). They are found in both prokaryotic and eukaryotic cells. Hsps play essential roles in protein folding, degradation of misfolded proteins, and metabolism (2,3). They recognize exposed hydrophobic surfaces of nonnative proteins, which are buried inside proteins under native states. Hsps noncovalently interact with those hydrophobic surfaces and prevent proteins from undergoing irreversible multimeric aggregation.

The search for effective cancer immunotherapies has explored numerous vaccine approaches using Hsps. Hsps can present a broad repertoire of tumor antigens to dendritic cells (DCs). Immunization with endogenous or synthetic peptide-bound Hsp or Hsp-antigenic peptide-fusion protein can result in MHC class I antigen-processing pathways in DCs. After uptake by DCs, Hsp-antigen peptide complex leads to priming strong $CD8^+$ T-cell responses (4,5). Hsps also elicit innate immunity, which is characterized by the release of cytokines from DCs and by the activation of immune cells including natural killer cells (6). Furthermore, Hsps deliver maturation signals to DCs by upregulating the expression of costimulatory and antigen-presenting molecules, including B7-1, B7-2, and MHC class II molecules (7–9), in addition to functioning as cytokines that attract DCs and T cells to tumors.

Recently, it has been reported that Hsps are taken up by Hsp receptors, CD91 and LOX-1, on antigen presenting cells (APCs), which would lead to cross-priming. CD91 is a member of the lipoprotein receptor family and is expressed on a variety cells including macrophages, fibroblasts, smooth muscle cells, and hepatocytes (10). CD91 is also involved in Hsp-mediated peptide re-presentation by macrophages (11). In addition, LOX-1, one of the scavenger receptors, is reported to be the main Hsp binding protein on DCs (12). LOX-1 is mainly expressed on endothelial cells, fibroblasts, smooth muscle cells and macrophages, and DCs (13). These observations suggest that the targeting of Hsp-antigen peptide complex to immune cells via CD91 or LOX-1 is a useful approach to initiating protective MHC class I–dependent immune responses (12,14). However, these mechanistic studies on the cellular uptake of Hsps were carried out only *in vitro* using cultured APCs. To our knowledge, there are no reports on the *in vivo* disposition characteristics of Hsps and their cellular uptake mechanism.

To obtain effective immune responses and obtain the optimal molecular design for an Hsp-based vaccine, it is necessary to elucidate the *in vivo* fate of Hsps after administra-

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ABBREVIATIONS: α_2-M, α_2-macrogrobulin; AUC, the area under plasma concentration-time curve; CL, clearance; DTPA, diethylenetriaminepentaacetic acid; Hsp70, heat shock protein 70; PAGE, polyacrylamide gel electrophoresis.

tion to the body. Therefore, in the current study, we performed a pharmacokinetic analysis of recombinant mouse Hsp70, the most abundant and important form of Hsps, in mice after intravenous or subcutaneous injection. We demonstrated that the liver is the main organ involved in the uptake of Hsp70 and hepatocytes play a major role in this hepatic uptake. Involvement of CD91 has also been shown.

MATERIALS AND METHODS

Animals

Male ddY mice (26–28 g) were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Animals were maintained under conventional housing conditions. All animal experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocols for animal experiments were approved by the Animal Experimentation Committee of the Graduate School of Pharmaceutical Sciences of Kyoto University.

Purification of Recombinant Mouse Hsp70

Recombinant mouse Hsp70 protein was expressed from the pTrc99A plasmid constructed by cloning the 1.96-kb Nco I-Xba I fragment of the genomic mouse clone *hsp70.1*, which was kindly supplied by Dr. Paul Slusarewicz (Mojave Therapeutics, Inc., New York, NY, USA). The expression of Hsp70 in DH5α cells was induced by 1mM isopropyl-β-D-thiogalactopyranoside (IPTG), and the harvested cells were

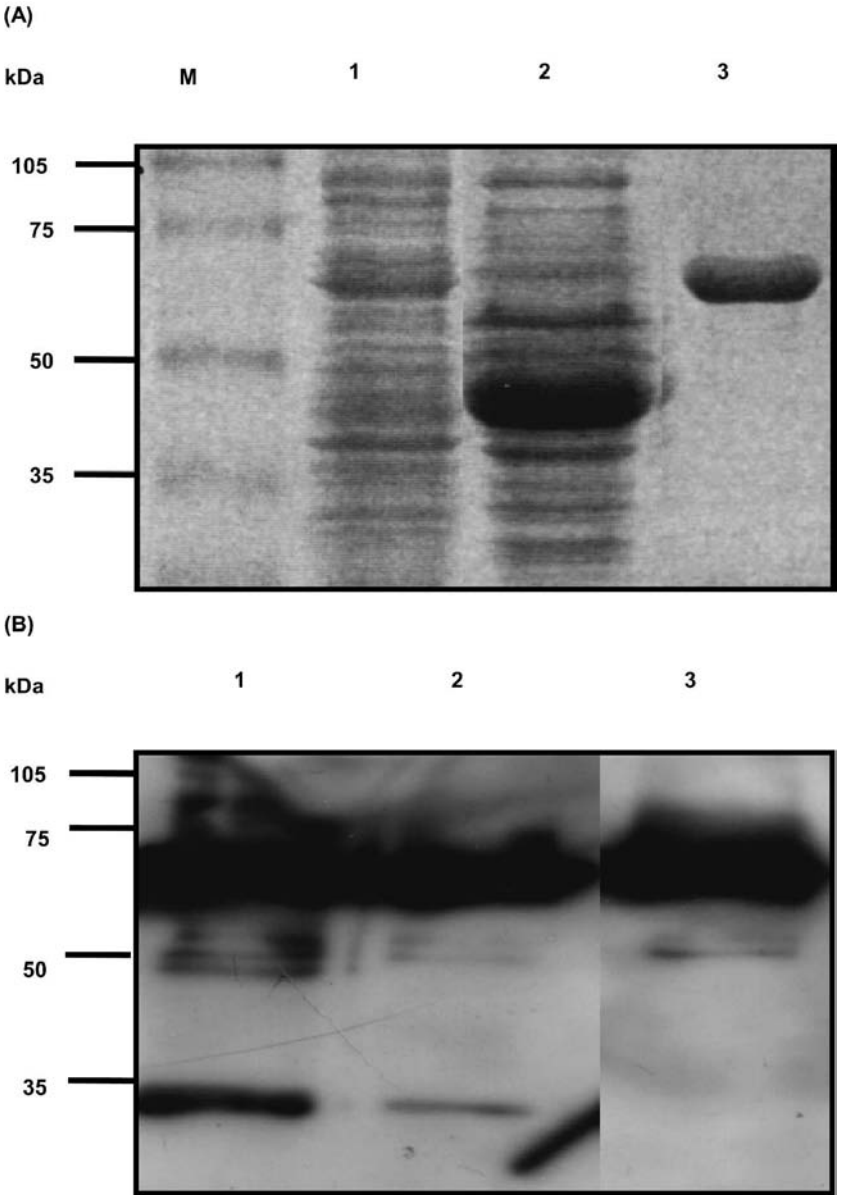

Fig. 1. SDS-PAGE (A) and Western blotting (B) of purified murine Hsp70. Lane M: marker proteins. Lane 1: *Escherichia coli* supernatant after sonication. Lane 2: after DEAE sephacel anion-exchange chromatography. Lane 3: after ATP affinity chromatography.

disrupted. The clarified supernatants were loaded onto a DEAE Sephacel column (Sigma Aldrich, USA), and Hsp70 was eluted by applying 20 mM Tris-HCl solution containing 100 mM NaCl. The eluate was precipitated by addition of $(NH_4)_2SO_4$ to 80% saturation followed by dialysis. Subsequently, the Hsp70 solution was loaded onto an ATP-agarose column (Sigma Aldrich) and eluted with 20 mM Tris-HCl solution containing 3 mM ATP. The eluate was concentrated by ultrafiltration, then lyophilized.

Western Blotting

Protein samples were run on 10% sodium dodecyl sulfate-polyacryl amide gel electrophoresis (SDS-PAGE). The proteins were transferred to a polyvinyldiene difluoride membrane (Immobilon P, Millipore, USA), blotted with antibody, and detected by enhanced chemiluminescence (ECL). Mouse anti-Hsp70 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used for the 1-h incubation of the

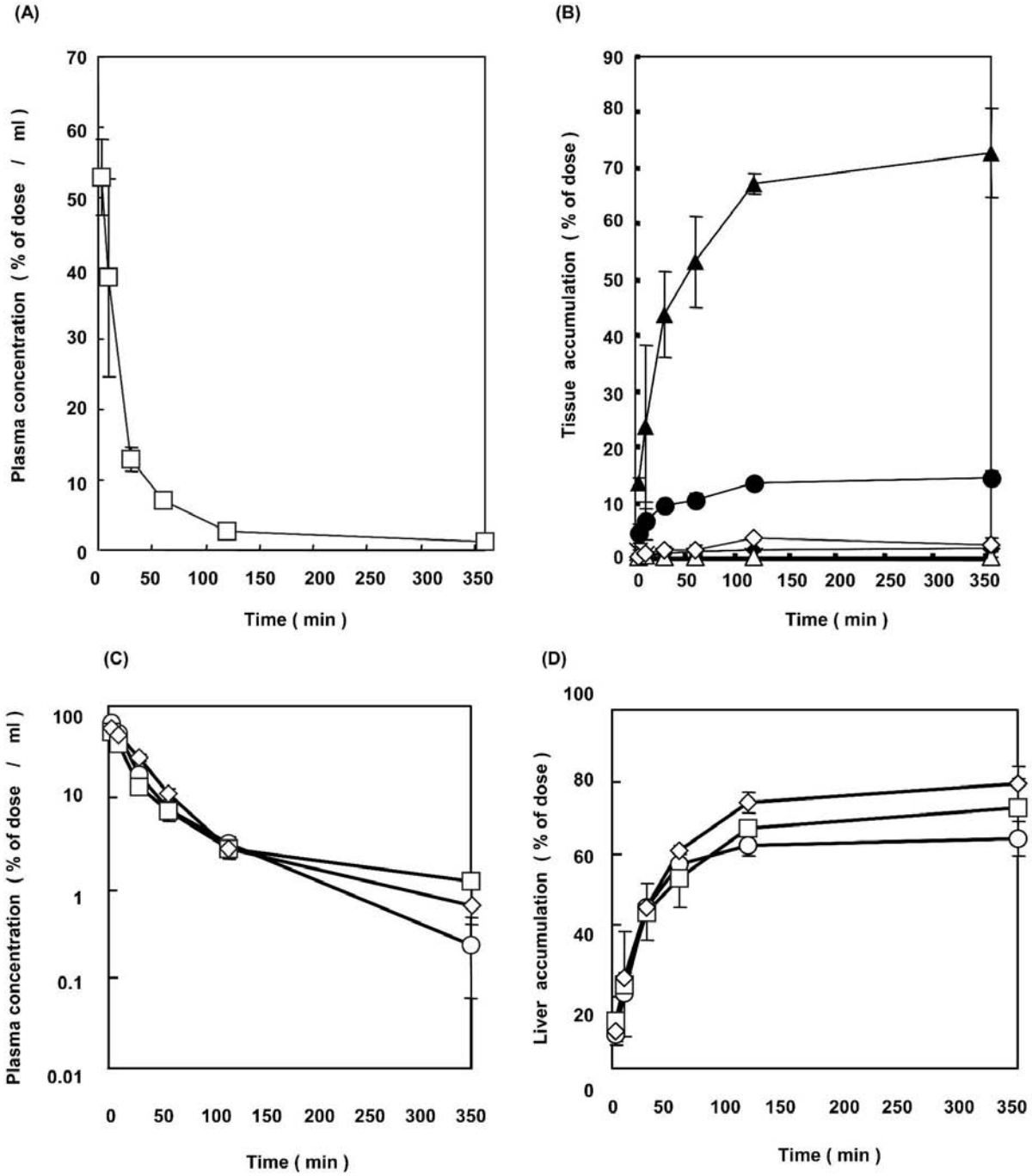

Fig. 2. Tissue distribution of ^{111}In-Hsp70. Time-course of plasma concentration (A) and tissue accumulation (B) of ^{111}In-Hsp70 in mice after intravenous injection at a dose of 10 μg/mouse: plasma (□), liver (▲), kidney (●), urine (◊), spleen (♦), heart (Δ), lung (×). Plasma concentration (C) and liver accumulation (D) of ^{111}In-Hsp70 after intravenous into mice at various doses: 2 μg/mouse (○), 10 μg/mouse (□), 100 μg/mouse (◊). Results are expressed as mean ± SD of three mice.

membrane transferred proteins. After washing with PBS containing 0.1% Tween 20, incubation with horseradish peroxidase (HRP) conjugated mouse anti-IgG antibody was carried out for 1 h. After washing with PBS containing 0.1% Tween 20, the labeled spots were visualized by an ECL kit (Amersham Pharmacia, Piscataway, NJ, USA).

Radiolabeling of Hsp70

For the tissue distribution experiments, Hsp70 was radiolabeled with ^{111}In using the bifunctional chelating agent DTPA anhydride according to the method reported (15). In brief, Hsp70 (1 mg) was dissolved in 0.2 ml 0.1 M HEPES buffer, pH 7, and mixed with 2-fold molar DTPA anhydride in 4 μl dimethyl sulfoxide. The mixture was stirred for 1 h at room temperature, and the radiolabeled product was purified by gel filtration at 4°C using a PD-10 column (Pharmacia, USA) to remove unreacted DTPA. The fractions containing the sample were collected and concentrated by ultrafiltration at 4°C. Then, 20 μl $^{111}InCl_3$ solution (37 MBq/ml) was added to 20 μl 0.1 M citrate buffer, pH 5.75, and 40 μl DTPA-coupled derivative solution was added to the mixture. After 30 min, the mixture was applied to a PD-10 column and eluted with 0.1 M citrate buffer containing 1.5 M NaCl, pH 6.0. The fractions containing Hsp70 were collected and concentrated by ultrafiltration at 4°C. This radiolabeling method is suitable for examining the distribution phase of macromolecules from plasma to various tissues because any radioactive metabolites produced after cellular uptake are retained within the cells where the uptake takes place (16,17). The radiochemical purity of ^{111}In-Hsp70 was confirmed by cellulose acetate electrophoresis, which was run at an electrostatic field of 0.8 mA/cm for 30 min in veronal buffer (I = 0.06, pH 8.6).

Tissue Distribution Experiment

^{111}In-Hsp70 was injected into the tail vein of mice at a dose of 2, 10, or 100 μg/mouse. Other mice were given a subcutaneous injection of ^{111}In-Hsp70 into the footpad of the left leg at a dose of 10 μg/mouse. At appropriate times after injection, blood was collected from the vena cava under ether anesthesia, and the mice were then killed. Heparin sulfate was used as an anticoagulant. Plasma was obtained from the blood by centrifugation. The liver, kidney, spleen, heart, lung, leg, and the lymph node behind the knee of the left leg were removed, rinsed with saline, and weighed. Urine was also collected. The radioactivity in each sample was measured using a well-type NaI-scintillation counter (ARC-500, Aloka, Tokyo, Japan). Groups of three or four mice were used for each measurement, and the results were expressed as mean ± SD of the mice.

The amount in the whole the liver was also calculated. Briefly, using the number of cells per gram of liver, we calculated the relative contribution of hepatocyte and nonparenchymal cell uptake (18).

Calculation of Pharmacokinetic Parameters

The ^{111}In radioactivity concentrations in plasma were normalized with respect to the % of the dose/ml and analyzed using the nonlinear least-squares program MULTI (19). The tissue distribution profiles were evaluated using the tissue uptake clearance (CL_{tissue}; ml/h) by means of an integration plot analysis. By dividing the amount in a tissue at time t (X_t; ng) and the area under the plasma concentration-time curve (AUC; ng h/ml) from time 0 to t ($AUC_{0\text{-}t}$) by the plasma concentration at time t (C_t; ng/ml), the CL_{tissue} was obtained from the slope of the plot of X_t/C_t *vs.* $AUC_{0\text{-}t}/C_t$.

Preadministration of Various Compounds to ^{111}In-Hsp70 into Mice

To examine the involvement of CD91 or LOX-1 in the hepatic and splenic uptake of ^{111}In-Hsp70, ligands for these receptors (200 μg/mouse) were intravenously injected into mice, followed by the injection of ^{111}In-Hsp70 (2 μg/mouse) with 1-min interval. At 10 min after the injection of ^{111}In-Hsp70, the plasma, liver, and spleen were collected, and the radioactivity in each sample was measured as above.

Isolation and Culture of Primary Mouse Hepatocytes

Mouse hepatocytes were isolated by collagenase digestion followed by differential centrifugation according to a previous report (20). Briefly, at 10 min after intravenous injection of ^{111}In-Hsp70 at a dose of 10 μg/mouse, mice were euthanized and the liver was perfused through the portal vein with pre-perfusion buffer (Ca^{2+}- and Mg^{2+}-free HEPES buffer, pH 7.2) and then with HEPES buffer (pH 7.5) containing 5 mM $CaCl_2$, 0.005% (w/v) soybean trypsin inhibitor and 0.05% (w/v) collagenase. The liver cells dispersed in ice-cold Hank's-HEPES buffer (pH 7.2) were incubated at 37°C for 10 min followed by filtration through a Falcon 100-μm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ, USA) to remove aggregates of dying cells and then fractionated into hepatocytes and nonparenchymal cells by differential centrifugation. The hepatocytes were suspended in William's medium E supplemented with ITS (+) and penicillin-streptomycin-L-glutamine and seeded on collagen-coated 24-well plates with/without cover slips at a density of 2×10^6 cells/well. After culturing for 24 h at 37°C, the cells were washed twice with HBSS and used in the cellular association experiments described below.

Cellular Association Experiments

The primary hepatocytes were cultured in 24-well plates. Then the cells were washed twice with HBSS and incubated with HBSS containing ^{111}In-Hsp70. After a 1-h incubation at 37°C, the cells were washed five times with HBSS and solubilized with 1 ml 0.3 M NaOH containing 0.1% TritonX-100. Aliquots were taken for the determination of ^{111}In radioactivity using a well-type NaI-scintillation counter.

Table I. AUC and Clearances of ^{111}In-Hsp70 after Intravenous Injection in Mice

Dose (μg/mouse)	AUC (ng · h/ml)	Clearance (ml/h)			
		CL_{Total}	CL_{Liver}	CL_{Kidney}	CL_{Urine}
2	254×10^4	2.82	1.86	0.36	0.12
10	130×10^5	2.76	2.28	0.54	0.06
100	160×10^6	2.28	1.98	0.30	0.06

AUC, area under the plasma concentration-time curve.

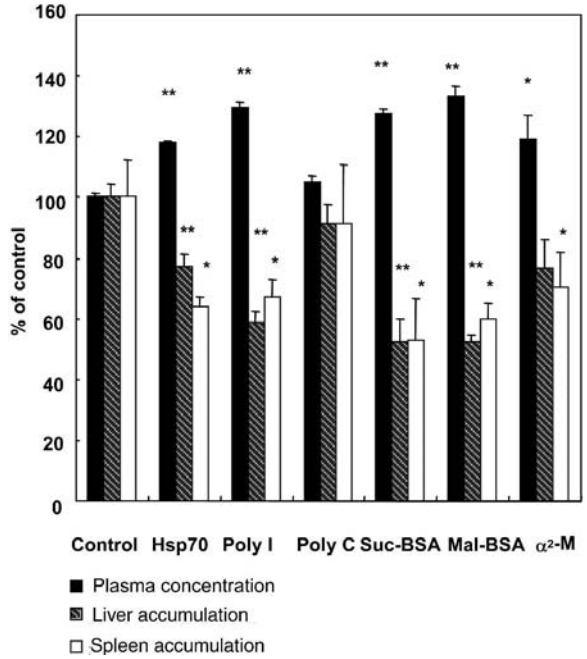

Fig. 3. Effects of preadministration of various compounds on the hepatic and splenic uptake of ^{111}In-Hsp70. ^{111}In-Hsp70 was injected at a dose of 2 μg/mouse, and the liver and spleen were collected at 10 min after the injection. Results are expressed as mean ± SD of three mice: plasma concentration (closed bar), liver accumulation (hatched bar), spleen accumulation (open bar). Statistically significant differences were assessed using Student's *t* test compared with controls (*$p < 0.05$, **$p < 0.01$).

RESULTS

Purification of Recombinant Mouse Hsp70

We used a protocol developed by Srivastava *et al.* (21) for the purification of Hsp70 by anion-exchange and ATP-affinity chromatography (21). Figure 1 shows the results of SDS-PAGE (A) and the Western blot (B) of the Hsp70. These pictures demonstrate that very pure Hsp70 was obtained. The isoelectric point (PI) of the purified Hsp70 was measured by isoelectric focusing under acidic pH and found to be about 5.2–5.3 (data not shown), which was identical to the reported value for mouse Hsp70 (22).

Tissue Distribution of ^{111}In-Hsp70

Figure 2 shows the time-course of the plasma concentration (Fig. 2A) and tissue accumulation (Fig. 2B) of ^{111}In-Hsp70 after intravenous injection at a dose of 10 μg/mouse. ^{111}In-Hsp70 rapidly disappeared from the plasma circulation and accumulated extensively in the liver (75% of the dose at 2 h). Figs. 2C and 2D show the plasma concentration and liver accumulation of ^{111}In-Hsp70 after intravenous injection into mice at various doses. As shown in these panels, the profiles of the plasma concentration and liver accumulation of ^{111}In-Hsp70, which were normalized to the dose, were almost identical at any dose studied, from 2 to 100 μg/mouse.

Calculation of AUC and Organ Uptake Clearances

Table I summarizes the clearance values for the total body, liver, kidney, and urine, as well as the AUC of ^{111}In-Hsp70, all of which were calculated based on the distribution data shown in Fig. 2. The clearances hardly changed with the dose, whereas the AUC increased proportionally to the increasing dose. These results suggest linear pharmacokinetics of ^{111}In-Hsp70 within the dose range examined.

Effect of Preadministration of Various Compounds on the Hepatic and Splenic Uptake of ^{111}In-Hsp70

To examine the involvement of any specific receptors in the tissue distribution of ^{111}In-Hsp70, several compounds were injected prior to the injection of ^{111}In-Hsp70 into mice. Ligands of Hsp receptors were used in the distribution study of ^{111}In-Hsp70 as possible competitors. These included succinylated-BSA (Suc-BSA), maleylated-BSA (Mal-BSA), poly I, poly C, and α_2-macrogrobulin (α_2-M) (Fig. 3). Suc-BSA and Mal-BSA are ligands for several scavenger receptors including LOX-1 (23), whereas α_2-M is a ligand for CD91 (24). The hepatic and splenic uptake of ^{111}In-Hsp70 was inhibited by a 100-fold excess of unlabeled Hsp70 (200 μg/mouse), suggest-

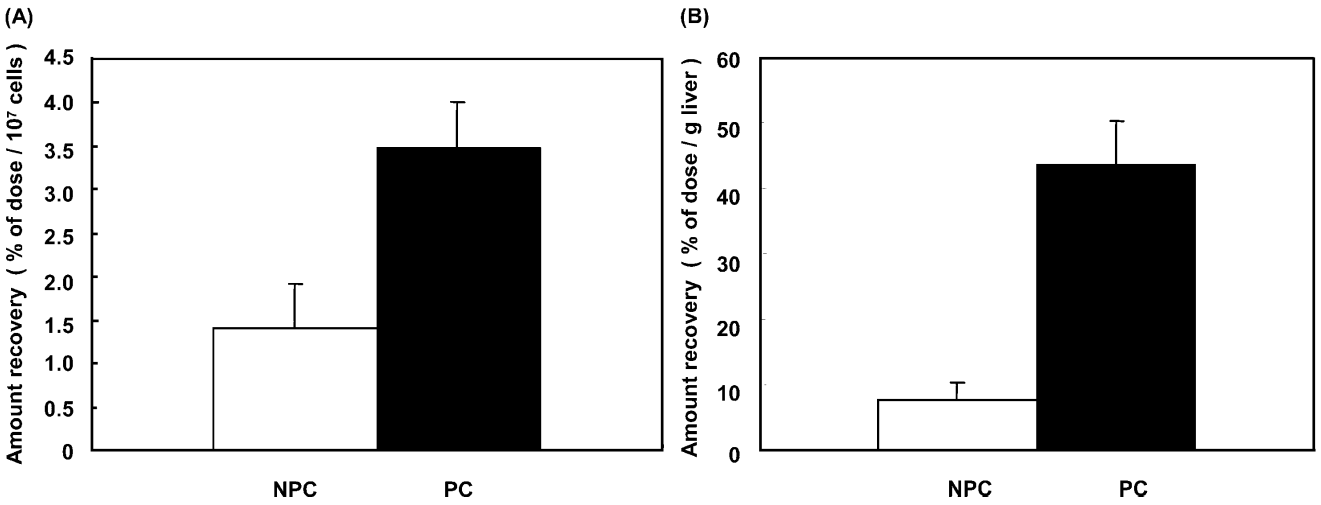

Fig. 4. Contribution of liver-constituting cells to the hepatic uptake of ^{111}In-Hsp70. ^{111}In-Hsp70 was injected at a dose of 10 μg/mouse, and the recovery in the liver was determined at 2 h after injection. Results are expressed on a cell number basis (A, % of dose/10^7 cells) and as the amounts in whole liver (B, % of dose/g liver). Both data are expressed as mean ± SD of four mice.

ing that specific mechanisms are involved in the uptake of Hsp70.

In addition, the uptake of ^{111}In-Hsp70 was also significantly inhibited by a 100-fold amount poly I, Suc-BSA, or Mal-BSA, but not by a similar amount of poly C. These results were quite similar to those obtained with ^{111}In-Suc-BSA (25), a typical ligand of scavenger receptors. Therefore, it appears that Hsp70 is taken up by the liver and spleen via scavenger receptors. An excess amount of α_2-M also inhibited the hepatic uptake of ^{111}In-Hsp70, suggesting that CD91 is also involved in this uptake. These competitors inhibited about 60–80% of ^{111}In-Hsp70 uptake by liver and spleen, therefore our data also suggested that other receptors could be involved in the uptake of Hsp70 by these organs.

Contribution of Liver-Constituting Cells to the Hepatic Uptake of ^{111}In-Hsp70

To examine the contribution of liver-constituting cells to the hepatic uptake of Hsp70, hepatocytes (PC) and nonparenchymal cells (NPC) were separated from the liver of mice receiving an intravenous injection of ^{111}In-Hsp70 at a dose of 10 μg/mouse. Figure 4 shows the intrahepatic distribution of ^{111}In-Hsp70 at 2 h after injection. The amount of ^{111}In-Hsp70 taken up by PC (3.5 ± 0.5% of dose/10^7 cells) was significantly greater than that by NPC (1.4 ± 0.5% of dose/10^7 cells) on a cell number basis. The amount recovered in the liver also showed that the uptake by PC (43.5 ± 6.7% of dose/g liver) was significantly greater than that by NPC (7.6 ± 2.7% of dose/g liver).

Inhibition of the Uptake of ^{111}In-Hsp70 by Mouse Hepatocytes

The tissue distribution experiments demonstrated that hepatocytes are the major cells involved in the uptake of intravenously injected mouse Hsp70. To examine the uptake characteristics of Hsp70 by hepatocytes in detail, *in vitro* studies were carried out using primary cultured mouse hepatocytes. The presence of CD91 on the hepatocytes was confirmed by immunostaining (data not shown).

Figure 5 shows the uptake of ^{111}In-Hsp70 by mouse hepatocytes in the absence or presence of inhibitors. α_2-M inhibited the cellular uptake of ^{111}In-Hsp70 depending on its concentration (Fig. 5A). The addition of anti-CD91 antibody also inhibited this uptake (Fig. 5B). These results indicate that CD91 is involved in the uptake of Hsp70 by hepatocytes.

Tissue Distribution of ^{111}In-Hsp70 after Subcutaneous Injection

After injection of ^{111}In-Hsp70 into the subdermal tissue of the left hind leg at a dose of 10 μg/mouse, ^{111}In-Hsp70 gradually disappeared from the injection site, and accumulated in the liver (Fig. 6). In addition to the hepatic accumulation, the approximately 3% of dose had accumulated in the primary lymph node at 8 h.

DISCUSSION

Endogenous Hsp70 has been reported to play an essential role as a molecular chaperon within cells, whereas exogenous Hsp70 is anticipated to act as a tumor vaccine. Aimed

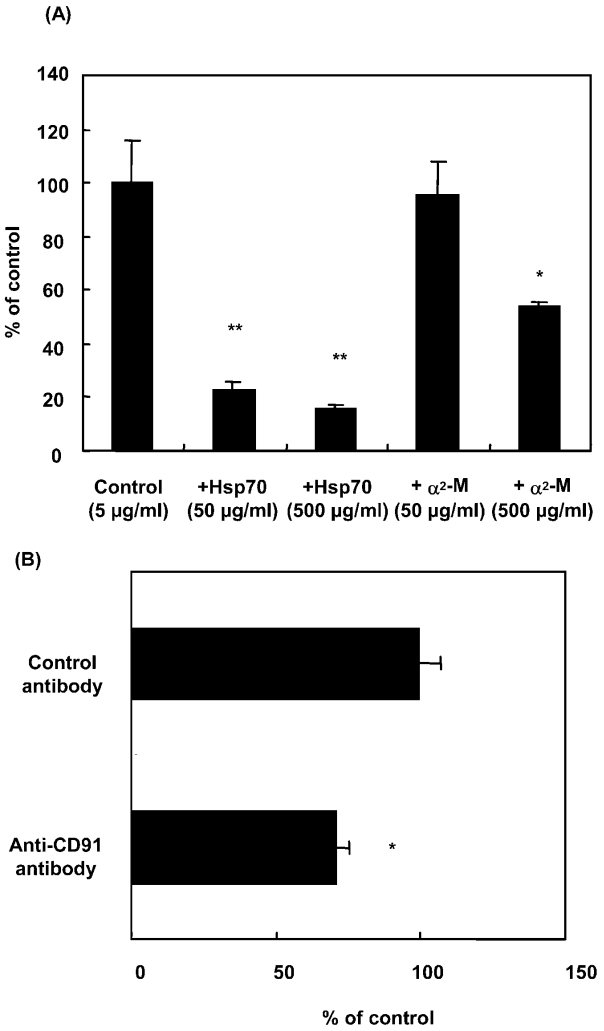

Fig. 5. Inhibition of the uptake of ^{111}In-Hsp70 by mouse hepatocytes. (A) Cells were incubated with ^{111}In-Hsp70 (5 μg/ml) in the presence of cold Hsp70 or α_2-M. (B) Cells were incubated with ^{111}In-Hsp70 in the presence of control or anti-CD91 antibody (40 μg/ml). Results are expressed as the mean ± SD of three determinations. Differences were statistically evaluated by Student's *t* test compared with the controls (*$p < 0.05$, **$p < 0.01$).

at immunization against infectious organisms or cancer, Hsp70 or its conjugates with antigen peptides have been administered to the body. It is important to deliver Hsp70-antigenic peptide to antigen presenting cells (APC) in order to induce efficient immunoresponses. To obtain a basic understanding of the pharmacokinetics of murine Hsp70, we investigated the tissue distribution of the Hsp70 after intravenous or subcutaneous injection in mice.

We have demonstrated clearly that the liver is the major organ involved in the uptake of ^{111}In-Hsp70 after intravenous injection (Fig. 2). Recently, it was reported that Hsps are recognized by CD91 and LOX-1. CD91 belongs to a family of LDL receptors, whereas LOX-1 belongs to a family of scavenger receptors (12,26). Previous studies have shown that several chemically modified proteins are vulnerable to capture by mononuclear phagocytes. Protein derivatives with a strong negative charge, such as succinylated or acconitylated proteins, are delivered to the liver, spleen, and kidney via scav-

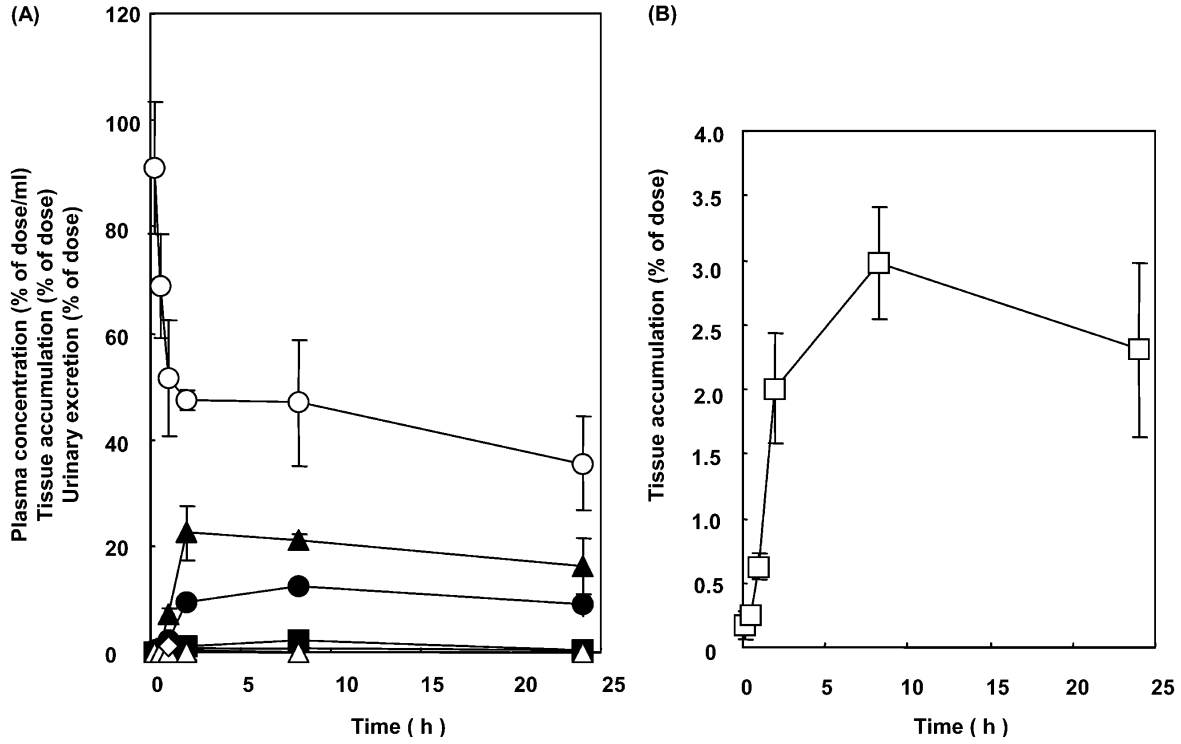

Fig. 6. Tissue distribution of ^{111}In-Hsp70 after subcutaneous injection in mice. Time-courses of the plasma concentration, tissue accumulation, and urinary excretion of ^{111}In-Hsp70 after subcutaneous injection in mice at a dose of 10 μg/mouse: (A) plasma (■), liver (▲), kidney (●), urine (◊), spleen (♦), heart (Δ), lung (×), leg (○), (B) primary lymph nude (□). Results are expressed as mean ± SD of three mice.

enger receptors after intravenous injection (25,27–29). In the current study, preadministration of ligands of CD91 or scavenger receptors suggested that both CD91 and scavenger receptors are involved in the uptake of Hsp70 by the liver and spleen (Fig. 3). These results suggest that the negative charge on the surface Hsp70 and/or the sequence patterns of Hsp70 are recognized by these Hsp receptors.

The liver consists of various types of cells such as hepatocytes, endothelial cells, and Kupffer cells, any of which is very important for determining the tissue distribution of proteins as well as other polymers and particles. Consequently, we investigated which type of cell was mainly involved in the hepatic uptake of ^{111}In-Hsp70, because the detailed mechanisms of the hepatic uptake are poorly understood. The separation of the different type of liver cells revealed that the recovery of ^{111}In-Hsp70 was greater in hepatocytes than in nonparenchymal cells. Hepatocytes are known to express CD91, but not LOX-1, so it is speculated that CD91 largely contributes to the hepatic uptake of Hsp70 *in vivo*. The estimated total recovery of the radioactivity injected into the liver was calculated from the data in Fig. 4 using the cell numbers per 1 g liver and an estimated mouse liver weight of 1.4 g. It was approximately 70% of dose, which was in good agreement with the direct measurement of the hepatic accumulation of ^{111}In-Hsp70 (Fig. 2).

In addition, the cellular uptake experiments using mouse hepatocytes supported our hypothesis that CD91 is the main clearance receptor of Hsp70 *in vivo* (Fig. 5). CD91 has been recently been reported to acts as a sensor for necrotic cell death (11). The release of Hsps in the blood as a result of severe tissue injury and lysis would not lead to a systemic and lethal proinflammatory cytokine cascade. Our data also support the idea that Hsp70 released from necrotic cells is removed and cleared from the circulation by CD91 on hepatocytes without activating the immune system. This phenomenon might be of physiologic significance in the body.

LOX-1, another well-known receptor for Hsp70, is expressed on several cells including Kupffer cells. In the present study, we found that LOX-1 ligands such as Suc-BSA, Mal-BSA, and poly I, inhibited the hepatic uptake of ^{111}In-Hsp70 after intravenous injection into mice. However, the uptake by NPC including Kupffer cells was much smaller than that by PC (Fig. 4), indicating that these cells dose not play a major role in the uptake of intravenously injected Hsp70. The contribution of CD91- and LOX-1–mediated cellular uptake of Hsp70 will depend on various parameters, such as affinity, the number of receptors and the accessibility of the receptors. The current study demonstrated that, even although LOX-1 interacts with Hsp70, LOX-1- mediated uptake is a minor determinant of the tissue distribution of Hsp70.

Subcutaneous injection is an important route for administration when Hsp70-based vaccination is attempted. ^{111}In-Hsp70 gradually disappeared from the injection site, and a fraction of the ^{111}In-Hsp70 accumulated in the liver (Fig. 6). It is important that the subcutaneous ^{111}In-Hsp70 is efficiently delivered to the lymph nodes where many APCs are present. It appears that Hsp70 was taken up by APCs in lymph nudes via CD91 and LOX-1 receptors, when we injected Hsp70 subcutaneously as a vaccine.

In conclusion, the current study has demonstrated that exogenous Hsp70 is recognized and cleared by the liver via CD91 and scavenger receptors. Furthermore, Hsp70 was

mainly taken up by hepatocytes via CD91. These findings provide basic information on the tissue distribution characteristics of Hsp70, which should be useful for designing Hsp70-based therapeutic strategies.

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