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BIODISTRIBUTION AND CLEARANCE OF ^{125}I-LABELED C-REACTIVE PROTEIN AND ^{125}I-LABELED MODIFIED C-REACTIVE PROTEIN IN CD-1 MICE

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ABSTRACT:

Iodinated forms of C-reactive protein (CRP), soluble modified CRP (mCRP-sol), and suspended mCRP (mCRP-susp) were injected iv into CD-1 mice, for analysis of their pharmacokinetics (PK) and biodistribution (BD). The plasma half-life of ^{125}I-CRP, measured as 4.7 hr, agrees closely with previous reports. The PK and BD characteristics for ^{125}I-mCRP-sol and ^{125}I-mCRP-susp were comparable to each other and were distinctly different from those measured for CRP. Whereas ~50% of ^{125}I-CRP was recoverable from plasma 5 min after injection, only ~5% of ^{125}I-mCRP was similarly recoverable. The estimated volume of distribution at steady state calculated for either form of ^{125}I-mCRP was ~10-fold greater than that calculated for ^{125}I-CRP (23.4–27.6 and 2.4 ml, respectively). The estimated mean residence times for ^{125}I-mCRP were ~2 times longer than that measured for ^{125}I-CRP (9.5–11.5 hr, compared with 4.9 hr). At both 4- and 24-hr time points, substantial amounts of ^{125}I-mCRP were selectively distributed in the bone marrow. At 24 hr, ~25% of the injected ^{125}I-mCRP-sol and ^{125}I-mCRP-susp was localized to the bone marrow (corresponding to 92% of injected dose/g of tissue). At this time point, only 8% (or 27%/g) of ^{125}I-CRP was localized to the bone marrow. Overall, the data presented indicate that 1) mCRP has PK and BD characteristics distinct from those of CRP; 2) injected mCRP, although it is rapidly cleared from the general circulation, accesses large body areas and is selectively localized to the bone marrow; and 3) all forms of CRP appear to be excreted in the urine.

CRP[1] is a major component of the acute-phase response. As part of the immediate defense system of the body, CRP serum concentrations can increase up to 1000-fold within 24–72 hr of an infection or tissue insult (Kushner, 1982; Pepys and Baltz, 1983). The *in vitro* biological activities of CRP include complement activation (Siegel *et al.*, 1974; Kaplan and Volanakis, 1974), opsonization (Nakayama *et al.*, 1982; Kilpatrick and Volanakis, 1991), and activation of macrophages for tumoricidal activity (Zahedi and Mortensen, 1986; Barna *et al.*, 1987, 1988). Both *in vitro* and *in vivo*, CRP has been shown to bind to and affect the clearance of nuclear antigens (DuClos, 1996). However, no definitive *in vivo* function has been identified for CRP.

Our laboratory has established that CRP can exist in a conformationally and antigenically distinct form that we call mCRP (Potempa *et al.*, 1983, 1987). mCRP has been shown to be a naturally occurring protein in various tissues throughout the body (Rees *et al.*, 1988; Egenhofer *et al.*, 1993; Radosevich *et al.*, 1996). mCRP has immunostimulatory activities that are different from those reported for the native pentameric CRP. These include modulation of leukocyte, monocyte, and platelet activities *in vitro* (Potempa *et al.*, 1988), potentiation of megakaryocyte growth *in vitro*, and stimulation of thrombopoietic activity *in vivo* (Potempa *et al.*, 1996).

One of the major physical differences between CRP and mCRP is the difference in their solubility characteristics. Whereas CRP is soluble in buffers of physiological ionic strength and pH, mCRP is maximally soluble in solutions of low ionic strength and more alkaline pH. When added to solutions of physiological pH and ionic strength, mCRP forms an opalescent suspension. Our laboratory has studied both mCRP-sol and mCRP-susp forms as biological response modifiers in various animal disease models. In addition to assessment of the *in vivo* efficacy of mCRP as a potential therapeutic agent, it is necessary to determine *in vivo* distribution characteristics and clearance mechanisms.

The *in vivo* plasma clearance of ^{125}I-CRP was previously reported. The plasma half-life of ^{125}I-CRP was ~4 hr in mice and rats (Baltz *et al.*, 1985) and ~7 hr in rabbits (Challadurai *et al.*, 1983; Rowe *et al.*, 1984). Plasma and whole-body turnover of human ^{125}I-CRP has also been reported for normal and diseased volunteers (Vigushin *et al.*, 1993). The clearance closely approximated a monoexponential function, with ~90% of injected radioactivity being recovered in urine after 7 days. The half-life was 19 hr in normal volunteers and was unchanged in patients with rheumatoid arthritis, systemic lupus erythematosis, bacterial infections, or malignant neoplasia. Furthermore, scintographic analyses using ^{123}I-CRP in 10 patients with prominent inflammation and tissue damage revealed no selective localization of labeled CRP to any tissue or organ; the distribution of label was confined to the blood pool.

This is the first report of the PK/BD characteristics of mCRP. We used ^{125}I-labeled CRP to prepare both mCRP-sol and mCRP-susp for injection into normal male mice. The PK parameters and tissue BD for

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[1] Abbreviations used are: CRP, C-reactive protein; mCRP, modified C-reactive protein; mCRP-sol, soluble modified C-reactive protein; mCRP-susp, suspended modified C-reactive protein; PK, pharmacokinetics; BD, biodistribution; AUMC, area under the first moment curve; MRT, mean residence time; CL_T, total-body clearance; V_{ss}, volume of distribution at steady state; TCA, trichloroacetic acid.

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both forms of mCRP were then compared with those of the more widely studied CRP molecule.

Materials and Methods

Preparation of Labeled Proteins. CRP was isolated and purified from human ascitic fluids by calcium-dependent phosphorylcholine affinity chromatography, using phosphorylcholine-substituted Bio-Gel resin (Bio-Rad Laboratories, Richmond, CA), and ion exchange chromatography, according to previously published procedures (Potempa *et al.*, 1987). Purified CRP at 1 mg/ml was dialyzed into 25 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl and 2 mM $CaCl_2$. CRP was iodinated using Iodo-Bead technology (Pierce Chemical Co., Rockford, IL), as follows. For every 1 ml of CRP solution, six beads were washed with iodination buffer (25 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl and 5 mM $CaCl_2$,). $Na^{125}I$ was diluted to 3 mCi/ml of iodination buffer and added to the beads. After 5 min, 1 ml of CRP (at 1 mg/ml) was added and allowed to incubate for 5 min at room temperature. The iodinated protein was removed and passed through a Sephadex G-75 column (Pharmacia, Piscataway, NJ), to separate free label from protein-bound label and to equilibrate the ^{125}I-CRP solution in 10 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl and 2 mM $CaCl_2$.

^{125}I-mCRP-sol was prepared from ^{125}I-CRP by addition of 10 mM EDTA and solid ultrapure urea (final concentration, 8 M) and incubation at 37°C for 1–2 hr, followed by dialysis into 10 mM Tris-HCl, pH 8.0 (Potempa *et al.*, 1987). Specific activities for ^{125}I-CRP and ^{125}I-mCRP-sol were calculated based on the total protein concentration of each labeled protein (estimated based on an A_{280} value of 1.95 mg/ml) and the ^{125}I activity. An unlabeled sample of mCRP-sol was prepared from CRP by urea chelation/dialysis and was adjusted to 500 μg/ml. Unlabeled mCRP-susp was prepared by incubating CRP at 60°C for 16 hr in the presence of 3 mM EDTA. Unlabeled mCRP-susp was then mixed with ^{125}I-mCRP-sol for the PK/BD study of ^{125}I-mCRP-susp. For each of the protein samples, a stock mixture of labeled and unlabeled protein was prepared, so that each animal would receive a dose of 2.5 mg/kg protein, with ^{125}I activity of $\sim 0.5–1.0 \times 10^6$ cpm.

Labeled proteins were characterized by UV spectroscopy and radial immunodiffusion. UV wavelength scans obtained for ^{125}I-CRP and ^{125}I-mCRP were identical to those for unlabeled CRP, indicating that protein integrity was maintained during the iodination and conversion processes. Immunodiffusion analysis of radiolabeled CRP confirmed that the protein remained diffusable and retained CRP antigenicity.

Animals. Male CD-1 mice were purchased from Charles River Laboratories, Canada. The mice (age, 35–49 days; weight, 26–31 g) were quarantined for approximately 2 weeks and then examined for signs of disease or injury before the start of the study. Mice were randomized into six groups. Each mouse received a single bolus injection (volume, 0.14–0.31 ml), *via* the tail vein, of a mixture of unlabeled and labeled CRP, mCRP-sol, or mCRP-susp. Dose volumes were calculated based on the pretreatment body weight and were rounded to the nearest 0.01 ml. For each form of CRP studied, one group of mice had blood samples taken at 5 min, 2 hr, and 4 hr, urine and feces collected over 0–4 hr, and tissue samples obtained, after sacrifice, at 4 hr. Another group of mice had blood samples taken at 30 min, 8 hr, and 24 hr, urine and feces samples collected over 0–24 hr, and tissue samples obtained at 24 hr.

Whole-blood samples of approximately 300–500 μl were collected from the retroorbital sinus of each animal, under CO_2 anesthesia, into EDTA-containing tubes. Samples were processed to yield plasma and were evaluated for ^{125}I activity in a Packard model 5650 γ-scintillation counter. After determination of plasma ^{125}I activity, an equal volume of 20% TCA was added to each plasma aliquot, to determine the amount of ^{125}I activity that remained associated with intact protein. The samples were briefly vortex-mixed and were placed on ice for 15 min. The aliquots were centrifuged at approximately 3000*g* for 10 min, and the supernatant, containing free label or label associated with fragmented protein, was aspirated from each sample. The resultant TCA-precipitated pellet was analyzed for ^{125}I activity. Wherever possible, duplicate samples were processed and the values were averaged.

Urine and feces samples were collected from mice that were individually housed in metabolism cages. Total excreted feces and total voided urine, if present, were collected at 4 or 24 hr. Urine samples collected from each animal were kept separate. The volume of each collected sample was recorded, and, when possible, duplicate 0.05-ml aliquots of each sample were analyzed for ^{125}I activity in a γ-scintillation counter.

Fecal samples were processed and evaluated for ^{125}I activity. Each sample was weighed before homogenization with sufficient water to permit reasonably accurate pipetting. When possible, duplicate 0.2-ml aliquots of each homogenate were analyzed for ^{125}I activity.

After collection of the final blood samples, the animals were anesthetized by injection of sodium pentobarbital. The mice were then injected ip with approximately 1000 units/kg heparin and, immediately before euthanasia, were perfused with a minimum of 1 blood volume of 0.9% saline solution containing heparin. After perfusion and exsanguination, tissues/organs were collected, trimmed of extraneous fat and connective tissue, emptied and cleaned of all contents, and individually weighed before determination of ^{125}I activity. For tissues too large to evaluate *in toto*, a representative sample of $\leq$0.5 g was evaluated.

Data Analysis. PK parameters were calculated for each form of CRP by noncompartmental analysis using statistical moment theory (Riegelman and Collier, 1980). The AUC and AUMC were calculated by the linear trapezoidal rule, using the following equations.

$$AUC_{0-\infty} = (C_n + C_{n-1})/2(t_n - t_{n-1}) + C_z/K_e$$

$$AUMC_{0-\infty} = (t_nC_n + t_{n-1}C_{n-1})(t_n - t_{n-1})/2 + t_zC_z/K_e + C_z/K_e^2$$

Because of the nature of the plasma data, the elimination rate constant (K_e) could only be estimated as the slope of the line formed by the last two blood collection time points. The estimated MRT was calculated from the AUMC/AUC ratio. The estimated half-life of elimination was calculated as $0.693/K_e$. The estimated CL_T was calculated as the mean dose/AUC ratio. The estimated V_{ss} was calculated as $CL_T \times$ MRT.

Tissue Distribution Analysis. The tissue and organ accumulation of radiolabeled protein in 4 and 24 hr after iv administration was estimated by assuming that the ^{125}I-labeled protein complex was stable. The distribution was expressed either as the percentage of the injected dose recovered in each collected tissue or organ or as the percentage of the injected dose recovered in each tissue or organ divided by the weight of that tissue or organ (in grams). The values therefore have units of percent or percent per gram, respectively. For plasma, urine, or feces, the volume (in milliliters) was used instead of the weight, *i.e.* values of percent per milliliter are reported instead of percent per gram.

Results

PK. The PK clearance of ^{125}I-CRP was biexponential (fig. 1), with a rapid early phase and a slower later phase. The half-life of ^{125}I-CRP was calculated as 4.7 hr. At the first time point measured, *i.e.* 5 min, $\sim$50% of injected ^{125}I-CRP was recovered in plasma. In contrast, the clearance of both ^{125}I-mCRP-sol and ^{125}I-mCRP-susp was very rapid; at the 5-min time point, $<$5% of the injected label could be recovered in plasma (fig. 1, *inset*).

Plasma samples were treated with TCA to precipitate protein-bound label and separate it from label that might have leached off the protein or remained attached to fragmented protein. At the 4-hr time point, 75% of the label was recovered in the plasma compartment as TCA-precipitable CRP; similarly, 49 and 67% of label was found to be TCA-precipitable with mCRP-sol and mCRP-susp, respectively.

Because plasma was not collected before 5 min, the PK parameters for mCRP could only be estimated based on the residual $<$5% of the label that remained in plasma after this initial time point. The estimated values for the PK parameters are presented in table 1 to show that, even with the limitations in data analysis, the values for mCRP-sol and mCRP-susp are in close agreement with each other and are markedly different from the values for the more widely studied and more fully understood CRP molecule. Because our calculated values for the CRP conformer agree closely with those from previously reports (Baltz *et al.*, 1985), we consider our model system and methods to be valid and our estimated values for the mCRP conformer to be meaningful.

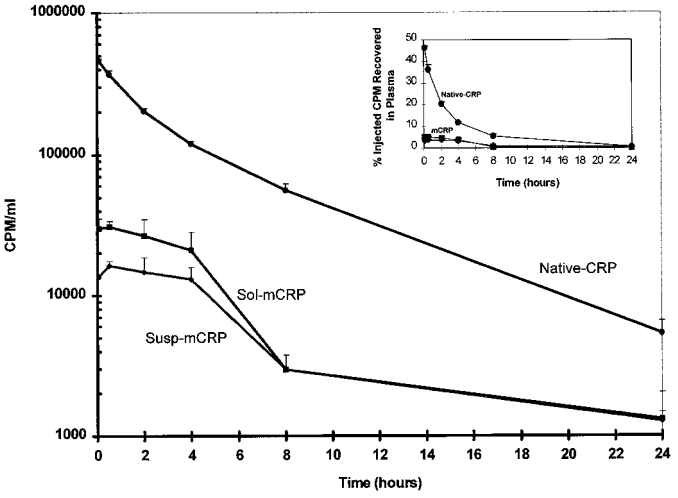

FIG. 1. Comparison of the plasma concentration-time curves for ^{125} I-mCRP, ^{125}I-mCRP-sol, and ^{125}I-mCRP-susp.

CD-1 mice were injected with 0.5–1.5 $\times$ 10^6 cpm of ^{125}I activity in a 2.5 mg/kg dose consisting of a mixture of labeled and unlabeled protein. Plasma aliquots obtained at 5 min, 30 min, 2 hr, 4 hr, 8 hr, and 24 hr were analyzed for ^{125}I activity. The mean values from three mice in each group were plotted. *Inset*, percentage of injected label recovered in plasma.

TABLE 1

PK parameters for ^{125}I-CRP, ^{125}I-mCRP-sol, and ^{125}I-mCRP-susp, estimated by noncompartmental analysis

Parameter	CRP	mCRP-sol[a]	mCRP-susp[a]
AUC (cpm/hr-ml)	2.14 $\times$ 10^6	0.25 $\times$ 10^6	0.18 $\times$ 10^6
AUMC (cpm-hr-hr/ml)	10.57 $\times$ 10^6	2.36 $\times$ 10^6	2.01 $\times$ 10^6
K_e (hr^{-1})	0.147	0.050	0.055
$t_{1/2}$ (hr)	4.71	13.83[b]	12.69[b]
V_{ss} (ml)	2.35	23.39	27.64
CL_T (ml/hr)	0.474	2.46	2.40
MRT (hr)	4.95	9.52	11.51
Mean dose (cpm)	1.01 $\times$ 10^6	0.61 $\times$ 10^6	0.421 $\times$ 10^6

PK parameters were calculated based on plasma ^{125}I activity for all time points (5 and 30 min and 2, 4, 8, and 24 hr).

[a] All values were estimated based on the <5% of injected label that was measured in plasma at all data collection points.

[b] Values describe only the subfraction of ^{125}I-mCRP remaining in circulation for the 24-hr experimental period.

Because the majority of ^{125}I-mCRP is cleared from the circulation before the first data collection point, the estimated half-life of elimination for ^{125}I-mCRP shown in table 1 describes only the subfraction of ^{125}I that remains in circulation during the period of 5 min to 24 hr (fig. 1). The AUC and AUMC were calculated to be 10- and 5-fold less, respectively, for mCRP, compared with CRP. The V_{ss} and CL_T were 10- and 5-fold greater, respectively, for mCRP, compared with CRP.

BD. Figs. 2 and 3 show the tissue distribution of ^{125}I-CRP and ^{125}I-mCRP-sol, at 4 and 24 hr after iv injection into mice, expressed either as the percentage of the injected dose or as the percentage of the injected dose per gram for each tissue (or organ). At 4 hr, the greatest amount of recovered ^{125}I-CRP was found in plasma (12% of injected dose). Skeletal muscle (7%), liver (6%), urine (6%), skin (5%), and femur bone (5%) were the other predominant sites for the uptake of ^{125}I-CRP (fig. 2*A*). The greatest concentrations of label (expressed as percentages of the injected dose per gram) were found in the urine and plasma (39%/ml and 14%/g, respectively) (fig. 3*A*). At 24 hr, the percentage of ^{125}I-CRP in urine was significantly increased (fig. 2*A*). The rank order of accumulation was urine (22%) > bone marrow (8%) > femur bone (6%) > skeletal muscle (5%). There was a notable increase in the concentration of radiolabel localized to the bone marrow at 24 hr (27%/g), relative to the 4-hr time point (4.9%/g) (fig. 3*A*).

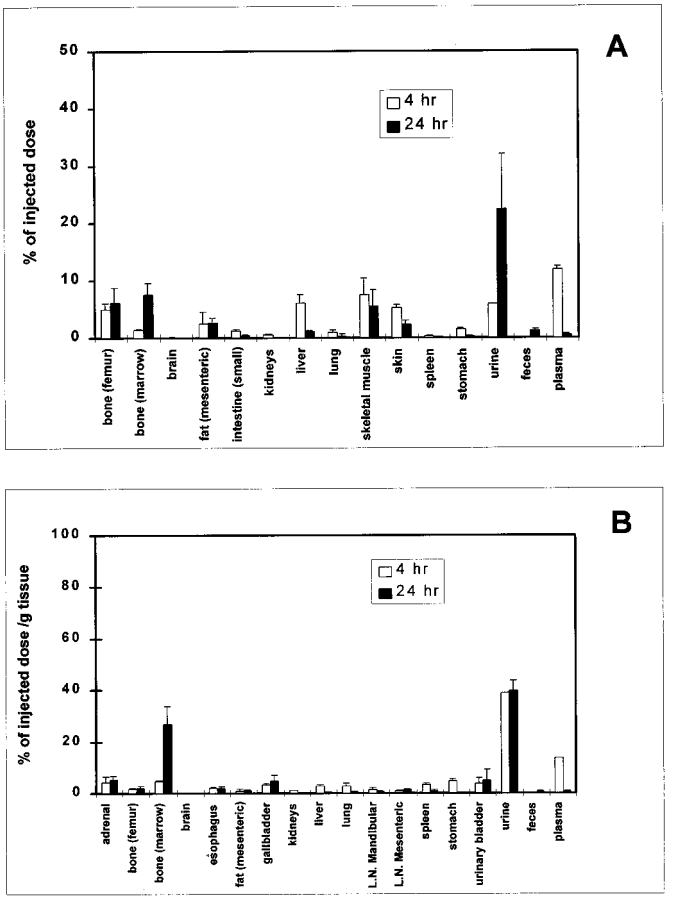

FIG. 2. *Tissue and organ accumulation of ^{125}I-CRP at 4- and 24-hr time points, expressed as percentages of the injected dose* (A) *or percentages of the injected dose per gram (plasma, urine, and feces values are expressed as percentages of the injected dose per milliliter)* (B).

L.N., lymph nodes.

At the 4-hr time point, the greatest amounts of recovered ^{125}I-mCRP were found in skeletal muscle (10%), skin (8%), bone marrow (6%), liver (6%), femur bone (5%), and plasma (3%) (fig. 2*B*). No experimental value for urine is reported for this time point because no useable sample was collected. The greatest concentrations of ^{125}I-mCRP-sol were recovered in the bone marrow (21%/g), esophagus (12%/g), spleen (9%/g), and stomach (8%/g) (fig. 3*B*). At the 24-hr time point, both the percentage and concentration of the radiolabel (fig. 3) indicated substantial localization to the bone marrow (25% and 92%/g, respectively). In addition, a high percentage and a high concentration of radiolabel were recovered in urine (38% and 78%/ml, respectively).

The BD pattern for injected ^{125}I-mCRP-susp was very similar to that described for ^{125}I-mCRP-sol (data not shown). At 4 hr, a higher percentage of injected ^{125}I-mCRP-susp (16%) was recovered in the bone marrow than was measured for ^{125}I-mCRP-sol (6%). There was also greater selective distribution of radiolabel to the bone marrow (56%/g) and urine (36%/ml) at this time point. At 24 hr, further substantial distribution of ^{125}I-mCRP-susp to the bone marrow (92%/g) and urine (55%/ml) was observed. Small amounts of radiolabel were concentrated in the adrenal glands, the gallbladder, and the esophagus.

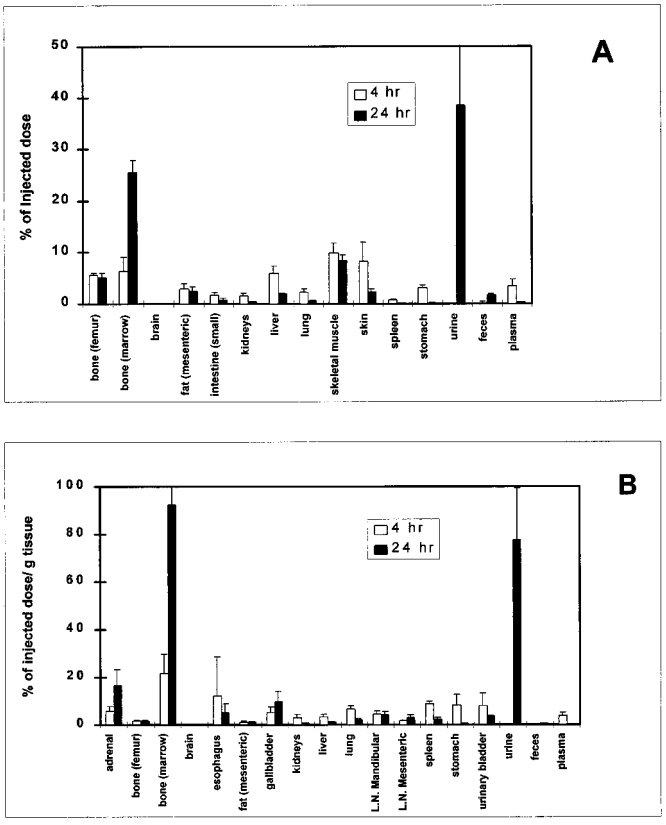

FIG. 3. *Tissue and organ accumulation of ^{125}I-mCRP-sol at 4- and 24-hr time points, expressed as percentages of the injected dose (*A*) or percentages of the injected dose per gram (plasma, urine, and feces values are expressed as percentages of the injected dose per milliliter) (*B*).*

No urine sample was obtained for analysis at the 4-hr time point. *L.N.*, lymph nodes.

Discussion

The present study compares the clearance characteristics of ^{125}I-mCRP-sol and ^{125}I-mCRP-susp with those of ^{125}I-CRP after iv injection of each protein into normal CD-1 mice. CRP is widely known as the prototypical acute-phase reactant; its plasma levels are markedly increased within hours after acute, tissue-damaging, inflammatory events (Kushner, 1982; Pepys and Baltz, 1983). mCRP is a conformationally modified form of CRP that is naturally occurring, primarily as a tissue-based (rather than a serum-based) form of CRP (Egenhofer *et al.*, 1993; Potempa *et al.*, 1983, 1987; Rees *et al.*, 1988; Radosevich *et al.*, 1996).

The plasma clearance of ^{125}I-CRP measured here is biexponential, with a half-life of 4.7 hr (282 min); this value closely agrees with previous reported values for the CRP molecule (Baltz *et al.*, 1985). The half-life for CRP injected into human subjects was reported to be 19 hr (Vigushin *et al.*, 1993), and the same plasma half-life values were measured in normal control subjects and in patients documented to have infections, neoplasms, rheumatoid arthritis, or systemic lupus erythematosis. This result was surprising, because maladies such as those described are known to stimulate increased blood levels of CRP; it was thought that localized disease would produce increased binding sites for CRP, which would therefore be cleared more quickly.

In contrast to ^{125}I-CRP, ^{125}I-mCRP was cleared from the plasma very rapidly; >95% of the injected protein was removed from plasma at the 5-min time point. The short plasma half-life of the drug is not the result of rapid clearance from the body, because 80–100% of the radiolabel injected with mCRP could be accounted for in samples collected at the 24-hr time point. These data suggest that mCRP, in contrast to CRP, is immediately taken up into tissues within the first few passages through the circulation.

mCRP is a protein that is soluble in low-ionic strength solutions and forms self-aggregates when placed in buffers of physiological ionic strength (Potempa *et al.*, 1983, 1987). Antigens cross-reactive with mCRP are found to be abundantly expressed at the intima, media, and adventitia of a number of normal blood vessels. Immunohistochemical reactivity is also found in the fibrous capsule of the adrenal glands and tonsils, in the fibrous trabeculae of the spleen, and in skin and skeletal muscle (Radosevich *et al.*, 1996). These data are consistent with the view that mCRP is a natural component of the extracellular matrix of reticuloendothelial tissues. The fact that the majority of injected ^{125}I -mCRP is rapidly removed from the circulation may reflect a strong tendency for the mCRP conformer to partition into tissues where naturally occurring mCRP is found. This result is supported by the large V_{ss} values calculated for mCRP (23–27 ml) and by the BD results. Drugs that are sequestered by tissues exhibit large V_{ss} values (Benet and Sheiner, 1988). CRP, in contrast, is primarily a protein of the circulatory system, having a comparatively small V_{ss} (2.35 ml).

mCRP exhibits an ~10-fold larger V_{ss} than does CRP, and estimated CL_T and MRT values were calculated to be ~5- and ~2–3-fold greater, respectively, than those for CRP. These data, together with the finding that all radiolabel associated with mCRP could be accounted for at the 24-hr time point, indicate that, by accessing tissues, mCRP is cleared at a slower rate than CRP.

TCA precipitation of plasma samples showed that there was a generally uniform recovery of ~72–84% of ^{125}I-CRP from plasma through the 8-hr time point after treatment. However, TCA precipitation of ^{125}I-mCRP-sol or ^{125}I-mCRP-susp plasma samples obtained up to the 4-hr time point showed only 49 and 63%, respectively, of the label remaining protein bound (data not shown). The lower percentage of TCA-recoverable label in the <5% subfraction of injected ^{125}I-mCRP recovered in the plasma suggests that the label might have dissociated from the protein, that mCRP in plasma might have been degraded faster than mCRP partitioned into tissues, or that nonlinear protein binding might have occurred *in vivo*. These data suggest that the ^{125}I-mCRP recovered in plasma at 4 hr might be a distinctive subpopulation of ^{125}I-mCRP or a metabolic breakdown product of the prepared reagent.

Tissue distribution of ^{125}I-CRP, ^{125}I-mCRP-sol, and ^{125}I-mCRP-susp was generally unremarkable, except for the interesting selective BD of both forms of mCRP to the bone marrow. The early distribution measured at 4 hr after iv injection of all forms of ^{125}I-(m)CRP tested was found in the skeletal muscle, liver, and skin. At 24 hr, whereas 7% (equivalent to 27%/g) of ^{125}I-CRP was distributed to the bone marrow, ~25% (equivalent to 92%/g) of both ^{125}I-mCRP-sol and ^{125}I-mCRP-susp was accumulated in the bone marrow. Thus, the BD results corroborate the PK estimates that mCRP, to a greater extent than CRP, is rapidly cleared from the blood compartment and enters tissue, especially bone marrow, where a large portion of it remains for at least 24 hr.

It is noteworthy that a key component of the bone marrow is the stroma, which is composed, in part, of reticular proteinaceous fibers that are similar to those found in fibrous tissues of the systemic reticuloendothelial system (Allen *et al.*, 1990; Mohammad and Asai, 1993). The physical characteristics of mCRP as a protein that can self-associate into a matrix-like protein (Motie *et al.*, 1996) may relate to its physiological role as a component of the extracellular matrix of tissues.

The finding that mCRP accumulates in the bone marrow for up to 24 hr after injection is noteworthy in light of our report that mCRP can

stimulate megakaryocytopoiesis in mice (Potempa *et al.*, 1996). Drugs that accumulate in a given tissue are believed to serve as a reservoir for prolonged drug action in that tissue or at another site (reached through the circulation) (Benet and Sheiner, 1988). The accumulation of radiolabeled mCRP in the bone marrow agrees with this organ being the site of increased hematopoietic activities and this drug having stimulatory effects on megakaryocytopoiesis. Furthermore, because mCRP can be formed from CRP under a variety of conditions (Potempa *et al.*, 1987), the accumulation of ^{125}I-CRP *in vivo* in the bone marrow at 24 hr might indicate that some injected CRP was converted *in situ* to mCRP, which was then localized to this organ. The fact that there was increased accumulation of label in the bone marrow at 24 hr, although there was a very low level of radioactivity associated with mCRP in the circulation, may reflect capacity-limited clearance of mCRP. Because of its low aqueous solubility, injected mCRP may be sequestered (as a noncovalently associated aggregate) into tissues, where it serves as a reservoir for circulating protein. The tissue-associated mCRP would be in equilibrium with the plasma mCRP, which would slowly and continuously be sequestered into specific (*e.g.* bone marrow) tissues.

Hutchinson *et al.* (1994) reported the accumulation of ^{125}I-tyramine cellobiose-labeled CRP in hepatocytes of mice and rabbits 24 hr after iv injection. They suggested that the liver is the main organ for pentraxin catabolism. The same group reported that the kidney is the primary organ for excretion of CRP-associated radiolabel in humans (Vigushin *et al.*, 1993). Our results indicate that the final excretory pathway for all forms of radiolabeled CRP appears to be through the urinary tract, presumably after filtration through the kidney. At the 24-hr time point, 40, 78, and 55%/ml of ^{125}I-CRP, ^{125}I-mCRP-sol, and ^{125}I-mCRP-susp, respectively, were found in the urine. We propose that the lack of substantial accumulation of radiolabel in the kidneys may be a sign of the low toxicity associated with the injection of mCRP in both soluble and suspended forms.

This study demonstrates that CRP and both forms of mCRP can be safely injected iv into mice, where they are cleared from the body and excreted in the urine. The selective localization of mCRP to tissues, especially the bone marrow, provides a clue to the bioactivity of this protein as a thrombopoietic factor (Potempa *et al.*, 1996), as well as an amplifying factor in the leukocyte and platelet responses (Potempa *et al.*, 1988). The therapeutic applications of mCRP in the treatment of various immunological diseases are under study.

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