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# Effects of Recombinant Drug-Specific Single Chain Antibody Fv Fragment on [$^3$H]-Desipramine Distribution in Rats

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**ABSTRACT.** Tricyclic antidepressant overdose can be reversed in rats by drug-specific antibody Fab fragments, but the required Fab dose may itself be toxic. We studied the potential use of a smaller, recombinant desipramine (DMI)-specific single chain Fv fragment (B9-sFv) for this purpose. Anesthetized rats received a tracer (subtoxic) dose of [$^3$H]-DMI followed in 15 min by B9-IgG, B9-Fab, B9-sFv (0.1 μmol of binding sites), or BSA. Each of the active treatments produced a rapid and substantial increase in the serum radiolabel concentration, whereas BSA did not ($P < 0.001$). The increase in serum radiolabel concentration 1 min after treatment was 13.3-fold with B9-IgG, 10.0-fold with B9-Fab and 7.3-fold with B9-sFv. Serum antibody concentrations were also highest after B9-IgG and lower with B9-Fab or B9-sFv. The 24-hr urinary excretion of radiolabel did not differ among groups, but was extensive even in the BSA group and probably represented the excretion of DMI metabolites. B9-sFv concentrations in urine or buffer at 37° declined by >90% over 24 hr, but this fragment was much more stable in serum, retaining 70% of its activity after 96 hr. These data demonstrate that B9-sFv can alter markedly the distribution of [$^3$H]-DMI *in vivo*. The rapidity of this effect, and its magnitude in comparison with Fab fragment or IgG, suggest that further study of B9-sFv as a treatment of DMI overdose is warranted. BIOCHEM PHARMACOL 51;4:531–537, 1996.

**KEY WORDS.** antibody; sFv fragment; tricyclic antidepressant; immunotoxicology; overdose; immunotherapy

Drug-specific antibodies represent a versatile new class of antidotes for drug or chemical toxicity. Their clinical value has been well established by the use of digoxin-specific antibody Fab** fragment for the treatment of acute digoxin cardiotoxicity [1]. The Fab fragment binds digoxin in serum and extracellular fluid, redistributes the drug out of tissues (including the heart), and rapidly reverses toxicity [2]. This approach to management of drug overdose is appealing because it should be readily generalizable, in that it is possible to produce high affinity antibodies to most drugs. A patient with colchicine overdose has been treated successfully in this manner [3], and initial animal data suggest that drug-specific antibody fragments may also be useful for the treatment of phencyclidine [4], paraquat [5], or tricyclic antidepressant [6] toxicity.

In the treatment of digoxin toxicity, Fab fragment (50 kDa) is used rather than IgG (150 kDa) because it is eliminated more rapidly and is less immunogenic [7]. Although digoxin-specific Fab has proven clinically effective and safe, most drugs of clinical interest have toxic doses several orders of magnitude higher than that of digoxin and require correspondingly higher doses of antibody or Fab (up to several g/kg) to reverse toxicity. These very high doses of Fab may themselves be toxic. Nonspecific polyclonal Fab administered to dogs at a dose of 5.3 g/kg produced transient renal failure [8]. DMI-specific polyclonal Fab administered to rats at doses of 1–4 g/kg over 20 min was effective in reducing DMI cardiotoxicity, but some deaths occurred after initial improvement [9]. Lethality has also been observed with the rapid infusion of drug-specific Fab to rats for colchicine toxicity.†† Thus, drug-specific antibody fragments can be effective as antidotes to drug overdose, but Fab may not be the ideal antibody fragment for this purpose, particularly when large doses are needed.

The mechanisms responsible for Fab toxicity are not clear. It is possible that Fab toxicity is due, in part, to the large

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** *Abbreviations:* Fab, 50 kDa antibody fragment produced by papain digestion; Fab′, 55 kDa antibody fragment produced by pepsin digestion and reduction; sFv, 27 kDa recombinant antibody fragment consisting of $V_H$-linker-$V_L$; B9-sFv, DMI-specific sFv; $V_H$, $V_L$, variable domains of antibody heavy or light chains; DMI, desipramine; and PCR, polymerase chain reaction.

†† Scherrmann JM, personal communication. Cited with permission.

protein load administered and the resulting intravascular volume expansion [10]. If this is the case, smaller antibody fragments could provide a substantial benefit by decreasing the protein load. If specific protein regions contribute to Fab toxicity, the reduced size of a smaller fragment might serve to eliminate such regions and thereby reduce toxicity. Smaller fragments should also be less immunogenic than Fab, a property of potential interest because patients might overdose more than once and require repeated antibody administration.

Smaller fragments may also have pharmacokinetic advantages over Fab. A smaller protein may potentially have a larger volume of distribution than Fab, and may reverse DMI toxicity more rapidly owing to more extensive distribution to the site(s) of toxicity [11]. Smaller molecules may also be excreted more rapidly and more extensively by the kidneys. This would shorten the exposure of animals or patients to both the antibody fragment and the drug–antibody complex, and thereby reduce the opportunity for antibody-induced toxicity.

Because the hapten-binding domains (complementarity determining regions, or CDRs) of IgG are contained within the N-terminal half of the Fab fragment (Fig. 1), the production of still smaller fragments that retain the ability to bind hapten is feasible. The smallest fragment containing all six CDRs, designated Fv, consists of the variable portions of the heavy and light chains ($V_H$ and $V_L$). Production of Fv that fully retains the affinity of the parent IgG for hapten, by enzymatic digestion of IgG, has been achieved [12], but for most antibodies the yield of Fv is too low to be useful. Fv fragment can be produced by cloning and separately expressing recombinant $V_H$ and $V_L$, but the subsequent association of $V_H$ and $V_L$ is inefficient because they lack the disulfide bonds that link the heavy and light chains of IgG or Fab.

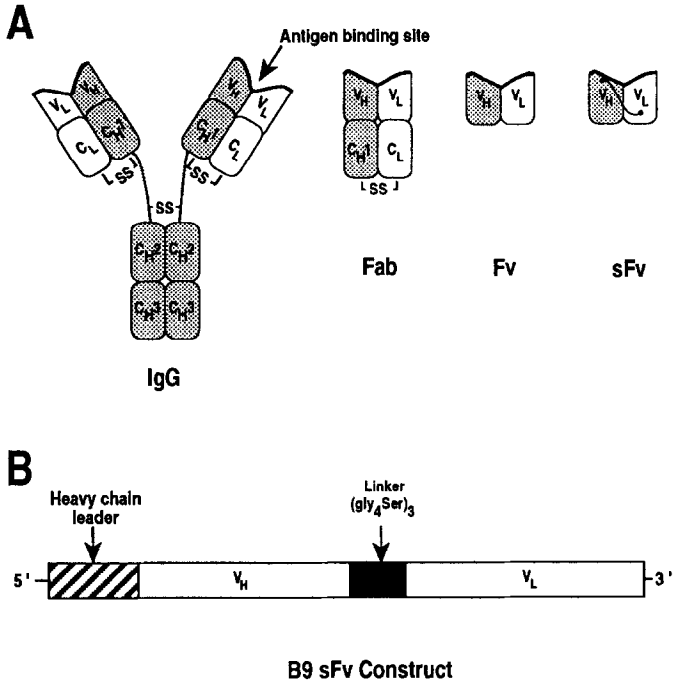


**FIG. 1. (A) Diagrammatic representation of IgG, Fab, Fv, and sFv. Because the complementarity determining regions responsible for antigen binding are contained in the variable regions of the heavy and light chains of IgG ($V_H$ and $V_L$), smaller fragments can be produced that fully retain its antigen binding affinity. $C_H$ and $C_L$ represent the constant regions of the heavy and light chains. (B) B9-sFv construct. The leader peptide is cleaved prior to secretion of sFv into the culture medium. (Adapted with permission from *Int Rev Immunol* 10: 195–217, 1993. Copyright (1993) Gordon & Breach Science Publishers, S.A. [Ref. 51].**

An alternative that has proven useful is a single chain Fv fragment (sFv) consisting of $V_H$ and $V_L$ covalently joined by a flexible amino acid linker that serves to facilitate their proper association [13–15] (Fig. 1). The sFv fragment generally retains the hapten binding activity of the parent immunoglobulin but is approximately one-half the size of Fab (27 vs 50 kDa). Preliminary data suggest that sFv distributes out of serum more rapidly than Fab, has a shorter elimination half-life, and undergoes more extensive renal excretion [16–18]. Thus, by virtue of its size and pharmacokinetics, sFv is of considerable interest as an alternative to Fab for the treatment of drug overdose. An additional advantage of sFv is that, being a recombinant protein, its structure can potentially be engineered to alter or improve its therapeutic properties.

Tricyclic antidepressant toxicity is the leading cause of death from intentional drug overdose in the United States [19]. Drug-specific Fab′ or Fab fragments have been shown to rapidly reduce toxicity due to the tricyclic antidepressant DMI in rats [6, 9], but the high dose of antibody fragment required may itself have adverse effects [10, 20]. In the current study, we report an initial investigation of the *in vivo* activity of a recombinant DMI-specific sFv fragment. To establish a pharmacokinetic basis for its potential use as an antidote, we studied its effects on the distribution of a tracer dose of [$^3$H]-DMI in rats, in comparison with the corresponding Fab fragment and IgG.

## MATERIALS AND METHODS

### *Preparation of B9-sFv*

The cloning, expression, and purification of sFv will be described in detail elsewhere [21]. In brief, sFv was cloned from a hybridoma line designated B9, which secretes a monoclonal antibody of the $IgG_{2a}$ (kappa) subclass specific for DMI [22]. The $V_H$ region of the B9 heavy chain and the entire light chain cDNA were amplified from total RNA using PCR and cloned into pGEM3Zf(+) for sequencing. These sequences were used to design primers for cloning the sFv construct depicted in Fig. 1 by overlapping PCR with a $(Gly_4Ser)_3$ linker. The B9 heavy chain leader sequence was inserted upstream of $V_H$ to direct the secretion and proper refolding of the sFv construct (the protein sequence corresponding to the leader is cleaved prior to secretion of sFv into the medium). The 800 bp fragment corresponding to B9-sFv was ligated into pGEM3Zf(+), subcloned into a mammalian expression vector, and used to transform NS0 myeloma cells. Clones were screened for the presence of B9-sFv mRNA by reverse transcriptase PCR amplification from total RNA using the B9 5′-$V_H$ and 3′-$V_L$ primers, followed by agarose gel electropho-

resis. B9-sFv activity (binding of DMI) *in vitro* was measured by analysis of the culture supernatant using the competitive ELISA described below. Several NS0 sFv secreting clones were grown in a reduced serum medium in 100-mL shake cultures or a 1L glass-jacketed bioreactor. Medium was harvested aseptically, cells were removed by centrifugation, and the supernatant was stirred at 4° for no more than 1 week. B9-sFv was isolated by affinity purification using a DMI-Sepharose column [9]. The pH of the eluted material was raised immediately to 6 by addition of 1 M Tris, and stored at −20°. The yield and purity of B9-sFv were determined by ELISA and SDS–PAGE. The affinity of B9-sFv for DMI was measured by competitive ELISA [23].

### *Preparation of B9-IgG and Fab*

B9-IgG was produced in mouse ascites, and purified by sodium sulfate precipitation and affinity chromatography. B9-Fab fragment was prepared from IgG by papain digestion of the sodium sulfate precipitate [9]. Fab was purified by affinity chromatography and protein G chromatography (Pharmacia, Piscataway, NJ) to remove undigested IgG. The purity of B9-IgG, Fab, and sFv was determined by SDS–PAGE.

### *Antibody Assay*

B9-IgG, Fab, and sFv concentrations were measured by a modification of a previously described ELISA [22]. Wells were coated with a BSA–DMI conjugate. Samples were added with various concentrations of DMI followed by chicken anti-B9-Fab (0.1 μg/well) and rabbit anti-chicken IgG peroxidase conjugate (100 μL/well of 1:30,000 dilution; Sigma Chemical Co., St. Louis, MO). Because this assay is based upon the ability of antibody or antibody fragments to bind to immobilized BSA–DMI, pilot studies were performed to demonstrate that the very low concentration of DMI present in serum after [$^3$H]-DMI administration did not interfere with the ELISA assay.

### *Stability Studies*

B9-sFv was placed in NS0 culture medium, affinity column elution buffer, rat serum, or rat urine and stored at 37° for 96 hr. Two separate experiments were performed. In the first experiment, equal concentrations of sFv (0.2 mg/mL) were placed in each solution. In the second experiment, different sFv concentrations were used in each solution. These concentrations were intended to correspond to the relevant concentrations of sFv found in each of these solutions, e.g. 2.4 mg/mL in elution buffer, 0.2 mg/mL in serum, and 0.02 mg/mL in medium. Six samples were stored in each condition. B9-sFv concentrations were determined by competitive ELISA, using freshly prepared affinity-purified B9-sFv as a standard. Affinity column elution buffer was 5% acetonitrile/1% propionic acid. NS0 culture medium was Dulbecco's Modified Eagle's Medium containing 4.5 g/L dextrose (Gibco-BRL), 25 mM HEPES (Sigma), 2.5% heat-inactivated fetal bovine serum (Biocell, Rancho Domingo, CA), 0.5% Nutridoma-NS (Boehringer Mannheim, Indianapolis, IN), 100 μM non-essential amino acids (Sigma), 1 mM sodium pyruvate (Sigma), 0.5 mM L-glutamic acid, 0.5 mM L-asparagine, 50 μM monothioglycerol, 30 μM each of adenosine, guanosine, cytidine, and uridine, and 10 μM thymidine.

Single samples of B9-sFv (0.5 mg/mL) in buffer were also stored for 6 months at 25, 4, −20 and −70° and assayed by ELISA. Because of the long duration of this experiment, and because freshly prepared B9 sFv was not available at each time point to use as a standard, B9-sFv activity was compared instead with that of a reference solution of 0.5 mg/mL B9-Fab, which is stable for at least 6 months at −20° (unpublished data). B9-sFv activity was recorded as the ratio of the sFv sample absorbance to that of the B9-Fab.

### *Dose Preparation*

Protein concentrations were measured by dye binding [24]. Doses were prepared in 10 mM phosphate buffer (pH 6) and concentrated to a final volume of 1 mL by ultrafiltration (Centriprep-3, Amicon Corp., Danvers, MA) for administration. The doses of B9-IgG, -Fab, and -sFv were 7.5, 5, and 2.7 mg, respectively, each representing 0.1 μmol of DMI binding sites. Assumptions used in calculating these doses were (1) molecular weights of 150, 50, and 27 kDa for B9-IgG, B9-Fab, and B9-sFv, and (2) two binding sites per IgG, one binding site per Fab or sFv. Doses represented the amount of antibody or antibody fragment administered (calculated as the total protein concentration, determined by dye binding, multiplied by the percent homogeneity as determined by SDS–PAGE densitometry). BSA (7.5 mg) was used as a control protein that does not alter DMI pharmacokinetics *in vivo* [25].

### *Animal Preparation*

Male Holtzman rats weighing 150–225 g were anesthetized i.m. with a mixture of droperidol (2 mg/mL) and fentanyl (0.04 mg/mL), 1 mL/kg initially followed by 0.5 mL/kg as needed [25]. Both femoral veins were cannulated. [$^3$H]-DMI and antibodies were administered via the right femoral vein, and blood samples were obtained via the left femoral vein.

### *Protocol*

Groups consisted of 5 rats each. All groups received 10 μCi ($10^7$ dpm) of [$^3$H]-DMI ($1.7 \times 10^{-4}$ μmol DMI) in 0.25 mL of 0.9% NaCl by rapid i.v. infusion. Fifteen minutes later, animals received B9-IgG, B9-Fab, B9-sFv, or BSA in a volume of 1 mL administered i.v. over 2 min. Blood samples (0.3 mL) were collected as indicated in Fig. 4. Serum was separated immediately by centrifugation, and 0.1 mL was placed in scintillation fluid for measurement of [$^3$H]-DMI concentrations. After the final blood collection, 2 mL of 0.9% saline was infused i.v. to help assure urine output, the veins were tied off, and the wound was stapled. Rats were placed in metabolic cages with *ad lib.* food and water. All urine was collected for 24 hr, and 0.1-mL aliquots of the 0–3, 3–6 and 6–24 hr collec-

tions were placed in scintillation fluid for measurement of [$^3$H]-DMI concentrations.

### *Statistical Analysis*

The percent change in the serum radiolabel concentration (dpm/mL) was compared among groups by repeated measures ANOVA. Comparisons at individual time points were performed using one-way ANOVA with Scheffe's contrast. *P* values of <0.05 were considered significant.

## RESULTS

### *Antibody Characterization*

SDS–PAGE of B9-IgG, -Fab, and -sFv is shown in Fig. 2. Their previously determined affinities for DMI were 3.3, 1.8, and 1.4 $\times 10^7$ $M^{-1}$, respectively [21]. The yield of affinity-purified B9-sFv from NS0 medium ranged from 10 to 40 mg/L.

### *sFv Stability*

At 37° and an sFv concentration of 0.2 mg/mL, sFv concentrations decreased over 96 hr in all solutions (Fig. 3A). The decrease was most rapid in urine and buffer. The measured sFv concentration in serum increased slightly initially and then decreased, but the decrease was slower than that of sFv stored in buffer or urine. The stability of sFv in medium was intermediate between that of serum and buffer. Similar results were obtained with sFv studied at concentrations representative of those found occurring in the various solutions in the current study (Fig. 3B). Urine was not included in this experiment.

Stability data of sFv stored in buffer at other temperatures

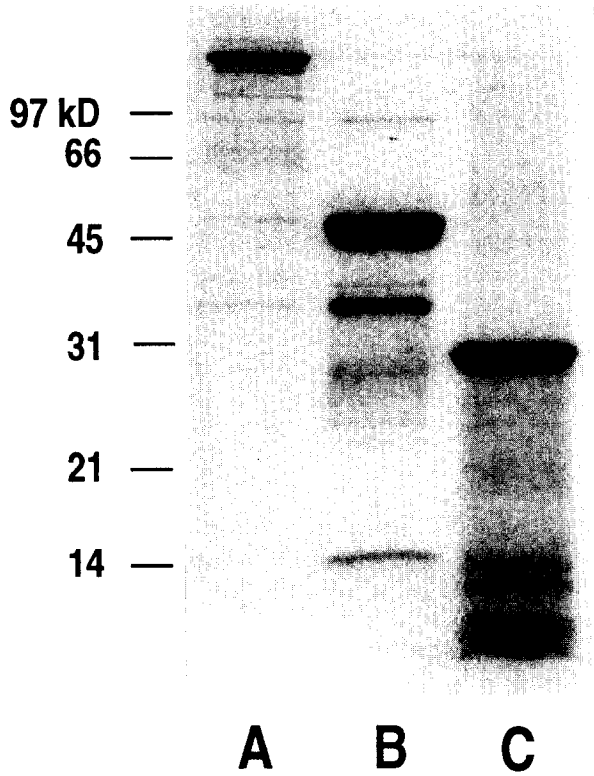


**FIG. 2. Nonreducing SDS–PAGE of (A) B9-IgG (150 kDa), (B) B9-Fab (50 kDa), and (C) B9-sFv (27 kDa). It is common for sFv fragments to migrate on SDS–PAGE with an apparent size that is slightly higher than calculated [26]. The 35 kDa band associated with Fab has not been identified.**

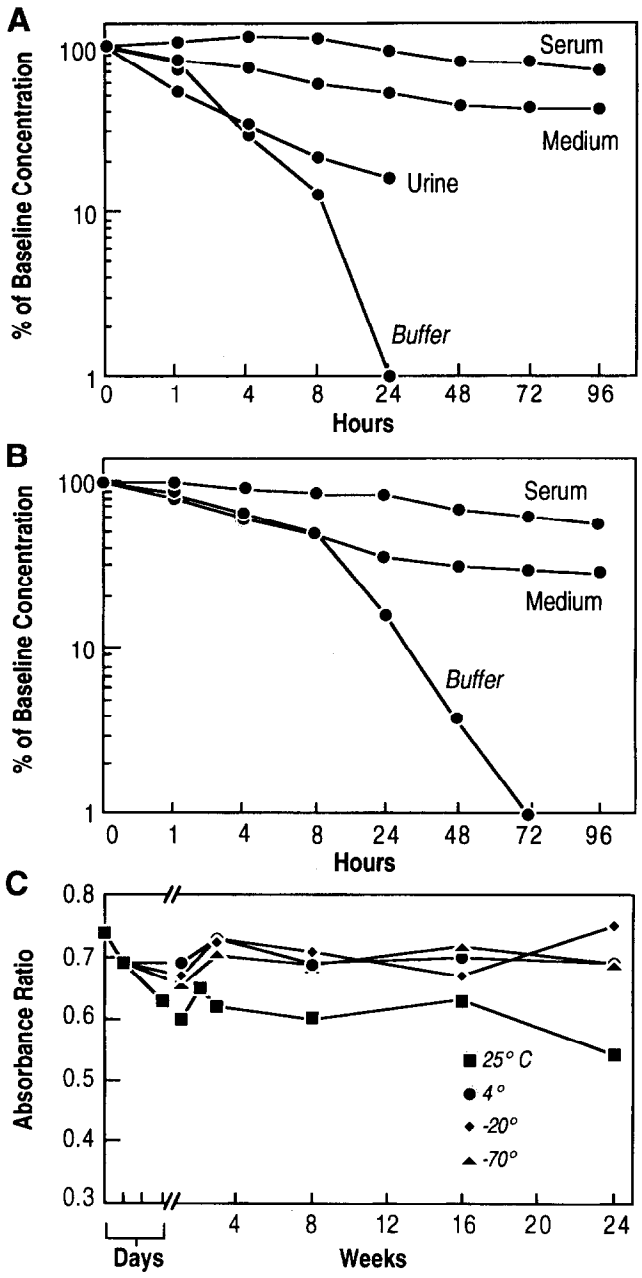


**FIG. 3. (A) Stability of B9-sFv at 37° and a concentration of 0.2 mg/mL in various solutions. (B) Stability of B9-sFv at 37°, at concentrations corresponding to those typically found in each solution in the current study; 2.4 mg/mL in elution buffer, 0.2 mg/mL in serum, and 0.02 mg/mL in medium. N = 6 for both panels A and B. (C) Stability of sFv at lower temperatures (data represent single samples at each time point). Note that the time scale differs from that of A and B, and that sFv activity was measured as the ELISA absorbance ratio rather than as concentrations.**

are shown in Fig. 3C. Samples stored at 25° showed a modest decrease in concentration over several months, whereas samples stored at 4, −20, or −70° showed no decrease in concentration. sFv stored in urine at −20° showed a rapid decrease in measured concentration, with a decline of more than 4-fold over 24 hr (data not shown). For this reason, further studies of sFv stability in urine were not performed.

### *Effects of Treatments on Radiolabel Distribution and Excretion (Fig. 4)*

All three forms of antibody (B9-IgG, B9-Fab, and B9-sFv) rapidly and significantly increased the concentration of radiolabel in serum compared with the BSA control group ($P < 0.01$). The percent increase in serum radiolabel concentration was greatest for B9-IgG. The B9-IgG and B9-Fab groups were significantly different from the BSA group at 1, 5 and 15 min, while the B9-sFv group differed from the BSA group at 1 and 5 min. The B9-IgG group had a higher percent increase in radiolabel concentration than the B9-Fab and B9-sFv groups at all time points ($P < 0.01$), while the B9-Fab and B9-sFv groups did not differ from each other at any time.

Urinary radiolabel excretion over 24 hr ranged from 28.9 to 38.9% of the administered dose, with most excretion occurring during the first 6 hr (Table I). There were no significant differences between groups at any time ($P = 0.8$). Urine volumes ranged from 11.7 ± 2.8 to 14.1 ± 1.5 mL and did not differ among groups ($P = 0.9$).

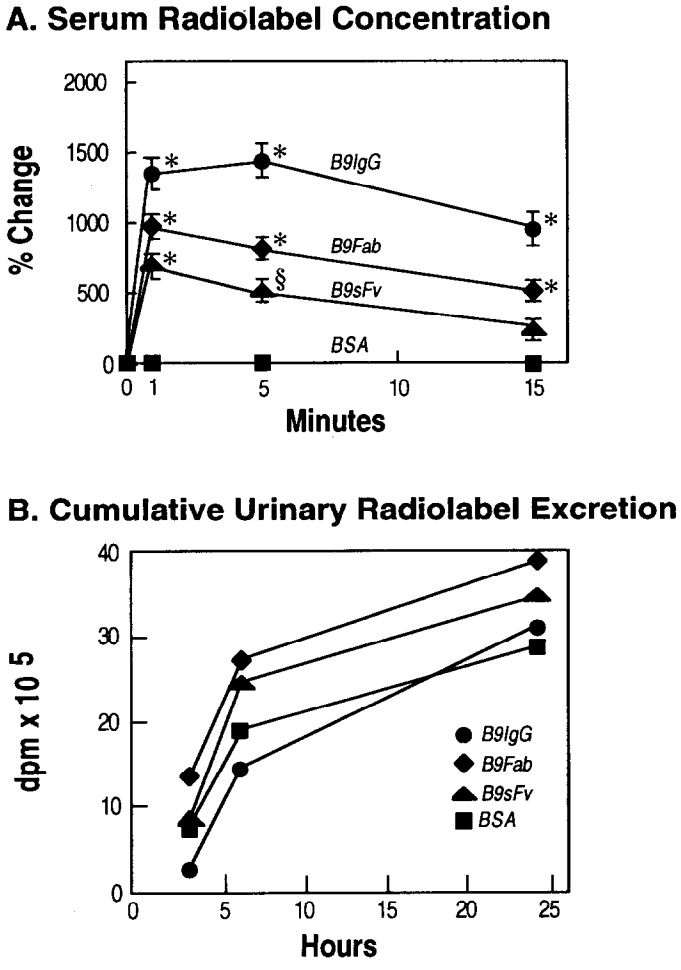


**FIG. 4. (A) Effects of antibodies on the percent increase in radiolabel concentration in serum (mean ± SD, N = 6). Antibody was administered at 0 min, and [$^3$H]-DMI was administered 15 min earlier. Key *$P < 0.01$ compared with the BSA control group, and §$P < 0.05$ compared with BSA. (B) Effects of treatments on cumulative urinary radiolabel excretion. Values are the means for 6 animals; standard deviations are omitted for clarity (see Table 1). There were no significant differences among groups.**

### *Serum Antibody Binding Site Concentrations*

Serum binding site concentrations were highest at all times after B9-IgG, followed by B9-Fab and B9-sFv (Table 2).

## DISCUSSION

The principal finding of this study is that DMI-specific B9-sFv administered to rats produced a rapid and substantial redistribution of radiolabel into serum. The measurement of radiolabel concentration does not distinguish between [$^3$H]-DMI and metabolites, but the elimination half-life of DMI in rats is long (29 hr) and only negligible amounts of metabolites are formed in the first few hours after a single dose [6, 27]. Therefore, the increase in serum radiolabel concentration produced by B9-sFv administration was likely due to [$^3$H]-DMI. Because the red blood cell:plasma DMI concentration ratio is only 2:1 [27], the 7.3-fold increase in serum radiolabel concentration produced by B9-sFv must have resulted from redistribution of radiolabel out of tissues. The redistribution of DMI by antibodies or antibody fragments has been observed with other DMI-specific Fab or Fab′ fragments in rats, and is predictive of their ability to reduce DMI toxicity. A different monoclonal DMI-specific IgG (G5-IgG) produces a dose-related redistribution of radiolabel in a protocol similar to that of the current study, using a subtoxic dose of [$^3$H]-DMI [28]. The same G5-IgG produces a similar redistribution of a toxic dose of DMI, while simultaneously preventing its cardiotoxicity [29]. Redistribution of DMI, and concurrent reversal of DMI toxicity, have also been reported with G5-Fab′ fragment, and with a polyclonal DMI-specific Fab fragment [6, 9]. Thus, the redistribution of DMI out of tissues (represented by an increase in the serum DMI concentration) is a pharmacokinetic marker for its ability to

**TABLE 1. Cumulative urinary radiolabel excretion after administration of treatments**

| Treatment | Radiolabel excretion (dpm × $10^5$) | | |
|---|---|---|---|
| | 3 hr | 6 hr | 24 hr |
| BSA | 7.7 ± 11.3 | 19.0 ± 9.2 | 28.9 ± 11.2 |
| B9-IgG | 2.7 ± 3.9 | 14.5 ± 12.1 | 31.0 ± 11.4 |
| B9-Fab | 13.4 ± 13.6 | 27.2 ± 4.8 | 38.9 ± 9.7 |
| B9-sFv | 8.6 ± 11.4 | 24.9 ± 9.2 | 34.9 ± 10.9 |

Values are means ± SD, N = 6 per group. There were no significant differences among groups.

**TABLE 2. Serum concentrations of drug-binding sites after administration of treatments**

| Treatment* | Binding sites† (μM) | | |
|---|---|---|---|
| | 1 min | 5 min | 15 min |
| B9-IgG | 23.5 ± 3.1 | 22.3 ± 3.9 | 21.2 ± 3.1 |
| B9-Fab | 17.4 ± 2.2 | 16.0 ± 1.6 | 12.2 ± 4.6 |
| B9-sFv | 13.7 ± 12.6 | 11.5 ± 11.1 | 8.1 ± 7.8 |

* Treatments were administered at 0 min.

† Values means ± SD, N = 6 per group represent the concentration of drug-binding sites (two sites per molecule of IgG, one site per Fab or sFv).

reverse DMI toxicity, consistent with the hypothesis that redistribution of drug out of target tissues is the mechanism by which drug-specific antibodies act as antidotes to drug overdose. The redistribution of radiolabel produced by B9-sFv in the current study suggests that, like its larger counterparts, this antibody fragment has the potential to reverse DMI toxicity.

The magnitude of increase in the serum radiolabel concentration was greatest with IgG, and was somewhat lower with Fab or sFv. It is possible that these differences were due to the affinities of these antibodies for DMI, but B9-IgG has only a 2-fold higher affinity than B9-Fab or B9-sFv. The higher radiolabel concentrations in serum after IgG might also be due to the higher measured serum IgG concentrations compared with Fab or sFv concentrations. The rates of initial distribution of IgG, Fab, and sFv out of serum differ markedly, with the smaller fragments cleared from serum more rapidly. For example, Milenic *et al.* [17] found the initial distribution half-lives for $^{125}$I-labeled B72.3 tumor-specific IgG, Fab′ or sFv in mice to be 39, 9.1, and 3.7 min, respectively. Similar results were reported with another (B6.2) $^{125}$I-labeled tumor-specific Fab′ and sFv, which had initial serum half-lives of 14.8 and 2.4 min in mice [16], and for fibrin-specific IgG and sFv, which had initial serum half-lives of 90 and 10 min [18]. The measured serum antibody concentrations in the current study are consistent with this difference in distribution kinetics, with B9-IgG having the smallest and B9-sFv the largest decrease in serum concentration over the 15-min sampling period. Thus, the lower serum concentrations of B9-sFv compared with Fab or IgG may be explained by its more rapid initial distribution kinetics. Further studies of the B9-sFv, specifically designed to understand its pharmacokinetics, will be of interest to help understand these observations.

A large variability was noted in the measured sFv concentrations in serum. The reason for this variability is not clear. An additional 29 kDa band was observed on SDS–PAGE of some batches of B9-sFv (data not shown), perhaps representing sFv with the heavy chain leader peptide retained. This additional band clearly binds DMI because it is retained by the DMI affinity column during purification. However, the possibility that this material interferes with the ELISA assay used to quantitate sFv is being studied. Interestingly, the percent increase in serum radiolabel concentration of the B9-sFv group showed much less variability (coefficient of variation 23% at 1 min) than the measured serum sFv concentrations (coefficient of variation 92% at 1 min), also suggesting that there may have been some inaccuracy in the measurement of serum sFv concentrations unrelated to the redistribution of DMI.

It is worthwhile noting that a smaller increase in serum radiolabel concentration after sFv administration compared with that observed after Fab or IgG, if it is due to more rapid distribution of sFv out of serum, does not necessarily imply that it will be less effective as an antidote to DMI toxicity. On the contrary, in the case of digoxin toxicity, digoxin-specific Fab is more rapidly effective than IgG despite producing a lesser increase in the serum digoxin concentration, suggesting that more rapid distribution out of serum enhances its ability to redistribute drug out of target tissues [11].

Urine radiolabel excretion did not differ among treatments in this study. This result was likely due to the use of radiolabel to estimate DMI excretion rather than a more specific assay (which was not possible because of the very low DMI dose administered and the resulting low concentration of DMI in urine). DMI is excreted only sparingly in urine as parent compound; for example, only 2.1% of a toxic dose of DMI administered to rats was excreted in urine [30]. The high percentage of the administered radiolabel excreted in the current study, even in BSA control animals, suggests that most of the radiolabel represented DMI metabolites. This large background of radiolabel excretion could have masked any differences in DMI excretion in the groups receiving active antibody. The measurement of B9-sFv concentrations in urine would also have been interesting. However, the poor stability of B9-sFv in urine (see below) precluded its accurate measurement in the current study.

Antibody sFv fragments, in general, are not as stable as their corresponding Fab or IgG [31, 32]. Instability of sFv could produce losses in processing and purification of the fragment, or affect its pharmacokinetics and efficacy as an antidote. B9-sFv showed some loss of activity in elution buffer at 25° but no loss of activity when stored at 4° or lower for up to 6 months. Therefore, processing of B9-sFv for this study was carried out at 4°, and losses in processing were minimal. B9-sFv was much less stable at 37°. Stability was greatest in serum, perhaps owing to the higher concentration of unrelated proteins. While loss of activity was measurable over 96 hr, losses over the time frame relevant to the treatment of DMI overdose (6–24 hr in humans) were small and unlikely to compromise its efficacy. In the current study, very little loss of activity would be expected over the 15-min time period when blood radiolabel concentrations were sampled, and should not have affected the ability of B9-sFv to redistribute DMI.

In summary, a recombinant 27 kDa DMI-specific sFv fragment was shown to rapidly redistribute a subtoxic dose of DMI in rats. These data suggest that larger doses of B9-sFv may be effective as an antidote for DMI toxicity. sFv may have pharmacokinetic or toxicologic advantages over larger antibody fragments as an antidote to drug overdose. Moreover, as a recombinant protein, its structure can potentially be altered to modify or improve its therapeutic properties. Further study of sFv as an antidote for DMI or other drug toxicity is therefore of interest.

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## References


1. Smith TW, Review of clinical experience with digoxin immune Fab (ovine). *Am J Emerg Med* **9**(Suppl 1): 1–6, 1991.
2. Ochs HR and Smith TW, Reversal of advanced digitoxin toxicity and modification of pharmacokinetics by specific antibodies and Fab fragments. *J Clin Invest* **60:** 1303–1313, 1977.
3. Baud FJ, Sabouraud A, Vicaut E, Taboulet P, Lang J, Bismuth C, Rouzioux JM and Scherrmann J-M, Brief report: Treatment of severe colchicine overdose with colchicine-specific Fab fragments. *New Engl J Med* **332:** 642–645, 1995.
4. McClurkan MB, Valentine JL, Arnold L and Owens SM, Disposition of a monoclonal anti-phencyclidine Fab fragment of immunoglobulin G in rats. *J Pharmacol Exp Ther* **266:** 1439–1445, 1993.
5. Chen H, Bowles MR and Pond SM, Prevention of paraquat toxicity in suspensions of alveolar type II cells by paraquat-specific antibodies. *Hum Exp Toxicol* **13:** 551–557, 1994.
6. Brunn GJ, Keyler DE, Pond SM and Pentel PR, Reversal of desipramine toxicity in rats using drug-specific antibody Fab′ fragment: Effects on hypotension and interaction with sodium bicarbonate. *J Pharmacol Exp Ther* **260:** 1392–1399, 1991.
7. Smith TW, Lloyd BL, Spicer H and Haber E, Immunogenicity and kinetics of distribution and elimination of sheep digoxin-specific IgG and Fab fragments in the rabbit and baboon. *Clin Exp Immunol* **36:** 384–396, 1979.
8. Keyler DE, Salerno DM, Murakami MM, Ruth G and Pentel PR, Rapid administration of high-dose human antibody Fab fragments to dogs: Pharmacokinetics and toxicity. *Fundam Appl Toxicol* **17:** 83–91, 1991.
9. Pentel PR, Ross CA, London J, Sidki A, Shelver WL and Keyler DE, Reversal of desipramine toxicity in rats using polyclonal drug-specific antibody Fab fragments. *J Lab Clin Med* **123:** 387–393, 1994.
10. Keyler DE, Shelver WL, Landon J, Sidki A and Pentel PR, Toxicity of high doses of polyclonal drug specific antibody Fab fragments. *Int J Immunopharmacol* **16:** 1027–1034, 1994.
11. Lloyd BL and Smith TW, Contrasting rates of reversal of digoxin toxicity by digoxin-specific IgG and Fab fragments. *Circulation* **58:** 280–283, 1978.
12. Hochman J, Inbar D and Givol D, An active antibody fragment (Fv) composed of the variable portions of heavy and light chains. *Biochemistry* **12:** 1130–1135, 1973.
13. Huston JS, Levinson D, Mudgett-Hunter M, Tai MS, Novotny J, Margolies MN, Tidge RJ, Bruccoleri RE, Haber E, Crea R and Oppermann H, Protein engineering of antibody binding sites: Recovery of specific activity in an anti-digoxin single-chain Fv analog produced in *Escherichia coli*. *Proc Natl Acad Sci USA* **85:** 5879–5883, 1988.
14. Bird RE, Hardman KD, Jacobson JW, Johnson S, Kaufman GM, Lee SM, Lee T, Pope SH, Riordan GS and Whitlow M, Single-chain antigen-binding proteins. *Science* **242:** 423–426, 1988.
15. Huston JS, McCartney J, Tai MS, Mottola-Hartshorn C, Jin D, Warren F, Keck P and Oppermann H, Medical applications of single-chain antibodies. *Int Rev Immunol* **10:** 195–217, 1993.
16. Colcher D, Bird R, Roselli M, Hardman KD, Johnson S, Pope S, Dodd SW, Pantoliano MW, Milenic DE and Schlom J, *In vivo* tumor targeting of a recombinant single-chain antigen-binding protein. *J Natl Cancer Inst* **82:** 1191–1197, 1990.
17. Milenic DE, Yokota T, Filpula DR, Finkelman MAJ, Dodd SW, Wood JF, Whitlow M, Snoy P and Schlom J, Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancarcinoma monoclonal antibody CC49. *Cancer Res* **51:** 6363–6371, 1991.
18. Laroche Y, Demaeyer M, Stassen JM, Gansemans Y, Demarsin E, Matthyssens G, Collen D and Holvoet P, Characterization of a recombinant single-chain molecule comprising the variable domains of a monoclonal antibody specific for human fibrin fragment D-dimer. *J Biol Chem* **266:** 16343–16349, 1991.
19. Litovitz TL, Clark LR and Soloway RA, 1993 Annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. *Am J Emerg Med* **12:** 546–584, 1994.
20. Moran RJ, Struthers AD and Hewick DS, Digoxin-specific Fab fragments impair renal function in the rat. *J Pharm Pharmacol* **46:** 854–856, 1994.
21. Kitchin K, Lin G, Shelver WL, Murtaugh MP, Pentel PR, Pond SM, Janelle CO, Humphrey JE, Smith JM and Flickinger MC, Cloning, expression and purification of an anti-desipramine single chain antibody in NS/0 myeloma cells. *J Pharm Sci* **84:** 1184–1189, 1995.
22. Bowles M, Johnston SC, Schoof DD, Pentel PR and Pond SM, Large scale production and purification of paraquat and desipramine monoclonal antibodies and their Fab fragments. *Int J Immunopharmacol* **10:** 537–545, 1988.
23. Hogg PJ, Johnston SC, Bowles MR, Pond SM and Winzor DJ, Evaluation of equilibrium constants for antigen–antibody interactions by solid-phase immunoassay: The binding of paraquat to its elicited mouse monoclonal antibody. *Mol Immunol* **24:** 797–801, 1987.
24. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* **72:** 248–254, 1976.
25. Pentel PR and Keyler DE, Effects of high dose *alpha*-1-acid glycoprotein on desipramine toxicity in rats. *J Pharmacol Exp Ther* **246:** 1061–1066, 1988.
26. McCartney JE, Lederman L, Drier EA, Cabral-Denison NA, Wu GM, Batorsky RS, Huston JS and Oppermann H, Biosynthetic antibody binding sites: Development of a single-chain Fv model based on antidinitrophenol IgA myeloma MOPC 315. *J Prot Chem* **10:** 669–683, 1991.
27. DeVane CL, Simpkins JW and Stout SA, Cerebral and blood pharmacokinetics of imipramine and its active metabolites in the pregnant rat. *Psychopharmacology* **84:** 225–232, 1984.
28. Pentel PR, Keyler DE, Brunn GJ, Milavetz JM, Gilbertson DG, Matta SG and Pond SM, Redistribution of tricyclic antidepressants in rats using a drug-specific monoclonal antibody: Dose–response relationship. *Drug Metab Dispos* **19:** 24–28, 1991.
29. Pentel PR, Brunn GJ, Pond SM and Keyler DE, Pretreatment with drug-specific antibody reduces desipramine cardiotoxicity in rats. *Life Sci* **48:** 675–683, 1991.
30. Keyler DE, Le Couteur DG, Pond SM, St. Peter JV and Pentel PR, Effects of specific antibody Fab fragments on desipramine pharmacokinetics in the rat *in vivo* and in the isolated perfused liver. *J Pharmacol Exp Ther* **272:** 1117–1123, 1995.
31. Glockshuber R, Malia M, Pftizinger I and Pluckthun A, A comparison of strategies to stabilize immunoglobulin Fv-fragments. *Biochemistry* **29:** 1362–1367, 1990.
32. Brinkmann U, Reiter Y, Jung S, Lee B and Pastan I, A recombinant immunotoxin containing a disulfide-stabilized Fv fragment. *Proc Natl Acad Sci USA* **90:** 7538–7542, 1993.