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SHORT COMMUNICATIONS

Redistribution into plasma of tracer doses of desipramine by anti-desipramine antiserum in rats

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Tricyclic antidepressant (TCA) toxicity is the leading cause of death from intentional drug overdose in the United States [1]. Available therapies such as hypertonic sodium bicarbonate are of some benefit [2, 3], but they are often ineffective in seriously poisoned patients [4]. Enhancing the elimination of the TCAs from the body is difficult because of their extensive distribution to tissues [5]. Extracorporeal methods such as hemodialysis or hemoperfusion have access only to drug that is in blood, and can remove only a small fraction of a toxic dose [6, 7].

Drug-specific antibodies have been shown recently to alter the distribution of drugs that are extensively bound to tissues and to be of value in treating drug toxicity [8]. Administration of digoxin-specific IgG or Fab fragments to dogs with digoxin toxicity rapidly redistributes digoxin from tissues into serum and reverses digoxin-induced ventricular arrhythmias [9, 10]. Digoxin toxicity in patients has been successfully treated using anti-digoxin Fab fragments [11].

The potential use of drug-specific antibodies to treat TCA toxicity is of interest because the TCAs resemble digoxin in their extensive distribution to tissues and their propensity to produce lethal cardiotoxicity. Fatalities due to TCA toxicity usually occur within hours of TCA ingestion. If TCA-specific antibodies are to be therapeutically useful, they must be capable of removing TCAs from the target organs for toxicity (heart and brain) rapidly enough to be of clinical benefit. As a preliminary step in determining the feasibility of using TCA antibodies therapeutically, we studied the effects of anti-desipramine antiserum (DMI-As) on the distribution of tracer doses of [^{3}H]desipramine ([^{3}H]DMI) in rats.

Materials and methods

[^{3}H]DMI (77 Ci/mole, 260 μCi/μg) was obtained from New England Nuclear. Identity of the labeled compound with unlabeled DMI was confirmed by thin-layer chromatography on silica plates in two solvent systems: ethanol–ammonium hydroxide (29/1) and ethanol–ammonium hydroxide–ethyl acetate (70/10/10). Purity of [^{3}H]DMI was >95%.

DMI-As, a gift of the SYVA Corp., was an ammonium sulfate cut of pooled sheep antiserum with total protein content of 60 mg/ml. A similarly prepared antiserum directed against tocainide served as the control antiserum. The intrinsic affinity constant (K_a) and the binding capacity of DMI-As for [^{3}H]DMI were determined by the method of Muller [12]. In brief, [^{3}H]DMI, DMI-As and various concentrations of inhibitor (unlabeled DMI) were incubated in buffer for 2 hr. The DMI-As concentration used was that which produced 50% binding of [^{3}H]DMI in the absence of inhibitor. Saturated ammonium sulfate was added, and the mixture was centrifuged to separate bound and free [^{3}H]DMI. The average affinity constant and binding capacity of DMI-As were determined by Scatchard analysis.

Male Sprague–Dawley rats weighing 200–350 g were anesthetized with Innovar Vet (droperidol 0.2 mg/ml, fentanyl 0.04 mg/ml) using 1 ml/kg i.m. initially and 0.3 mg/kg every 20 min thereafter. This regimen maintained adequate anesthesia with normal blood pressure, heart and respiratory rate. Cannulae were placed in both femoral veins and kept patent with heparinized (100 units/ml) saline.

Two groups of six animals each received 33 μCi/kg [^{3}H]DMI via cannula no. 1, followed by 0.5 ml of normal saline flush. This represents 0.13 μg/kg DMI, or less than 0.001% of the toxic dose. Treatments were administered 15 min later to allow time for [^{3}H]DMI distribution to tissues. Six animals received 5 ml/kg DMI-As over 2 min via cannula no. 2 followed by 0.5 ml of normal saline flush, and six animals received control antiserum. Blood samples (0.4 ml) were removed from cannula no. 2 just before and at 2, 20, 30, 60 and 90 min after antiserum administration. An equal volume of normal saline was infused after each sample. Blood samples were immediately centrifuged and 0.1 ml plasma was placed directly in Scint-A (Amersham) for assay of radiolabel concentration. Quenching due to plasma was <2%. Animals were killed 90 min after administration of antiserum by decapitation. Organs were rapidly removed, weighed, and frozen at −70°F until assay. Organs were homogenized in 7 ml water; the suspension was alkalinized with 0.3 ml methoxyethylamine and extracted into 3 ml ethyl acetate. One ml of the extract was placed in Scint-A for assay of radiolabel concentration. Efficiency of [^{3}H]DMI extraction from spiked tissue samples was 95%, and quenching <3%. Concentration of radiolabel in organs was corrected for organ blood content by determining the tissue blood content of six separate animals using ^{51}Cr-labeled red blood cells. A red blood cell/plasma [^{3}H]DMI ratio of 2:1 [13] was assumed. Blood and plasma volumes were also measured in animals receiving ^{51}Cr-labeled red blood cells and were used to calculate the percentage of administered ^{3}H-radiolabel remaining in plasma at various times after [^{3}H]DMI administration:

$$\%\ \text{Dose in plasma} = \frac{\text{cpm/ml plasma} \times \text{plasma volume (ml/kg)}}{\text{dose administered (cpm/kg)}} \times 100$$

Plasma radiolabel concentrations at the various sampling times were compared using Student's one-tailed *t*-test. Tissue radiolabel concentrations were compared using Student's two-tailed *t*-test.

Results and discussion

Binding studies of DMI-As and DMI demonstrated a high-affinity component with K_a of 1.1×10^9 l/mole and binding capacity of 2.15×10^{-6} moles/l of DMI-As. The dose of DMI-As used in this study was chosen to provide as much DMI-As as possible without causing adverse effects due to hypervolemia. At the selected dose of 5 ml/kg, the DMI binding capacity administered to each animal was 1.1×10^{-8} moles/kg. Because of the relatively limited DMI-binding capacity of DMI-As, tracer doses of [^{3}H]DMI were used in this study rather than the toxic doses; the total DMI dose administered was <0.001% of the toxic dose for rats [2].

Redistribution of the radiolabel from tissues to plasma following administration of DMI-As was demonstrated by a rapid, 50-fold increase in the plasma radiolabel concentration (Fig. 1). Because of the extensive distribution of [^{3}H]DMI to tissues, only 0.3% of the administered radiolabel was present in plasma prior to administration of DMI-As. Thirty minutes after DMI-As, 15% of administered radiolabel was present in plasma. The measurement

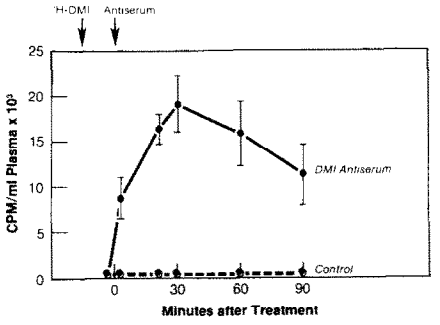

Fig. 1. Time course of plasma radiolabel concentrations. DMI-As or control antiserum was administered at time = 0, 15 min after administration of [^{3}H]DMI. $P < 0.001$ compared to control for all values after time = 0.

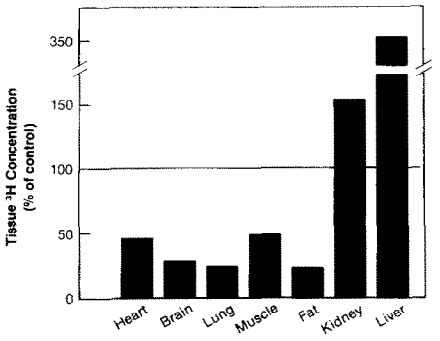

Fig. 2. Tissue radiolabel concentration of DMI-As-treated animals expressed as percent of control animals. Absolute values are shown in Table 1.

of plasma radiolabel concentration cannot distinguish between [^{3}H]DMI and its metabolites. However, the elimination half-life of DMI in rats is long (29 hr) and only negligible amounts of DMI metabolites are formed in the rat in the first few hours after administration of a single dose [2, 13]. In the current study, less than 2 hr elapsed from the time of [^{3}H]DMI administration to killing the animals. The increase in plasma radiolabel concentration was probably, therefore, largely due to redistribution of [^{3}H]DMI. Direct measurement of metabolite concentrations in this study by liquid chromatography was precluded by the small molar dose of [^{3}H]DMI administered. The binding of DMI metabolites to DMI-As *in vitro* has not been examined.

Redistribution of [^{3}H]DMI from tissues to plasma is supported by the observed decrease in radiolabel concentration in most tissues of DMI-As-treated animals compared to control animals (Fig. 2, Table 1). Decreases in radiolabel concentration were substantial, ranging from 51% in muscle to 77% in fat. Comparable decreases were found in brain (74%) and heart (54%), the target organs for DMI toxicity. The decreases observed in these organs support the potential use of DMI-As to reverse DMI toxicity. It must be emphasized, however, that the dose of [^{3}H]DMI used in this study was less than <0.001 of the toxic dose.

Further support for the potential therapeutic use of DMI-As is provided by the rapid time course of redistribution of radiolabel. In humans, DMI toxicity from overdose progresses rapidly and most deaths occur within hours of drug ingestion [4]. If DMI-As is to be of use in treating DMI toxicity, antagonism of toxicity must be correspondingly rapid. In the current study, nearly half of the maximum increase in plasma radiolabel concentration occurred within 2 min of DMI-As administration. This observation suggests that the pharmacokinetic effect of DMI-As on [^{3}H]DMI distribution is prompt enough to warrant further study as a therapeutic agent.

In contrast to the decrease in radiolabel concentration in most tissues of DMI-As-treated animals, concentrations of radiolabel in kidney and liver were greater in treated animals than in controls. This increase could have been due to uptake of [^{3}H]DMI–antibody complexes into reticuloendothelial cells in these organs. Radioactivity of the spleen, which also contains considerable reticuloendothelial tissue, was not measured. Because liver and kidney are both organs of drug elimination, a second possibility is that DMI-As altered the metabolic fate of [^{3}H]DMI. The tricyclic antidepressants, including DMI, are normally reversibly bound to alpha-1-acid glycoprotein in serum and are highly extracted by the liver; both bound and unbound drug are metabolized, and intact alpha-1-acid glycoprotein is returned to the circulation [14]. It is possible that binding of [^{3}H]DMI by DMI-As served to increase delivery of [^{3}H]DMI to the liver, thereby increasing its rate of metabolism. If this were true, an increase in the formation of conjugated metabolites that are excreted in urine might account for the increase in radiolabel concentration in the kidney. An increase in [^{3}H]DMI metabolism due to DMI-As would be of considerable interest, since it might serve to "recycle" the available antibody and thereby reduce the dose of antibody required to effect drug redistribution. Examining this hypothesis will require methods that can distinguish parent drug from metabolites.

The magnitude of increase in plasma radiolabel concentration in the current study was comparable to that reported in a previous study of [^{3}H]dihydromorphine distribution in mice [15]. Administration of tracer doses of [^{3}H]dihydromorphine followed by specific antiserum resulted in a 100-fold increase in plasma radiolabel concentration and a 75% reduction in brain radiolabel concentration. The dose of antiserum administered was estimated to be approximately equimolar to the body burden of [^{3}H]dihydromorphine. The administration of digitoxin-specific antibodies to dogs pretreated with digitoxin likewise resulted in a 10-fold increase in plasma drug con-

Table 1. Tissue radiolabel concentrations

	^{3}H-Radioactivity (cpm/0.1 g tissue*)						
	Heart	Brain	Liver	Lung	Muscle	Kidney	Fat
Control	1,360 ± 340	1,680 ± 360	1,330 ± 150	16,700 ± 4,040	200 ± 60	4,290 ± 1,430	180 ± 130
DMI-As	620 ± 110	470 ± 80	4,620 ± 1,100	4,000 ± 1,180	100 ± 70	6,570 ± 1,120	40 ± 20
P value†	0.001	<0.001	<0.001	<0.001	0.018	0.011	0.028

* Corrected for blood content of organs.

† Two-tailed comparison using Student's *t*-test of control vs DMI-As-treated groups.

centration, and was effected by a dose of antibody equal to twice the molar body burden of digitoxin [9]. In the current study, however, the molar dose of [^{3}H]DMI binding sites administered was more than 33 times the molar body burden of [^{3}H]DMI. The larger dose of DMI-As required to reduce [^{3}H]DMI is of potential importance because the body burden of DMI in a toxic animal or patient is quite large (>10 mg/kg) [2, 4]. Even if therapeutic use of DMI-As requires only an equimolar dose of binding sites, the dose will be substantial. If a larger dose is required, the potential problems associated with producing and administering these large doses of antiserum or antibody will be magnified. The reason for the large antibody dose required in our study is not clear. The average K_a of the high-affinity component of the antiserum is comparable to some of the antibodies used to reverse digitoxin or digoxin toxicity [10]. Possibly the binding of [^{3}H]DMI to tissues is tighter than the binding of the digitalis glycosides, and an antiserum with higher affinity for [^{3}H]DMI than used in this experiment is required. The efficiency of [^{3}H]DMI redistribution might also be improved if DMI-specific Fab fragments are used. Fab fragments distribute more extensively to extracellular fluid, and anti-digoxin Fab fragments have been shown to reverse digoxin toxicity more rapidly than an equimolar dose of anti-digoxin IgG.

In summary, DMI-As has been shown to redistribute tracer doses of [^{3}H]DMI from tissues to plasma. The rapid time course of drug redistribution and substantial reduction of radiolabel concentration in the target organs of DMI toxicity (heart and brain) support the potential clinical use of DMI-As to treat DMI toxicity. The increase in radiolabel concentration in liver and kidney after DMI-As could be due to altered distribution or metabolism of antibody–[^{3}H]DMI complexes. This possibility warrants further study because it might influence the amount of DMI-As required to effect drug redistribution and toxicity.

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Delivery of 5-aminosalicylate to the guinea pig cecum

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The effectiveness of sulfasalazine for the treatment and prophylaxis of ulcerative colitis [1] is considered to be mediated by 5-aminosalicylate (5-ASA), which is released by the intestinal flora [2–5]. That 5-ASA is the active component of sulfasalazine is consistent with its distribution to the colon [2–5] as well as its effectiveness when administered rectally to treat ulcerative diseases of the distal colon [6–10]. It appears likely, therefore, that sulfasalazine is merely a pro-drug which delivers 5-ASA to the colon where it is released by the intestinal bacteria [11].

In the search for an animal model of human ulcerative colitis, there has been particular interest in the observation that such rodents as the guinea pig respond to degraded carrageenan with a lesion in the lower bowel that resembles the human disease [12–14]. Unfortunately, it is not clear whether 5-ASA is effective in this guinea pig model [15–17]. Thus, while Jensen *et al.* [18] found that sulfasalazine administered by gastric fistula was quite effective in suppressing carrageenan-induced ulcers in the guinea pig, they could not demonstrate the effectiveness of a slow release preparation of 5-ASA. A possible explanation of this finding is that the preparation did not provide an adequate concentration of 5-ASA in the lower bowel. Previous work had shown that oral 5-ASA does not provide as high a concentration of 5-ASA in the colon of a rat as an equimolar dose of sulfasalazine [3].

In an attempt to determine whether 5-ASA can be administered more reliably in high concentrations to the cecum