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The pharmacokinetics of two novel antiviral agents, carobocyclic 3-deazaadenosine (Cc3Ado) and 3-deazaneplanocin (3-DA) was investigated in female BALB/c mice. For the former drug, there was rapid and extensive tissue distribution, an elimination half-life of 23 and 38 min for i.v. and orally-administered drug, respectively. For 3-DA, an elimination half-life of 26 min was found for iv administered drug. 3-DA was less extensively distributed into tissues. Cc3Ado was not found to bind to plasma proteins, while 3-DA, at low concentrations of drug bound extensively. Both compounds were metabolized to labeled products, which have not yet been identified. For Cc3Ado, one major metabolite eluted later than the parent compound, whereas in the case of 3-DA, the major labeled compound eluted earlier than the parent. We conclude provisionally that these labeled compounds represent metabolic conversion products. Tissues from mice dosed with either Cc3Ado or 3-DA contained detectable parent drug, but the majority of label represented chromatographic peaks other than the parent drug, indicating that the drugs were significantly metabolized.

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PAC In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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INTRODUCTION

Carbocyclic 3-deazaadenosine and 3-deazaneplanocin A (Cc3Ado, 3-DA, **APPENDIX** Figure 1) are antiviral agents with broad activity against a variety of DNA and RNA viruses. Little is known of the antiviral activities of 3-DA. The parent compound of Cc3Ado, 3-deazaadenosine has similar antiviral activities, but exhibits less selectivity and greater toxicity (DeClercq and Montgomery, 1983). The *in vitro* replication of a number of viruses, such as vaccina, reo, measles, parainfluenza and vesicular stomatitis was shown to be inhibited by Cc3Ado *in vitro* (De Clercq and Montgomery, 1983). In addition, Cc3Ado at 20-500 μg increased survival rates in newborn mice treated with lethal doses of vesicular stomatitis virus (De Clercq and Montgomery, 1983).

In another study, Cc3Ado markedly inhibited parainfluenza type 3 (PIV3) and respiratory syncytial (RSV) virus infections in HEp2 cells *in vitro* (Wyde *et al.* 1990). Cotton rats experimentally infected with RSV or PIV3 then treated with ≥ 1 mg/kg/day Cc3Ado had markedly lower virus titers than did controls (Wyde *et al.* 1992). In both of these studies, Cc3Ado exhibited little host toxicity *in vivo* and *in vitro*. Little has been published on the antiviral effects of 3-DA.

The antiviral effects of Cc3Ado and 3-DA probably arises from its inhibition of S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1), which catalyzes the reversible hydrolysis of S-adenosyl-L-homocysteine (SAH; AdoHcy) to adenosine and homocysteine (Chiang, *et al.* 1977; Montgomery *et al.*, 1982; De Clercq, 1987, and references therein). When this enzyme is inhibited, SAH accumulates in cells, which leads to a perturbation of methylation reactions. Accordingly, addition of homocysteine markedly potentiates the antitumor and antiviral activity of Cc3Ado *in vitro*, presumably by increasing intercellular levels of SAH (De Clercq *et al.* 1989). An additional, although reportedly less important mechanism of the antiviral activity of Cc3Ado and its congeners, is their ability to perturb intracellular concentrations of inosine and adenine nucleotides (Bennett *et al.* 1988).

A necessary prerequisite for exploring possible clinical applications for these drugs is to study its pharmacokinetic behavior in appropriate animal models. In this study, we characterized the absorption, distribution and elimination of these related drugs in female BALB/c mice. To our knowledge, this is the first investigation into the pharmacokinetic fate of these novel antiviral agents.

BODY: MATERIALS AND METHODS

Animals. Female BALB/c mice (approximately 18 g) were purchased from Simonsen Laboratories (Gilroy, CA) and housed in polycarbonate cages with corn cob bedding. The AAALAC-accredited facility operated on a 12 hr light-dark cycle, and animals were allowed free access to water and food (Wayne Lab Blox). Care and use of the animals adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Chemicals. Cc3Ado was kindly supplied by Dr. John Montgomery, Southern Research Institute, Birmingham, AL, and 3-DA was supplied by Dr. John Driscoll, National Cancer Institute. Both were tritium labeled by Moravek Biochemicals (Brea, CA). Radiochemical purity of the final products were greater than 98% as assessed by HPLC. ^{51}Chromate was purchased from Dupont NEN, Boston, MA. Labeled Cc3Ado was added to unlabeled Cc3Ado to achieve the desired specific activity, whereas labeled 3-DA was not diluted with unlabeled drug. For the final dose, the compounds were prepared in sterile saline.

Treatments and Sample collection. Animals were dosed (Cc3Ado: 10 mg/kg in 100 µl saline; 10µCi; 3-DA: 0.1 mg/kg; 10µCi) either intravenously (iv) via tail vein, or orally using an 18 ga. feeding needle. In the case of Cc3Ado, this was within the therapeutic dose range in cotton rats against experimental RSV and PIV3 infections (Wyde *et al.* 1990). Blood and tissue samples were collected at selected intervals for up to 72 hr. Blood was sampled from the retro-orbtial sinus using heparinized 70 µl capillary tubes, and was then stored at 4°C until the plasma was separated by centrifugation. Animals were lightly anesthetized by halothane to facilitate blood collection. The recovered plasma, generally 25-35 µl, was added to a microfuge tube containing 25 µl 2 M $HClO_4$. Animals were divided

into six groups of 4 animals each, and 3-4 samples were taken from each animal. Twenty-five μl H_2O was added to the sample, and the mixture was then mixed and centrifuged 10 min at 5000 x g. In the case of tissue, the tissue was homogenized in an equal volume of saline (Tissue tearor, BioSpec Products, Inc., Bartlesville, OK) before adding the perchlorate. This mixture was then centrifuged, and to 55 μl of the supernatant was added 0.1 M $NaHPO_4$, pH 10.6 (to approximately pH 4) and the mixture was then filtered through a 0.2 μm pore cellulose filter (RC-58 Bioanalytical Systems, West Lafayette, IN). Generally, about 80 μl was injected directly into the HPLC.

Analytical. Concentrations of Cc3Ado were determined using a modification of the HPLC method of Montgomery *et al.* (1982). Separations were made on a Beckman System Gold HPLC (Beckman, Fullerton, CA) equipped with a model 126 pump, and a model 506a refrigerated autosampler which was set at 4 °C. The column used was a Keystone #305 ODS/A C_{18} 5 μm particle size reversed-phase column (Keystone Scientific, Bellafonte, PA), that was was kept at 35 °C with a Beckman model 235 column heater. Cc3Ado was eluted with the following linear gradient program. From 0-13 min, a solvent mixture of 10 mM sodium phosphate, pH 4.5: acetonitrile (96:4) was pumped at 0.75 ml $min.^{-1}$ From 13-18 min, the flow rate was increased to 1.0 ml min^{-1} and the acetonitrile linearly increased from 4 to 60%. From 18-22 min, the flow rate returned to 0.75 ml min^{-1} by 22 min, and the acetonitrile concentration decreased from 60 to 4%. At 30 min, the run ended.

3-DA was separated using the above described HPLC instruments with the exception that the column was an Alltech Nucleosil C_{18} 5μm column. The solvent system was the following. Solvent A, which was an isocratic mixture of 4% acetonitrile, 96% of 1% acetic acid, was adjusted to pH 4.6 with ammonium hydroxide. Parent 3-DA and all metabolites eluted by 15 min using solvent A. At 15 min, a column wash program began using solvent B, which consisted of increasing 100% acetonitrile to 96% over 5 min, held

for 4 min, then returned to 4% over 8 min. The system was equilibrated to solvent A for 10 min prior to injection of the next sample.

Elution of Cc3Ado and 3-DA were monitored both by uv absorbance at 263 nm (Beckman model 166 programmable uv detector) and by in-line radiochemical detection (Beta-One, Radiomatic, Inc. Meriden, CT). Preliminary experiments were conducted to determine recovery and stability of parent Cc3Ado in blood and tissues at a range of concentrations routinely encountered in dosed animals (13 ng/ml to 50 μg/ml). In this concentration range, the average recovery of Cc3Ado was 101.6% ± 2.8, that of 3-DA was 97.5% ± 4.56. Both drugs were stable for at least 8 hr at 37 °C in tissues or in plasma.

Tissue concentrations of drug were calculated from the specific activity of the labeled drug, taking into consideration the degree of vascularization in each tissue as determined in a preliminary experiment: Briefly, 1.0 ml blood was collected from the heart/posterior vena cava of female BALB/c mice (approximately 18 g) and incubated with 500 μCi of sodium ^{51}chromate (50 μCi /0.1 ml blood) at 37 °C for 30 min. Ascorbate (2.5 mg) was added to prevent further tagging. Erythrocytes were separated by centrifugation, washed twice and reconstituted to original hematocrit. These cells were then injected iv (0.1 ml/mouse), and the mice were sacrificed after 30 min by CO_2 asphyxiation. Blood was collected as above, and the contents of the capillary tubes were placed in scintillation vials and weighed. To this was added 1 ml of 1:1 Soluene 350 (Packard, Downers Grove, IL): isopropanol, and incubated at 40 °C for 40 min and then 0.5 ml of 0.5% $HClO_4$ was added dropwise while swirling the sample. The samples were digested 30 min at 25 °C, then 15 min at 40°C then overnight at 25°C. Tissues were removed, blotted, weighed and 0.1-0.3 g portions were placed in scintillation vials with 1 ml of Soluene 350 and incubated at 40 °C overnight. To all samples was added 2.5 ml of Hionic-Fluor (Packard) before counting (Packard Model 1900). The amount of drug contained in blood in each tissue, based on blood volume x drug concentration in blood at that time point, was subtracted from the total

concentration of Cc3Ado in the tissues. The resulting value was then adjusted for the non-perfused tissue mass.

Protein binding determinations were carried out by partially ultrafiltering (RC-58 filters, Bioanalytical Systems, West Lafayette, IN) mouse blood to which eight concentrations of [^{3}H] Cc3Ado within the range of 16-2000 pmol were added *in vitro*. Samples were centrifuged and activity of filtrate as well as that in unfiltered sample was measured. Unbound drug was quantitated in the filtrate by scintillation counting (Model 3801, Beckman, Fullerton, CA).

Data Analysis. Pharmacokinetic parameters were estimated with the assistance of PCNONLIN (SCI Software, Lexington, KY). The data were assigned to the most appropriate model by best-fit of the weighted sums of squared errors as estimated by the F test.

BODY: RESULTS

All tables and figures appear in the **APPENDIX**. The mean blood volume in various tissues in BALB/c mice are presented in Table 1. In the BALB/c mouse, the kidney was the tissue with the highest mean blood volume (43%), followed by the lung (29%), spleen (24%) and liver (14%). Other tissues examined, such as brain, fat, heart, small intestine, stomach and thymus, had lower blood volumes. These values, together with the ^{3}H activity of blood at that time point, were then used as a correction factor to determine absolute drug concentration in the various tissues.

A sample chromatogram of the both drugs are presented in Figures 2 and 3. As can be seen, the retention time for Cc3Ado was approximately 13 min; that of 3-DA was 9 min. From 50 to 2000 ng/ml, the uv response for both drugs was linear ($r^2 = 0.9995$) but coupled with radiochemical detection, the lower limit of quantitation was in the femtomolar range. In the range 15-2000 pM Cc3Ado added to mouse blood, there was no binding to plasma proteins detected. For 3-DA, there was signficant binding to plasma proteins in the range of 10-150 ng/ml, but was less the concentrations greater than 200 ng/ml (Figure 4).

Plasma concentration *vs* time curves for both oral and intravenous Cc3Ado are presented in Figure 5. The plasma concentration *vs* time curves for both iv and oral Cc3Ado was best approximated by a two-compartment open model with first-order elimination. The half-life following iv administration was 23 min, while that for oral Cc3Ado was 38 min (Table 2). The plasma concentration of orally administered Cc3Ado reached a maximum at 24 min. On the basis of area-under-curve calculations, oral Cc3Ado was approximately 20% bioavailable compared to iv Cc3Ado. The steady state volume of distribution was 7.5 ml for iv Cc3Ado (Table 2).

Figure 6 shows that plasma concentration *vs* time curves for 3-DA. As with Cc3Ado, the data best approximated a two-compartment open model with first-order elimination. The

half life of iv 3-DA was approximately 26 min (Table 3). Data for oral 3-DA was too variable to allow for reliable calculations of pharmacokinetic parameters or bioavialability.

The decline in the concentration of parent Cc3Ado and 3-DA in the plasma were coupled with the concomitant increase of a major and at least one minor labeled peak (Figures 7 and 8). For Cc3Ado, the major peak, which we presume represents a major labeled metabolite, eluted 2 min after Cc3Ado, and reached a maximum concentration approximately 45 min after iv dosing. The major 3-DA metabolite emerged from the column at approximately 5 min.

By either route, Cc3Ado distributed into a variety of tissues. Tissue concentrations of Cc3Ado reached their maximum by 30 min following oral administration, while following iv dosing, the drug generally reached its maximum by 120 min. In orally dosed animals, the drug concentration in the stomach was greatest shortly after dosing and declined thereafter. By either route, the highest Cc3Ado concentrations were found in the liver, followed by kidney, spleen and lung, stomach then brain (Table 4 and 5) By 24 hr, all tissues contained a similar amount of drug. In most cases, the tissue concentration of Cc3Ado declined over time.

Compared to Cc3Ado, 3-DA was only poorly distributed into tissues (Tables 6 and 7).

CONCLUSIONS AND DISCUSSION

Carbocyclic-3-deazaadenosine and 3-DA are compounds with antiviral activity that presumably act via inhibition of S-adenosyl-L-homocysteine hydrolase. Knowledge of the fate and metabolism of Cc3Ado in an appropriate animal model is a requisite step in the development of this compound as a chemotherapeutic agent for human diseases. With that in mind, we characterized the distribution and pharmacokinetics of these drugs in the mouse, a popular model for antiviral studies. To our knowledge, this is the first investigation of the pharmacokinetic behavior of these drugs.

Carbocyclic-3-deazaadenosine was rapidly distributed and eliminated from the plasma, and the plasma concentration *vs* time curve exhibited triexponential character. Many of the principal pharmacokinetic parameters of Cc3Ado were similar to those of other nucleoside analogues. For example, the half-life of iv Cc3Ado was about half that in mice for 50 mg/kg iv AZT (Doshi *et al.* 1989). The 20% bioavailability of oral Cc3Ado we observed in this study was in the range of many anti-HIV drugs. In the mouse, the oral bioavailability of 2',3'-dideoxyadenosine and 2',3'-dideoxyinosine were 38% and 13%, respectively (Russel and Klunk, 1989). In the rat, the oral bioavailability of 2',3'-dideoxyinosine was 1 to 9% of the oral dose of 25 mg/kg (Ray *et al.* 1990). In mice, only 3.8% of the oral dose of 500 mg/kg of carbovir was bioavailable (El Dareer 1990). In our study, the bioavailability of Cc3Ado was probably reduced by incomplete absorption from the gut, and/or by rapid first-pass metabolism in tissues such as the liver or gut. In any event, oral administration of Cc3Ado may be an acceptable route of administration of this drug to achieve therapeutic concentrations.

Following either oral or iv administration, Cc3Ado distributed into a variety of tissues. In fact, at the early time intervals following oral administration, some tissues contained a greater concentration of Cc3Ado than plasma. With some exceptions, the liver contained the highest concentrations of Cc3Ado over the time course examined. In iv

dosed mice, the kidney contained the next highest drug concentration. This probably was due to renal excretion of this drug, although no examination of routes of excretion of Cc3Ado was made. Interestingly, in orally dosed animals, the kidney contained much less drug concentration than in those receiving the drug iv. That 3-DA was distributed in tissues to a lesser degree was undoubtedly due to the lower dose administered (0.1 vs 10 mg/kg) and also due to it's ability to bind to plasma proteins.

Although Cc3Ado is of potential use against respiratory viruses such as RSV and PIV3 (Wyde *et al.* 1990), very little of this drug distributed into the lungs. By either route of administration, the brain contained the lowest amounts of drug of any tissue.

Drug concentrations in residual tissue blood has been identified as an often-neglected source of error in conventional and physiological pharmacokinetic modeling (Khor 1991). We therefore sought to measure the amount of residual blood in each tissue sampled to allow for a measurement of the absolute drug concentration in each tissue. With the exception of the kidney, where we reported a higher blood volume, the values obtained for mean blood volumes in the mouse were close to that published for the rat (Tsuji *et al.* 1983). It is possible that the high blood volume in the kidney may have been caused by an accumulation of radioactive chromium which might occur following lysis of injected erythrocytes. In such as case, Cc3Ado 3-DA concentrations in the kidney would be actually higher than that reported here. In any event, the values from our determinations may be of use to other laboratories involved in pharmacokinetic analysis intent on precise measurements of tissue drug concentrations in this species.

We interpret the presence of a major labeled peak in the plasma of dosed animals to represent metabolites of Cc3Ado and 3-DA. In the plasma, these peaks increased concomitantly with the decrease in the concentration of the parent compound for the first 45 min after dosing. Future studies should include an identification of these metabolites to confirm their identity as drug metabolites.

Our data indicated that both drugs were rapidly metabolized in the various tissues we examined. Using in-line flow detection, we were able to monitor the relative contribution of both parent and labeled metabolites to the overall tissue concentration of drug. In many cases, the majority of tissue activity were from labeled peaks not corresponding to parent drug. Thus, this drug was likely converted to a variety of labeled products in tissues. For example, 30 min after oral administration, nearly 80% of the activity in the liver and kidney were from chromatographic peaks other than Cc3Ado or 3-DA.

In this initial investigation, the pharmacokinetics of a single dose of the related drugs Cc3Ado 3-DA were characterized. The dose chosen for Cc3Ado was within the range shown in previous studies to have antiviral activity *in vivo* (DeClercq and Montgomery, 1983; Wyde *et al.* 1990). The for 3-DA was chosen by consultation with Dr. John Huggins, USAMRIID. Future investigations on the fate of these related adensine drugs might include a focus on the potential dose-dependency of the pharmacokinetics as well as a characterization of the pharmacokinetic behavior of this drug in other animal species. Had the contract not been prematurely terminated, the firm identity of the Cc3Ado and 3-DA metabolites might also have been determined.

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APPENDIX TO FINAL REPORT

Contents:

Table 1. Mean blood volumes in selected tissues in BALB/c mice[1]

ORGAN	MEAN BLOOD VOLUME (%)
Brain	2.60 (± 0.42)
Fat	5.02 (± 1.49)
Heart	11.53 (± 1.94)
Kidney	43.39 (± 16.92)
Liver	14.02 (± 2.32)
Lung	29.43 (± 2.43)
Muscle[2]	3.73 (± 1.07)
Small Intestine	4.36 (± 1.39)
Spleen	24.86 (± 1.22)
Stomach	9.14 (± 4.95)
Thymus	13.12 (± 3.48)

[1]values represent mean percentage of blood in tissue (w/w) ± SEM of six mice
[2]skeletal leg muscle

Table 2. Estimates of pharmacokinetic pharmacokinetic parameters of iv and orally administered Cc3Ado in BALB/c mice.[1]

Parameter	i.v.	oral
t1/2 (min)	23.0 (± 3.24)	38.5 (± 10.2)
AUC ($\mu g \cdot min\ ml^{-1}$)[2]	381.15 (± 23.18)	78.52 (± 16.76)
t_{max} (min)	--	24.68 (± 2.01)
Cls ($ml\ kg^{-1} min^{-1}$)[3]	26.23 (± 1.70)	N/A[5]
K_{12} (min^{-1})	0.116 (± 0.041)	0.003 (± 0.001)
K_{21} (min^{-1})	0.134 (± 0.040)	0.019 (± 0.009)
Vd (ml)[4]	7.532 (± 0.682)	N/A
C_{max} (µg/ml)	24.427 (± 2.214)	1.034 (± 0.060)

[1] values represent mean (± S.D.) of 3-4 mice at each time interval.
[2] Area-under-curve calculated by the trapezoidal rule
[3] systemic clearance from the equation Cls = D/AUC
[4]Volume of distribution of the central compartment
[5] N/A = not applicable due to incomplete drug absorption

Table 3. Estimates of pharmacokinetic pharmacokinetic parameters of iv and orally administered 3-DA in BALB/c mice.[1]

Parameter	i.v.	oral
t1/2 (min)	26.0 (± 37.27)	N/A
AUC ($\mu g \cdot min\ ml^{-1}$)[2]	3.37 (± 1.20)	N/A
t_{max} (min)	--	N/A
K_{12} (min^{-1})	0.364 (± 0.255)	N/A
K_{21} (min^{-1})	0.0405 (± 0.046)	N/A
Vd (ml)[4]	741.42 (± 84.52)	N/A
C_{max} (µg/ml)	2.42 (± 0.277)	N/A

[1] values represent mean (± S.D.) of 3-4 mice at each time interval.
[2] Area-under-curve calculated by the trapezoidal rule
[3] systemic clearance from the equation Cls = D/AUC
[4]Volume of distribution of the central compartment
[5] N/A = not applicable due to variability of drug concentration data

Table 4. Tissue distribution of Cc3Ado in BALB/c mice following iv administration[1]

Time After Dosing

Tissue	30 min	120 min	24 hr
Heart	0.32 ± 0.13[2] (24.0)[3]	0.55 ± 0.18 (38.8)	0.05 ± 0.00 (12.2)
Lung	0.15 ± 0.09 (56.8)	0.45 ± 0.13 (44.9)	0.01 ± 0.00 (3.8)
Stomach	0.60 ± 0.08 (32.7)	0.50 ± 0.14 (51.9)	0.07 ± 0.01 (24.0)
Spleen	2.26 ± 0.53 (42.7)	0.70 ± 0.20 (31.3)	0.10 ± 0.01 (19.2)
Kidney	3.69 ± 0.93 (34.1)	0.44 ± 0.24 (13.1)	0.04 ± 0.00 (7.8)
Liver	4.81 ± 1.12 (22.1)	1.41 ± 0.51 (14.7)	0.17 ± 0.03 (10.1)
Brain	0.02 ± 0.01 (25.8)	0.07 ± 0.04 (28.0)	0.05 ± 0.01 (33.7)

[1] animals were given a single dose of 10 mg/kg Cc3Ado
[2] each value is the mean μg Cc3Ado/g tissue ± S.E. of four animals
[3] values in parentheses are the percentage of total activity present as parent Cc3Ado

Table 5. Tissue distribution of Cc3Ado in BALB/c mice following oral administration[1]

Time After Dosing

Tissue	30 min	120 min	24 hr
Heart	0.31 ± 0.16[2] (23.6)[3]	0.28 ± 0.08 (13.8)	0.07 ± 0.00 (10.6)
Lung	0.49 ± 0.15 (34.4)	0.46 ± 0.13 (23.7)	0.08 ± 0.01 (18.6)
Stomach	5.99 ± 3.90 (100)	0.57 ± 0.17 (31.3)	0.06 ± 0.00 (23.1)
Spleen	6.48 ± 2.94 (23.6)	1.63 ± 0.47 (29.2)	0.14 ± 0.02 (21.1)
Kidney	0.99 ± 0.47 (18.8)	1.38 ± 0.41 (20.7)	0.04 ± 0.00 (6.9)
Liver	3.83 ± 1.31 (18.4)	5.40 ± 1.66 (26.5)	0.14 ± 0.01 (14.8)
Brain	3.15 ± 1.67 (11.8)	0.02 ± 0.01 (7.9)	ND[4] (1.4)

[1] animals were given a single dose of 10 mg/kg Cc3Ado
[2] each value is the mean μg Cc3Ado/g tissue ± S.E. of four animals
[3] values in parentheses are the percentage of total activity present as parent Cc3Ado
[4] not detected

Table 6. Tissue distribution of 3-DA in BALB/c mice following iv administration[1]

Time After Dosing

Tissue	30 min	120 min	24 hr
Heart	0.06 ± 0.00[2](24.0)[3]	0.05± 0.00 (38.8)	0.05 ± 0.00 (12.2)
Lung	0.08 ± 0.01 (56.8)	0.08 ± 0.01 (44.9)	0.06 ± 0.00 (3.8)
Stomach	0.07 ± 0.01 (32.7)	0.07 ± 0.00 (51.9)	0.04 ± 0.00 (24.0)
Spleen	0.07 ± 0.01 (42.7)	0.10 ± 0.01 (31.3)	0.10 ± 0.01 (19.2)
Kidney	0.93 ± 0.09 (34.1)	0.90 ± 0.07 (13.1)	0.56 ± 0.06 (7.8)
Liver	0.44 ± 0.04 (22.1)	0.52 ± 0.03 (14.7)	0.13 ± 0.03 (10.1)
Brain	0.00 ± 0.00 (25.8)	0.00± 0.00 (28.0)	0.00 ± 0.00 (33.7)

1 animals were given a single dose of 0.1 mg/kg 3-DA
2 each value is the mean µg 3-DA/g tissue ± S.E. of four animals
3 values in parentheses are the percentage of total activity present as parent 3-DA

Table 7. Tissue distribution of 3-DA in BALB/c mice following oral dministration[1]

Time After Dosing

Tissue	30 min	120 min	24 hr
Heart	0.00 ± 0.00[2](23.6)[3]	0.01 ± 0.00 (13.8)	0.00 ± 0.00 (10.6)
Lung	0.01 ± 0.00 (34.4)	0.02 ± 0.01 (23.7)	0.02 ± 0.01 (18.6)
Stomach	0.24 ± 0.05 (100)	0.02 ± 0.01 (31.3)	0.01 ± 0.00 (23.1)
Spleen	0.01 ± 0.00 (23.6)	0.01 ± 0.00 (29.2)	0.02 ± 0.01 (21.1)
Kidney	0.03 ± 0.01 (18.8)	0.07 ± 0.02 (20.7)	0.03 ± 0.01 (6.9)
Liver	0.30 ± 0.05 (18.4)	0.58 ± 0.09 (26.5)	0.26 ± 0.08 (14.8)
Brain	0.00 ± 0.00 (11.8)	0.00 ± 0.00 (7.9)	0.00 ± 0.00 (1.4)

1 animals were given a single dose of 0.1 mg/kg 3-DA
2 each value is the mean µg 3-DA/g tissue ± S.E. of four animals
3 values in parentheses are the percentage of total activity present as parent 3-DA

FIGURE LEGENDS

Figure 1. Chemical structures of Cc3Ado and 3-DA.

Figure 2. Sample chromatogram of [^{3}H]Cc3Ado simultaneously detected by absorbance at 263 nm and by in-line radiometric detection. Chromatographic conditions were as described in **Materials and Methods**.

Figure 3. Sample chromatogram of [^{3}H] 3-DA simultaneously detected by absorbance at 263 nm and by in-line radiometric detection. Chromatographic conditions were as described in **Materials and Methods**.

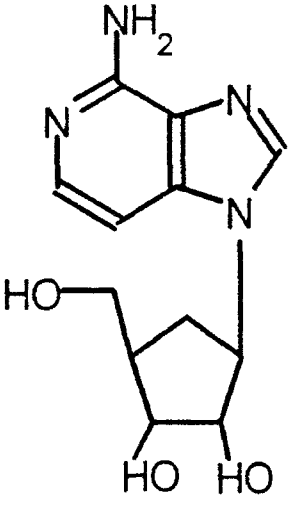
Figure 4. Binding of 3-DA to plasma proteins *in vitro*. The ultrafiltration method to determine protein binding is described in **Materials and Methods**.

Figure 5. Pharmacokinetic profile of 10 mg/kg intravenous and oral Cc3Ado in BALB/c mice. Each data point is the mean of 3-4 animals.

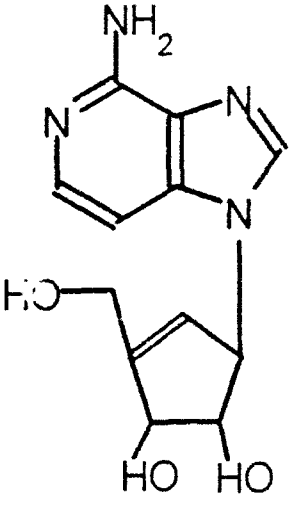
Figure 6. Pharmacokinetic profile of 0.1 mg/kg intravenous and oral 3-DA in BALB/c mice. Each data point is the mean of 3-4 animals.

Figure 7. Time-related disappearance of parent Cc3Ado and appearance of major labeled Cc3Ado metabolite in plasma taken from mice dosed with 10 mg/kg Cc3Ado iv.

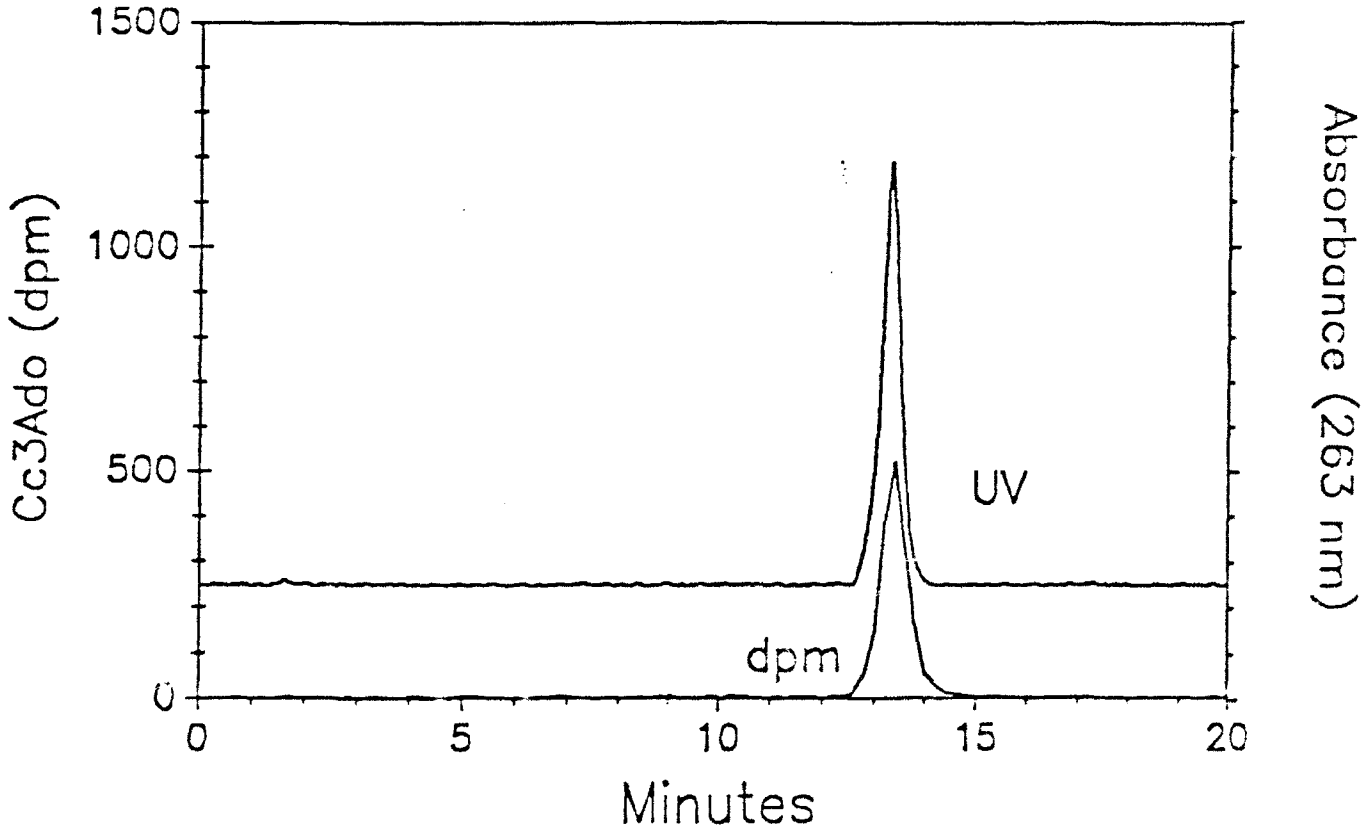
Figure 8. Time-related disappearance of parent Cc3Ado and appearance of major labeled 3-DA metabolite in plasma taken from mice dosed with 0.1 mg/kg 3-DA iv.

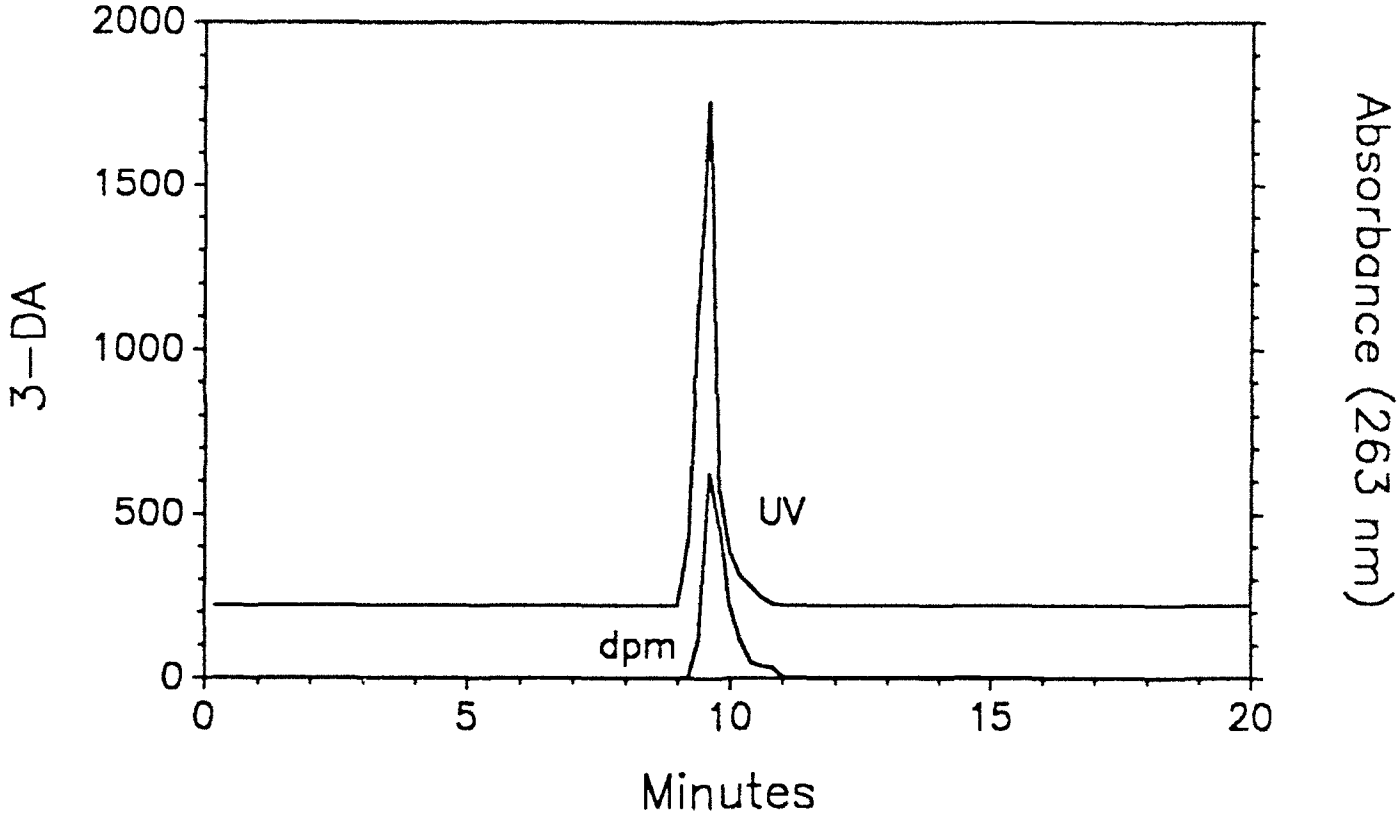


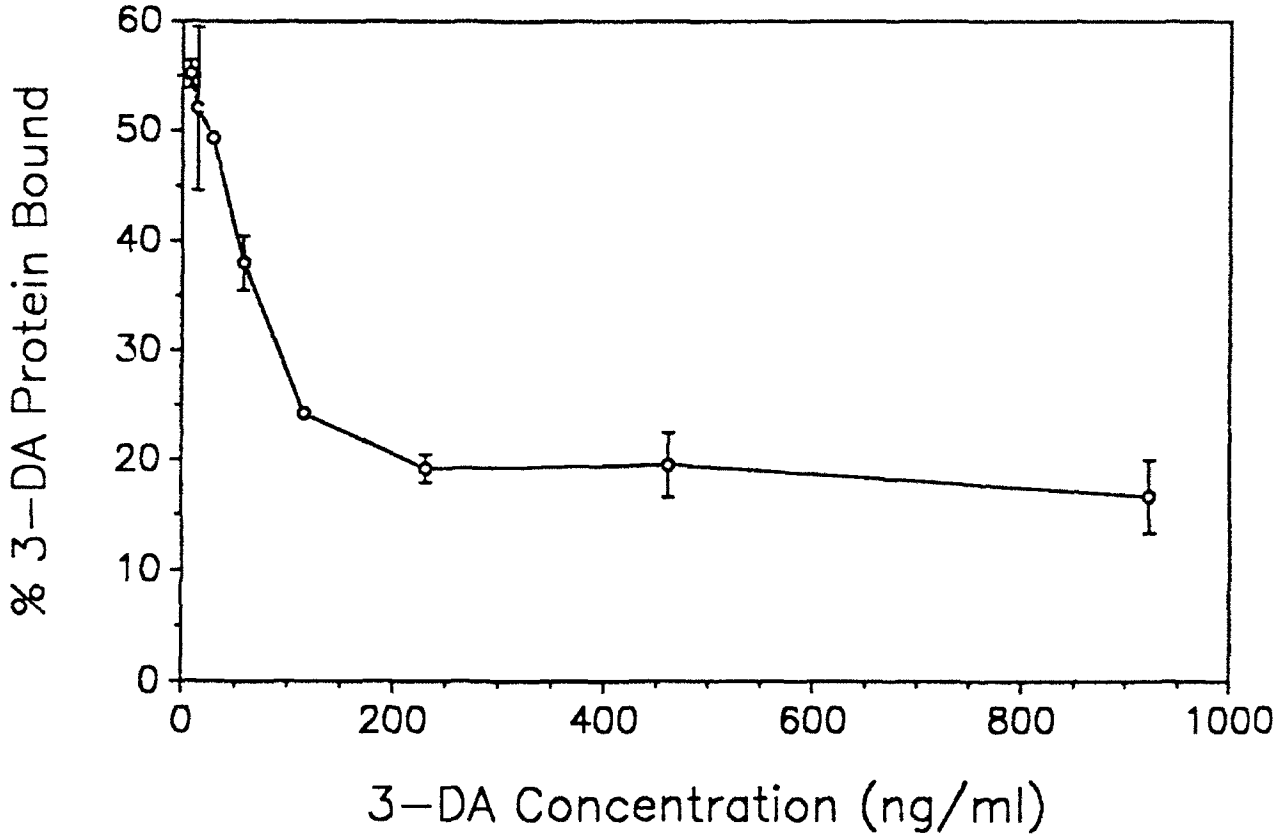
Cc3Ado

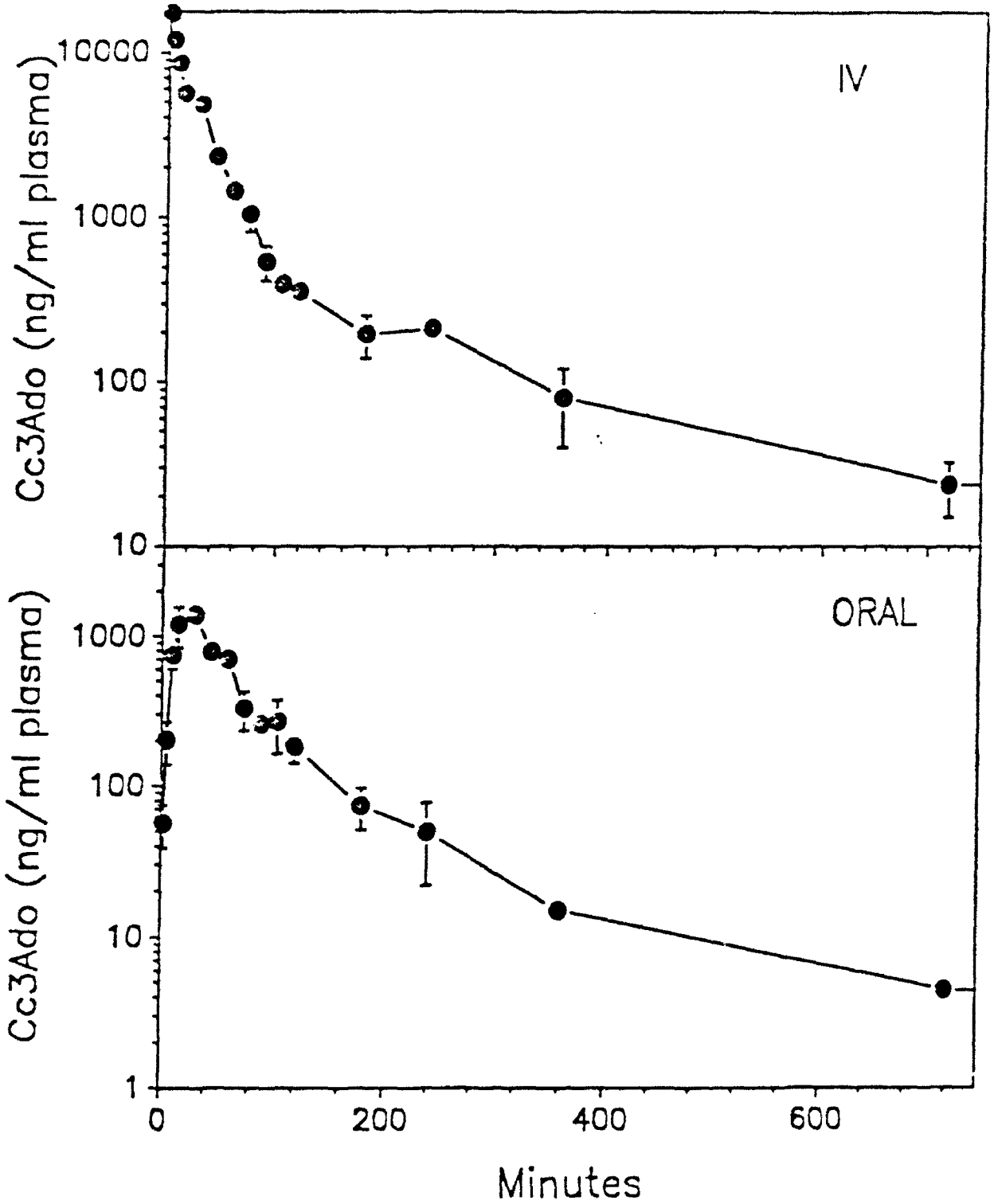


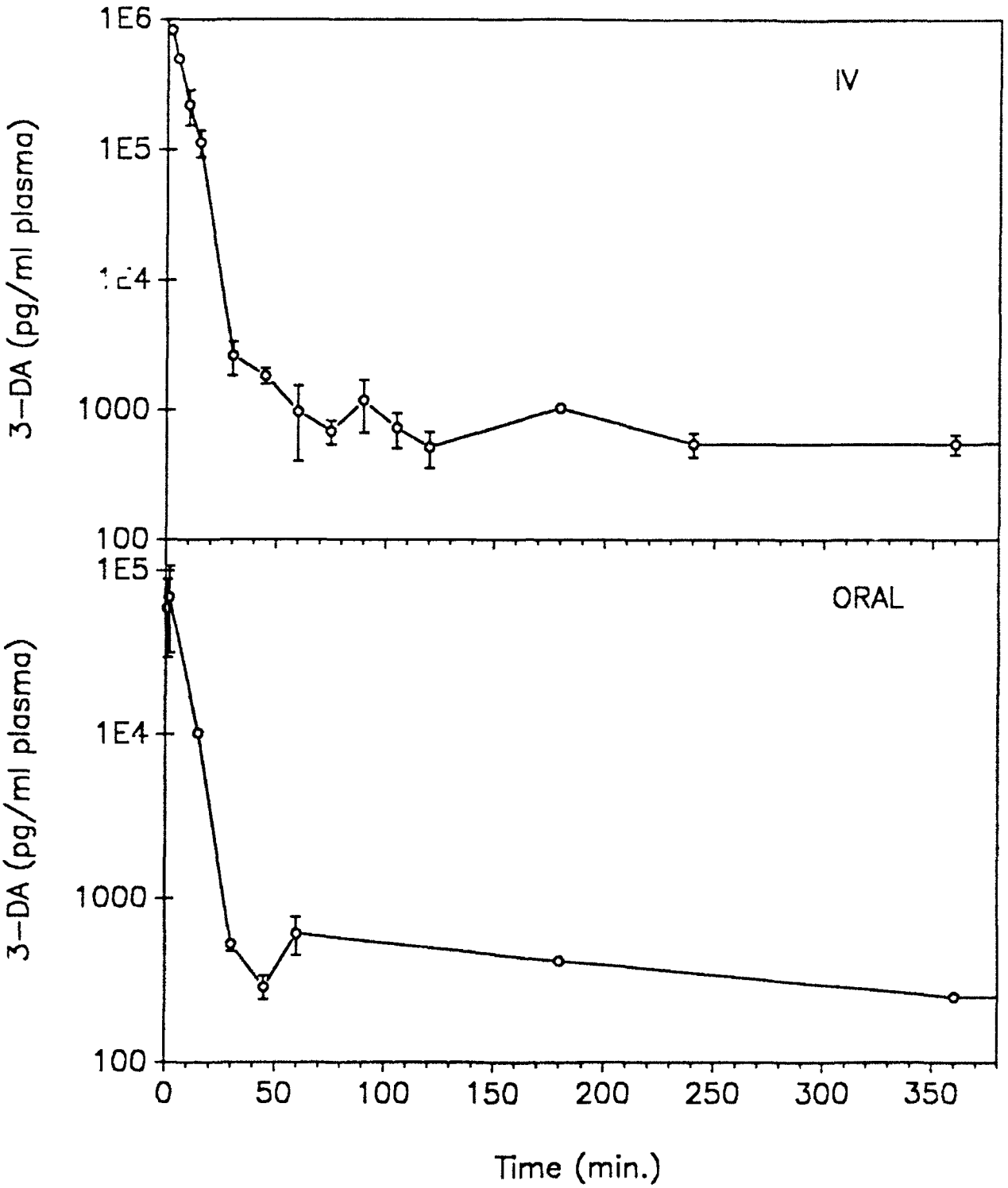
3-DA

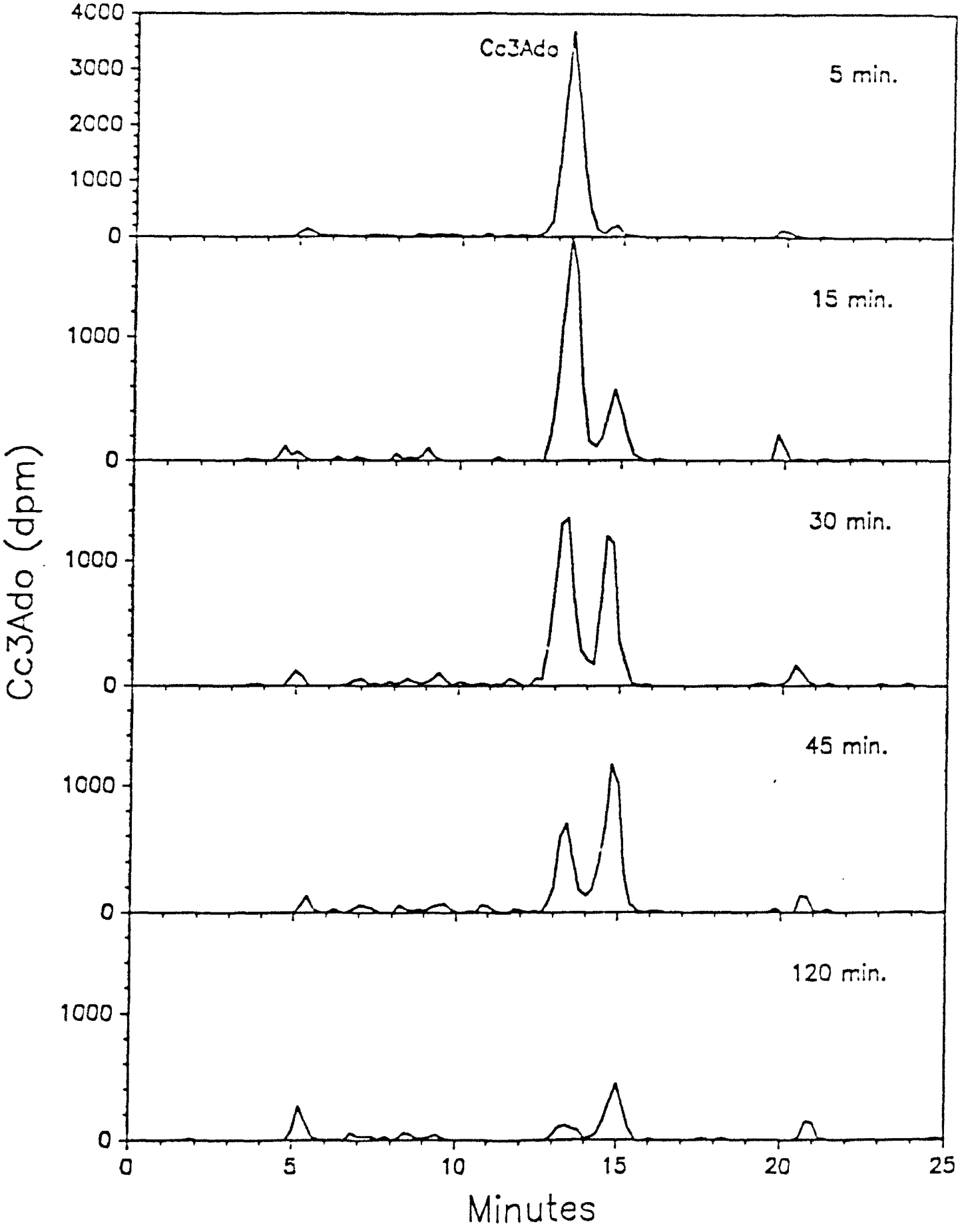

1500
1000
500
0
Cc3Ado (dpm)
Absorbance (263 nm)
UV
dpm
0
5
10
15
20
Minutes

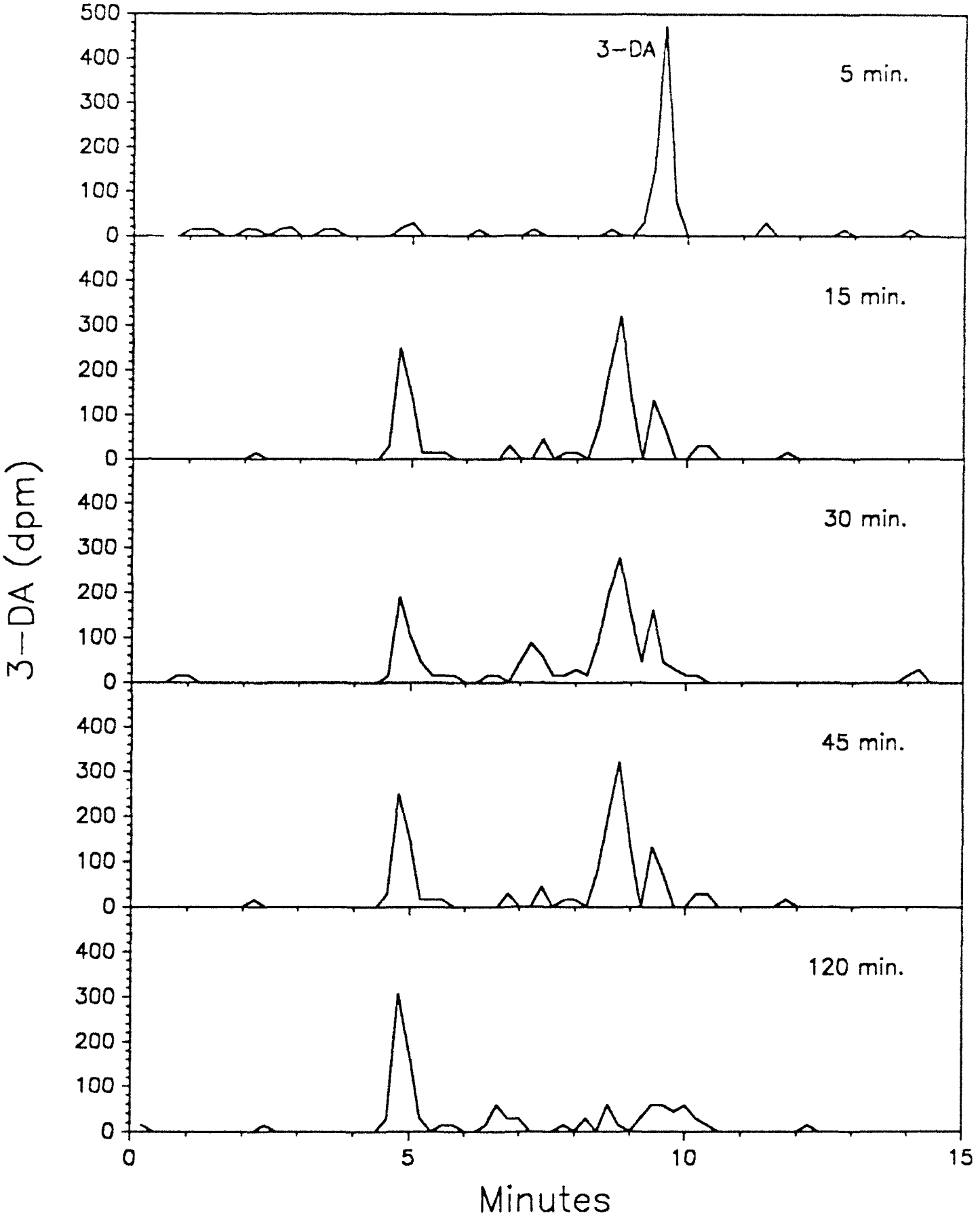

2000
1500
1000
500
0
3-DA
UV
dpm
0
5
10
15
20
Minutes
Absorbance (263 nm)


% 3-DA Protein Bound
60
50
40
30
20
10
0
0
200
400
600
800
1000
3-DA Concentration (ng/ml)


IV
ORAL
Cc3Ado (ng/ml plasma)
Cc3Ado (ng/ml plasma)
10000
1000
100
10
1000
100
10
1
0
200
400
600
Minutes


1E6
1E5
1E4
1000
100
3-DA (pg/ml plasma)
IV
1E5
1E4
1000
100
3-DA (pg/ml plasma)
ORAL
0
50
100
150
200
250
300
350
Time (min.)


Cc3Ado
5 min.
15 min.
30 min.
45 min.
120 min.
4000
3000
2000
1000
0
Cc3Ado (dpm)
0
5
10
15
20
25
Minutes


3-DA
5 min.
15 min.
30 min.
45 min.
120 min.
3-DA (dpm)
Minutes
0
100
200
300
400
500
5
10
15