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Esterification of cidofovir with alkoxyalkanols increases oral bioavailability and diminishes drug accumulation in kidney

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Abstract

Smallpox was eradicated by vaccination in the 1970s. However, concerns have arisen about the potential use of variola virus as a biological weapon. Most of the world's population has little residual immunity because systematic vaccination against smallpox ceased in the early 1970s. Vaccination of key elements of the population against smallpox is again being considered. However, there are now large numbers of persons who cannot be safely vaccinated with the current vaccine because of AIDS, immunosuppressive drugs, and certain common skin disorders. It would be useful to have a potent orally active drug as an alternative for these persons in case of an outbreak of smallpox.

Alkoxyalkyl esters of cidofovir (CDV) have been shown to be highly active and selective against poxviruses in vitro with activities several logs greater than the activity of unmodified CDV. This is due in large part to increased cellular penetration and conversion to CDV-diphosphate, the active antiviral. In this paper, the oral pharmacokinetics of ^{14}C-labeled hexadecyloxypropyl-cidofir (HDP-CDV), octadecyloxyethyl-cidofir (ODP-CDV), and oleyloxypropyl-cidofir (OLP-CDV) are examined and oral bioavailability and tissue distribution assessed and compared with parenteral CDV. The alkoxyalkyl CDVs are highly orally bioavailable and do not concentrate in kidney, the site of the dose-limiting toxicity of CDV. Plasma and tissue drug levels are many times greater than the in vitro EC_{50s} for variola, cowpox, and vaccinia viruses. Thus, the compounds are good candidates for further development for prevention and treatment of smallpox infection and the complications of vaccination.
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Keywords: Esterification; Alkoxyalkanols; Oral bioavailability

Cidofovir (CDV, Vistide®; 1-[(S)-9-(hydroxyphosphonomethyl-propyl)cytosine]), is a potent and selective inhibitor of viral DNA synthesis after conversion by cellular kinases to the diphosphate (Snoek et al., 1988; De Clercq et al., 1987; Ho et al., 1992; Cherrington et al., 1994; Xiong et al., 1996), and is FDA approved for the treatment of cytomegalovirus retinitis in AIDS patients (Lalezari et al., 1997; Studies of Ocular Complications Authors, 1997). However, owing to poor oral bioavailability of <5% (Wachsman et al., 1996; Cundy, 1999), CDV must be administered by intravenous infusion. In addition, CDV has exhibited treatment-limiting nephrotoxicity resulting in the requirement for prehydration, slow intravenous infusion and concomitant treatment with Probenecid.

CDV is a broad spectrum antiviral that shows in vitro activity against other DNA viruses including the herpes viruses, orthopoxviruses, polyomavirus, papillomavirus, and adenoviruses (reviewed in De Clercq, 2002). CDV is also active in vivo against lethal vaccinia and cowpox challenge experiments in rodents when given by injection including the intraperitoneal, intravenous, and subcutaneous routes of administration (De Clercq and Neyts, 1993; Bray et al., 2000; Martinez et al., 2000; Smee et al., 2001; Huggins et al., 2002).

We reported recently that the in vitro activity of CDV against herpes group viruses (HCMV and HSV) and orthopoxviruses (vaccinia and cowpox) could be increased

Abbreviations: CDV, cidofovir; HDP-CDV, hexadecyloxypropyl-cidofovir; ODE-CDV, octadecyloxyethyl-cidofovir; OLP-CDV, oleyloxypropyl-cidofovir

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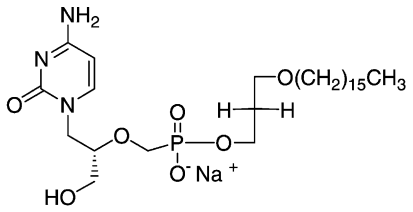
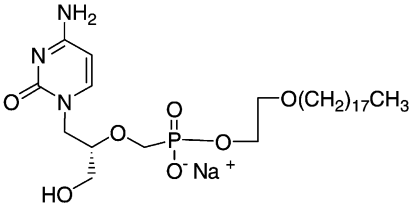
by several orders of magnitude by esterification with various alkoxyalkyl bromides (Kern et al., 2002; Beadle et al., 2002). Hexadecyloxypropyl-cidofir (HDP-CDV) and octadecyloxyethyl-cidofir (ODE-CDV) were 75–150 times more active against vaccinia virus and cowpox virus replication than unmodified CDV (Kern et al., 2002). Marked increases in activity were also noted in vitro against human CMV and HSV (Beadle et al., 2002). Studies with radiolabelled CDV and HDP-CDV show that the increased activity is due to the increased cellular penetration of HDP-CDV relative to CDV. This increased cellular penetration results in 100-fold higher intracellular levels of CDV diphosphate (Aldern et al., 2003).

The activity of CDV against smallpox has received much attention due to the potential use of the causative agent, variola major, as a biological weapon (Atlas, 1998; Henderson et al., 1999). If this virus is released into the general population, the majority of people who are exposed and those who are at risk of being exposed, can be protected by vaccination. However, up to 40 million Americans are not candidates for vaccination or are at greatly increased risk from the vaccine (Altman, 2002). This group includes: (1) individuals with a severely weakened immune systems (HIV, cancer chemotherapy, radiation therapy), (2) patients who have received organ transplants and are taking immunosuppressive drugs, (3) individuals with eczema or atopic dermatitis and anyone living in the same household with them, (4) pregnant women, and (5) infants. A possible added threat is suggested by the existence of genetically engineered interleukin-4-positive strains of mousepox virus which are able to kill despite prior vaccination (Jackson et al., 2001). This modification, if applied to variola, could produce a strain of virus that would cause illness or death even in vaccinated persons. Consequently, there is a compelling need for a safe, orally active antiviral drug for the prevention or treatment of smallpox infection to provide an alternative means of protection. In addition, if the drug shows activity against lethal vaccinia virus challenge models, it could also be used to treat life-threatening side effects resulting from vaccination. Preliminary studies indicate that the lipid esters of CDV have very high levels of activity against a lethal cowpox challenge when given orally to mice (Huggins et al., 2002).

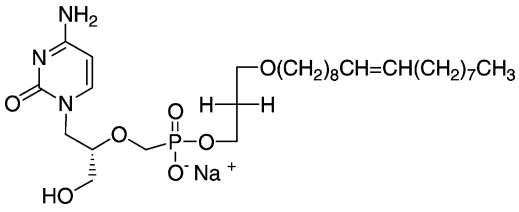
In this paper, we report the oral pharmacokinetics and the tissue distribution in mice of three alkoxyalkanol esters of CDV. Oral bioavailability was determined as the ratio of plasma area under the curve for drug administered by the oral and subcutaneous routes. The tissue distribution of the CDV esters given orally versus unmodified CDV given intraperitonealy were also examined. Tissue distribution was examined in mice in an effort to address two key issues: (1) does lipid esterification keep CDV out of the kidney and therefore eliminate or minimize the potential for the dose-limiting kidney toxicity and (2) is the ester delivered to the lung, liver, and spleen, critical tissues that must be treated in smallpox infection? The results indicate that, in contrast to CDV, the alkoxyalkanol esters of CDV are highly orally bioavailable, do not concentrate highly in the kidney and reach high levels in key target tissues.



Hexadecyloxypropyl-cidofovir (**HDP-CDV**)



Octadecyloxyethyl-cidofovir (**ODE-CDV**)



Oleyloxypropyl-cidofovir (**OLP-CDV**)

Fig. 1. Structures of HDP-CDV, ODE-CDV, and OLP-CDV.

1. Methods

1.1. Compounds and radiochemicals

HDP-CDV, ODE-CDV, and oleyloxypropyl-cidofir (OLP-CDV) were synthesized, purified and characterized as reported previously (Beadle et al., 2002; Kern et al., 2002). The structures are shown in Fig. 1. CDV and cyclic cidofovir were the generous gifts of Gilead Sciences, Foster City, CA. [2-^{14}C]CDV, specific activity 56 mCi/mmol, HDP-[2-^{14}C]CDV, ODE-[2-^{14}C]CDV, and OLP-[2-^{14}C]CDV, each with a specific activity of 50 mCi/mmol, were synthesized by Moravek Biochemicals, Brea, CA. For the oral pharmacokinetics and tissue distribution studies, [2-^{14}C]CDV and the three alkoxyalkanol esters of-[2-^{14}C]CDV were diluted with unlabeled compounds to provide final specific activities of 16.67–18.67 mCi/mmol, respectively. For oral or parenteral administration, the

radioactive compounds were dried under nitrogen gas and rehydrated with sterile 0.9% saline, followed by sonication to obtain clear solutions.

1.2. Pharmacokinetic studies

Female NIH Swiss mice (Harlan–Sprague–Dawley,) at a weight range of 20–25 g were treated by the routes and dosages indicated with [2-^{14}C]CDV or HDP-, ODE-, or OLP-[2-^{14}C]CDV. At times ranging from 1 to 72 h, blood was collected in heparinized tubes and the mice were euthanised. In tissue distribution experiments, spleen, liver, kidney, and lung tissues were removed at the indicated times, weighed, and solubilized with TS-2 (Research Products International, Mt. Prospect, IL) and processed for liquid scintillation counting. The blood was centrifuged and the drug content of a 50 μl aliquot was determined by liquid scintillation counting. Plasma and organ drug content is reported as nanomol drug per milliliter (plasma) and nanomol drug per gram (organs). Animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institute of Health.

1.3. Analysis of plasma

In some experiments with oral HDP-[2-^{14}C]CDV, plasma was subjected to lipid extraction to separate CDV from HDP-CDV (Folch et al., 1957). The aqueous and lipid phases were separated and the upper phase was washed twice with ideal lower phase (as defined in Folch et al., 1957). The original lower phase and the two washes were combined. Aliquots of the upper and the combined lower phases were removed for liquid scintillation counting. The lower phase was also analyzed by thin layer chromatography on silica gel G layers developed with chloroform:methanol:ammonia:water (70:58:8:8) by volume. The plates were scanned with a Radiomatic RTLC scanner (Radiomatic, Tampa, FL) and the peaks were compared with reference standards of HDP-[2-^{14}C]CDV and [2-^{14}C]CDV.

Plasma lipid extracts were also analyzed by HPLC as described previously (Aldern et al., 2003). Aliquots of the upper phases of the lipid extraction were applied to a 4.6 cm × 15 cm Partisil 10 SAX column with a SAX guard column. The column was eluted at a flow rate of 1 ml/min using a potassium phosphate buffer gradient of 20–700 mM, pH 5.8, beginning at 9 min for a duration of 20 min and a terminal hold of 5 min. Fractions were collected in 1 min/ml and FloScint IV scintillation fluid was added and the samples were analyzed by liquid scintillation counting. The lower phase from the lipid extractions were also analyzed by HPLC as follows. A small aliquot of the lower phase, 50–75 μl, was applied to a 4.6 cm × 15 cm Waters XTerra column with a 3.9 mm × 20 mm Waters XTerra guard column (Waters Corp, Milford, MA). The samples were eluted with 80% methanol at a flow rate of 0.5 ml/min for 15 min. One milliliter of fractions were collected and analyzed as described above. Radioactivity in each fraction was plotted versus time and the fractions containing HDP-CDV were determined by comparison with the retention time of an unlabeled HDP-CDV standard run under identical conditions.

2. Results

2.1. Dose dependence of plasma drug levels

HDP-[2-^{14}C]CDV was administered by oral gavage to mice at doses of 5, 10, and 20 mg/kg. For comparison, [2-^{14}C]CDV was given orally at 5.6 mg/kg (a dose which is the molar equivalent of the CDV delivered by 10 mg/kg of HDP-CDV). Plasma was obtained at times from 1 to 24 h postdose and drug levels determined as described in methods (Fig. 2). Plasma CDV levels were highest 1 h after oral dosing, 0.12 μM, and declined to 0.007 μM at 24 h. Oral administration of HDP-CDV produced substantially higher plasma levels. At 5 mg/kg, a peak value of 0.8 μM was reached at 6 h. At 10 mg/kg a plateau was observed between 3 and 12 h with a peak value of 2.37 μM at 12 h. At 20 mg/kg, delayed appearance of drug in plasma was noted relative to the results obtained with the 5 and 10 mg/kg doses. A peak level of 3.17 μM HDP-CDV occurred at 12 h, declining to 1.67 at 24 h.

Area under curve values ($AUC_{0-24\,h}$) were as follows: CDV 5.6 mg/kg, 0.81 units; HDP-CDV 5 mg/kg, 16.3 units; 10 mg/kg, 38.8 units, and 20 mg/kg, 16.5 units. At molar equivalent doses, the $AUC_{0-24\,h}$ of oral HDP-CDV in mouse plasma is 48 times greater than that of oral CDV. The $AUC_{0-24\,h}$ values for 5 and 10 mg/kg of HDP-CDV were roughly proportional to dose, but the $AUC_{0-24\,h}$ value for 20 mg/kg HDP-CDV was significantly lower than expected because much of the decline of drug in plasma occurred after 24 h and was not measured. Therefore, in the subsequent oral bioavailability experiments, plasma drug levels were measured out to 72 h.

2.2. Assessment of oral bioavailability

HDP-[2-^{14}C]CDV (10 mg/kg in normal saline) was administered to mice by the subcutaneous, intraperitoneal and oral routes and plasma was obtained at times ranging from 1 to 72 h. Similar experiments were carried out with ODE-[2-^{14}C]CDV and OLP-[2-^{14}C]CDV except that these drugs were only administered by the oral and subcutaneous routes (Fig. 3). The pharmacokinetic data derived from this data is shown in Table 1. When given by the intraperitoneal or subcutaneous routes, HDP-CDV drug levels in plasma peaked 3 or 6 h after administration at 3.1 and 2.7 μM, respectively. Oral HDP-CDV reached a plateau after 3 h with a peak of 2.4 μM at 12 h. After 12 h the oral plasma drug levels declined with a rate similar to that of the parenterally administered HDP-CDV. Comparison of the oral and parenteral

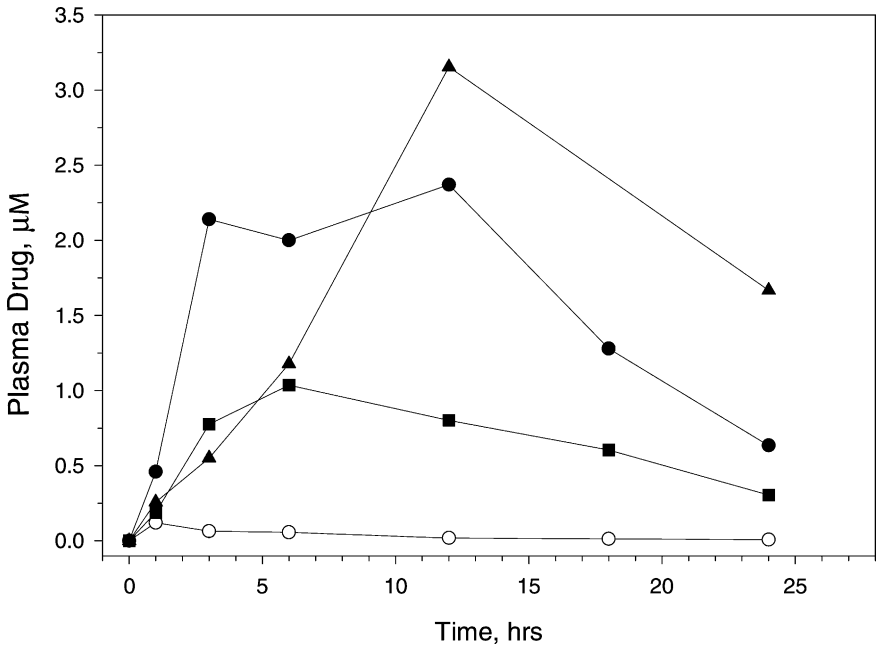


Fig. 2. Plasma drug levels after varying oral doses of HDP-[2-^{14}C]CDV or [2-^{14}C]CDV. The data represent all species of ^{14}C-labeled drug in plasma (μmol) at indicated times following oral administration. Symbols: open circle, 5.6 mg/kg CDV; square, 5 mg/kg HDP-CDV; closed circle, 10 mg/kg HDP-CDV; and triangle, 20 mg/kg HDP-CDV. Note that 5.6 mg/kg of CDV is the molar equivalent of 10 mg/kg HDP-CDV. The data represent the average of three separate determinations.

areas under curve ($AUC_{0–72\,h}$) indicated that the oral bioavailability of HDP-CDV was 93% (Table 1 and Fig. 3A). ODE-CDV administered orally peaked at 12 h at 3.03 μM, while subcutaneously administered ODE-CDV exhibited a plateau from 3 to 12 h in the range of 2.72–2.78 μM. After 12 h, both oral and subcutaneous drug levels declined similarly. The calculated oral bioavailability based on the $AUC_{0–72\,h}$ was 88% for ODE-CDV (Table 1 and Fig. 3B). OLP-CDV given subcutaneously peaked in plasma at 12 h at 4.46 μM versus oral OLP-CDV which did not peak until 24 h at 3.39 μM. The oral bioavailability of OLP-CDV was calculated to be 97% (Table 1 and Fig. 3C).

2.3. Analysis of drug species in plasma

The data above refer to all radioactive species of drug in plasma over time. However, it would be useful to know how much of the radioactive drug in plasma is HDP-CDV versus CDV and its metabolites. Therefore, we analyzed aliquots of plasma from an experiment in which 10 mg/kg of HDP-[2-^{14}C]CDV was administered orally to mice. The plasma was subjected to standard lipid extraction (Folch et al., 1957) and aliquots of the final chloroform and the aqueous methanol layers of the extraction were analyzed by liquid scintillation counting, analysis by thin layer chromatography scanning and HPLC. The material in the aqueous phase of the extraction was examined by Partisil SAX HPLC and determined to consist nearly entirely of CDV. A second small peak was noted which had a retention time consistent with that of HPMPU, the deamination product of CDV (HPMPC). The radioactive material in the lipid phase of the extractions was analyzed by thin layer chromatography and scanning. The lipid phase radioactivity was present in a single spot having the same rf as a pure HDP-CDV

Table 1
Plasma pharmacokinetic parameters

Drug	$T_{1/2}$	C_{max}	T_{max}	AUC_{oral}	AUC_{sc}	%F
CDV (i.p.)	16.9	0.84	1	–	4.37	–
HDP-CDV (oral)	14.9	2.37	12	53.2	60.5	88
ODE-CDV (oral)	13.9	1.37	6	41.4	44.5	93
OLP-CDV (oral)	9.9	3.40	24	117.7	120.6	97

Abbreviations: i.p., intraperitoneal; CDV, cidofovir; HDP-CDV, 1-*O*-hexadecyloxypropyl-CDV; ODE-CDV, 1-*O*-octadecyloxyethyl-CDV; and OLP-CDV, 1-*O*-oleyloxypropyl-CDV. AUC_{oral} and AUC_{sc} are the plasma areas under curve from 0 to 72 h in μmol h/l and the data represent all drug species in plasma. The unit for $T_{1/2}$ and T_{max} is hour and C_{max} is μmol. The oral bioavailability, %F, was determined by this ratio: $\%F = (AUC_{oral}/AUC_{sc}) \times 100$. The dose of CDV (i.p.) was 5.6 mg/kg and the oral doses of HDP-, ODE-, and OLP-CDV were 10 mg/kg.

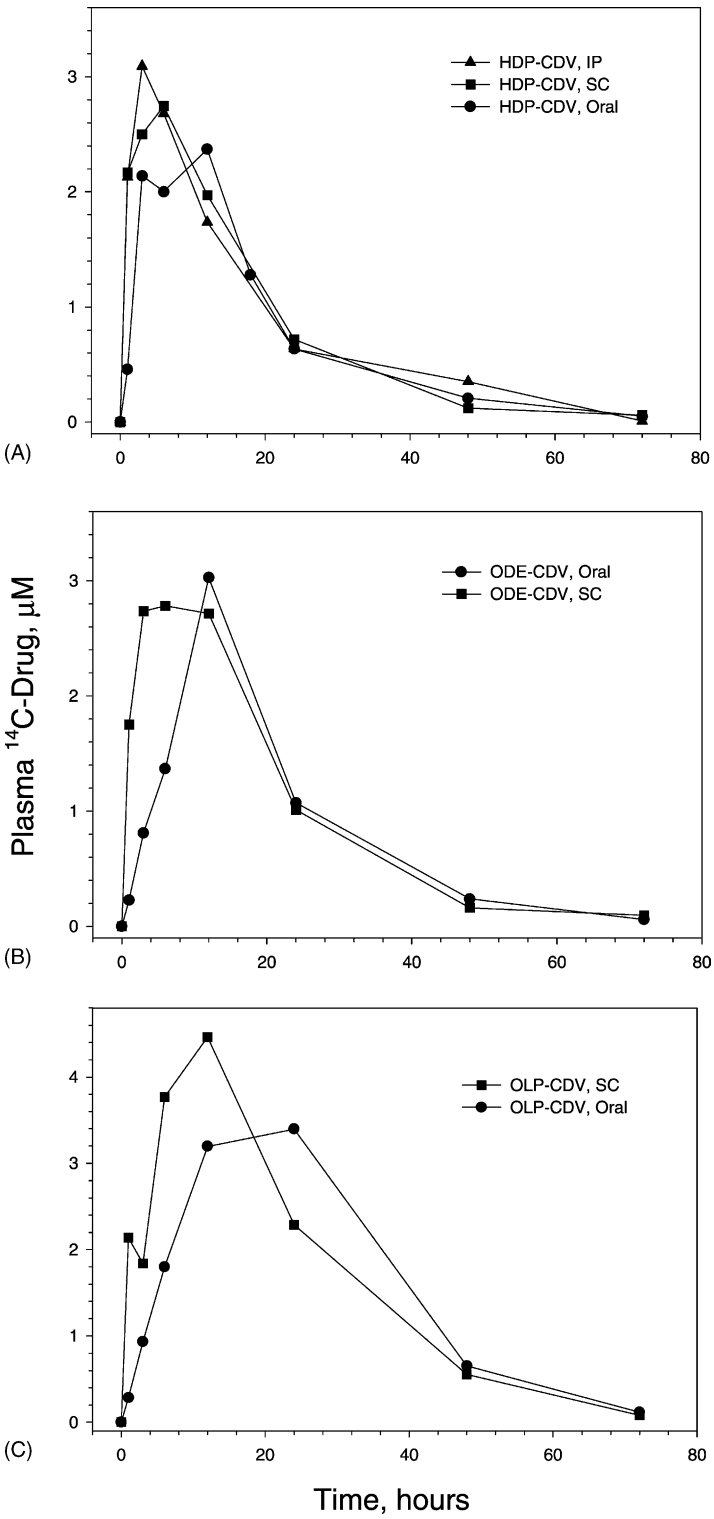


Fig. 3. Determination of the oral bioavailability of HDP-CDV, ODE-CDV, and OLP-CDV in mice. Mice were given 10 mg/kg of the indicated compounds by the orally (closed circles), subcutaneously (squares), or intraperitoneally (triangles). HDP-CDV (A), ODE-CDV (B), and OLP-CDV (C). The data represent all species of ^{14}C-labeled drug in plasma (μmol) at the indicated times following administration. The data are the average of three separate determinations. The pharmacokinetic data is summarized in Table 1.

Table 2
Tissue pharmacokinetic parameters

Drug	$T_{1/2}$	C_{max}	T_{max}	$AUC_{0-72\,h}$
Kidney				
Intraperitoneal CDV	7.36	180	1	1093
Oral HDP-CDV	44.7	5.84	12	203
Oral ODE-CDV	16.4	5.76	24	252
Oral OLP-CDV	19.7	6.89	12	213
Spleen				
Intraperitoneal CDV	46.6	0.44	1	9.92
Oral HDP-CDV	>72	0.56	12	53.2
Oral ODE-CDV	89.9	0.79	24	43.7
Oral OLP-CDV	27.4	0.96	12	32.1
Lung				
Intraperitoneal CDV	29.0	0.42	1	6.27
Oral HDP-CDV	>72	1.00	12	49.9
Oral ODE-CDV	71.7	1.19	24	60.1
Oral OLP-CDV	21.1	1.68	12	48.9
Liver				
Intraperitoneal CDV	27.5	1.6	1	39.6
Oral HDP-CDV	37.8	43.7	12	1366
Oral ODE-CDV	29.8	25.7	12	1213
Oral OLP-CDV	17.5	144	6	1465

Abbreviations as in Table 1. AUC unit is nmol g h. Doses of the compounds were as indicated in Table 1.

reference standard. The percentage of the total drug present as HDP-CDV and CDV was calculated. At 1 h postdose, HDP-CDV accounted for 84.1% of the total plasma radioactivity, declining slowly to 78% at 12 h and 68% at 48 h. CDV at 1 h represented 15.9% of plasma radioactivity and rose gradually to 22% at 12 h and 32% at 48 h.

2.4. Drug levels in tissue

In patients treated with intravenous CDV, the kidney is the site of dose-limiting toxicity. To evaluate the tissue distribution in mice of HDP-CDV given orally with that of CDV given by intraperitoneal injection, we compared equimolar doses of radiolabelled CDV (5.6 mg/kg intraperitoneal) with HDP-CDV (10 mg/kg oral) and determined the nanomoles of ^{14}C-labeled drug per gram of tissue (or nanomoles per milliliter of plasma) over time from 1 to 72 h postdosing (Fig. 4). CDV administration by the intraperitoneal route produced drug levels in kidney which were markedly higher than the levels following oral HDP-CDV (Fig. 4A). At 1 h CDV levels in kidney were 180 nmol/g versus only 1.17 nmol/g with HDP-CDV, a difference of 152-fold. HDP-CDV levels in kidney rose gradually to 5.9 nmol/g at 12 h and declined thereafter to 0.6 nmol/g at 72 h. Similar results were noted with oral ODE-CDV and OLP-CDV. For intraperitoneal CDV, the $AUC_{0-72\,h}$ was 4.7–5.8 times greater and the C_{max} was >30-fold greater than the corresponding values for the oral ether lipid analogs of CDV (Table 2).

Lung is a key tissue for poxvirus infection because disease transmission and mortality are related to viral pneumonitis (Martinez et al., 2000). In lung, oral administration of each of the ether lipid analogs of CDV produced much higher tissue levels of drug than measured with an equimolar dose of intraperitoneal CDV (Fig. 4B). With intraperitoneal CDV, a peak lung level of 0.43 nmol/g was noted at 1 h, declining to 0.037 nmol/g at 72 h. Peak levels in lung of 1.0 and 1.7 nmol/g, were observed at 12 h with HDP-CDV and OLP-CDV, respectively, declining to 0.54 and 0.24 nmol/g at 72 h, respectively. A peak lung level of 1.20 nmol/g was noted for ODE-CDV at 24 h, declining to 0.75 nmol/g at 72 h. In lung, AUC values for oral HDP-CDV, ODE-CDV, and OLP-CDV were 7.0-, 9.3- and 7.6-fold greater than that observed for an equimolar dose of intraperitoneal CDV (Table 2 and Fig. 4B). In spleen, results were generally similar to lung except that the peak levels of oral HDP-, ODE- and OLP-CDV were about 30% less, 0.56, 0.79, and 0.96 nmol/g, respectively. Intraperitoneal CDV gave a peak in spleen of 0.44 nmol/g, similar to lung. AUC values for oral HDP-, ODE-, and OLP-CDV were 3.1-, 4.3-, and 3.2-fold greater than intraperitoneal CDV (Table 2 and Fig. 4C).

Liver levels after oral administration of HDP-, ODE-, and OLP-CDV peaked at 12 h at 44, 27, and 26 nmol/g versus 1.55 nmol/g for intraperitoneal CDV at 1 h (Fig. 4D). Liver $AUC_{0-72\,h}$ values were 23–33 times greater with oral ether lipid analogs of CDV versus intraperitoneal CDV (Table 2).

3. Discussion

In the event of a smallpox outbreak resulting from biowarfare or bioterrorism, the availability of an oral antiviral therapy could be a key issue in ensuring rapid and effective treatment under emergency conditions. The ability to treat

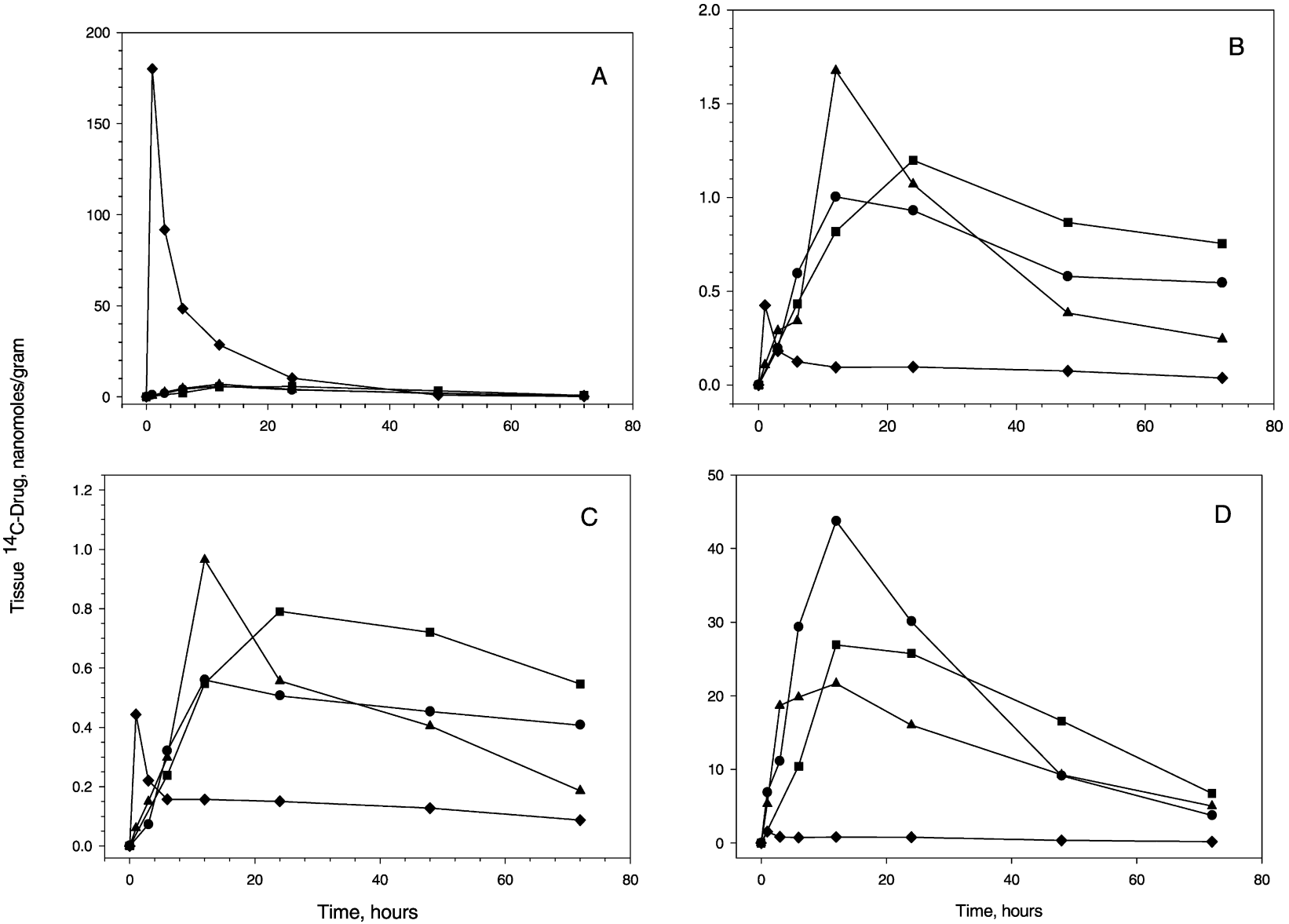


Fig. 4. Tissue levels of ^{14}C-drug following oral administration of three alkoxyalkanol analogs of CDV versus an equimolar dose of intraperitoneal CDV. Kidney (A), lung (B), spleen (C), and liver (D). Data are nanomole per grams of ^{14}C-labeled drug (all species) and represent the average of three determinations. Oral HDP-CDV, circles; oral ODE-CDV, squares; oral OLP-CDV, triangles; and intraperitoneal CDV. Alkoxyalkanol CDV doses were 10 mg/kg and the intraperitoneal dose of CDV was 5.6 mg/kg (the molar equivalent dose). Pharmacokinetic data is summarized in Table 2.

smallpox with a self administered oral dosage form could eliminate or minimize the need for the specialized medical infrastructure and individual patient care which would be necessary to provide supportive treatment with intravenous CDV.

Conjugation of an alkoxyalkanol to the phosphonate of CDV provides a high degree of oral bioavailability as indicated in Table 1, 88–97% compared with the reported value of <5% for CDV (Wachsman et al., 1996). The increased uptake from the gut that gives rise to these high values is believed to be due to the resemblance of the conjugates to lysolecithin (lysophosphatidylcholine), a dietary monoacyl phospholipid which is absorbed primarily intact in the small intestine (Hostetler et al., 1997). Once in the bloodstream, the conjugate remains intact and the ether lipid functionality helps to facilitate cellular uptake.

Another of the primary objectives of the conjugation of the alkoxyalkanols to CDV was to minimize exposure of the kidney to either the conjugate or the parent drug, CDV. As can be seen from the results in Table 2, this has been achieved. Exposure as indicated by $AUC_{0-72\,h}$ has decreased by 4- (ODE-CDV) to 5-fold (HDP-CDV) while C_{max} has decreased over 30-fold.

In order to treat smallpox effectively, adequate drug must be delivered to the liver, lungs, and spleen because these organs are key sites where the infection becomes established. As can be seen from both $AUC_{0-72\,h}$ and C_{max} values in Table 2, exposure is increased substantially in all the three organs compared to CDV. The highest levels are achieved in the liver. T_{max} values in the organs for the lipid-CDV conjugates are long, but comparable to the T_{max} values calculated for the plasma. This suggests that the rate limiting step in the distribution to and uptake by the target organs is governed by the rate of uptake from the gut.

The present studies demonstrate clearly that the oral bioavailability of CDV can be increased substantially by esterification of alkoxyalkanols to CDV. It is important to note that these findings are not unique to CDV. The alkoxyalkanol esterification approach appears to be generally applicable to other poorly absorbed pharmaceuticals. For example, esterification of phosphonoformate (PFA, foscarnet) to a thioalkylglycerol was previously shown to increase the oral bioavailability and antiviral activity of PFA (Beadle et al., 1998). Esterification of 1-*O*-hexadecyl-*sn*-3-glycerol or 1-*O*-hexadecyl-propane-3-ol to acyclovir monophosphate (HDP-P-ACV) or ganciclovir monophosphate (HDP-P-GCV) increased the low oral bioavailability of these unmodified nucleosides from 25–38% to 88–100% in rodents (Hostetler et al., 1997, 2001). In animal models of herpes virus disease, oral HDP-P-ACV was more active than oral ACV (Beadle et al., 2000) and the ether lipid analog of acyclovir, 1-hexadecyl-oxypropyl-3-phospho-acyclovir, was orally active against woodchuck hepatitis while large oral doses of acyclovir were ineffective (Hostetler et al., 2000).

The kidney toxicity of CDV is due to the accumulation of the drug in the renal cortex (Ho et al., 2000). This accumulation occurs because the convoluted proximal tubules which are localized in the superficial renal cortex contain an organic anion transport protein (hOAT1) which shows an extremely high affinity for CDV (Ho et al., 2000). The hOAT1 is in the antiluminal membrane of the proximal tubular epithelium (Tojo et al., 1999). CDV is picked up by the anion transporter so efficiently that the drug is removed from circulation faster than the glomuerular filtration rate. However, movement of the drug across the apical membrane and efflux into the tubular lumen is slow resulting in accumulation (Ho et al., 2000). We hypothesize that because one of the negative charges is masked by the covalent linkage to hexadecyloxy-propanol, the ether lipid conjugates of CDV do not appear to be good substrates for hOAT1 and accumulation in the kidney is minimal.

In summary, three alkoxyalkyl esters of CDV have been shown to have oral bioavailabilities ranging from 88 to 97% in mice versus <5% reported for CDV in man (Wachsman et al., 1996; Cundy, 1999). The lipid analogs are absorbed intact, circulate in plasma primarily as the intact prodrug and are converted slowly to CDV by the tissues. Drug levels in plasma and tissue are many-fold greater than the in vitro EC_{50} values against variola and vaccinia viruses. Oral administration of the alkoxyalkyl CDV analogs provides kidney AUC and C_{max} levels which are low versus those observed with parenteral administration of CDV. This is expected to improve tolerance to drug exposure. Preliminary studies in lethal cowpox virus challenge in mice indicate that HDP-CDV and ODE-CDV are highly protective when given orally (Huggins et al., 2002). These compounds are good candidates for further development and evaluation for possible use against smallpox and vaccinia virus infections.

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