PHARMACOKINETICS, BILIARY EXCRETION, AND TISSUE DISTRIBUTION OF NOVEL ANTI-HIV AGENTS, COSALANE AND DIHYDROCOSALANE, IN SPRAGUE-DAWLEY RATS

KAMESWARA R. KUCHIMANCHI, CHANDRASEKHAR UDATA, THOMAS P. JOHNSTON, AND ASHIM K. MITRA

Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, Kansas City, Missouri

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ABSTRACT:

Cosalane and dihydrocosalane are potent inhibitors of HIV replication with a broad range of activity. The purpose of this study was to investigate: 1) the pharmacokinetic disposition of both cosalane and dihydrocosalane in male Sprague-Dawley rats, and 2) biliary excretion, enterohepatic circulation, and tissue distribution of cosalane after i.v. and/or oral administration. Animals were administered i.v. (10 mg/kg) cosalane or dihydrocosalane through a jugular vein to obtain plasma profiles. Dose dependence of cosalane was studied over a dose range of 1.0 to 10 mg/kg. The extent of enterohepatic recycling, biliary excretion, and tissue distribution were studied after i.v. administration. Both cosalane and dihydrocosalane exhibited a biexponential disposition with very long half-lives of 749 ± 216 and 1016 ± 407 min, along with very large volumes of distribution 23.1 ± 4.4 and 24.4 ± 2.5 liter/kg, respectively. Both cosalane (nondetectable) and dihydrocosalane (<1%) showed very poor oral bioavailability. The biliary and renal excretions of cosalane were found to be negligible with no detectable metabolites either in urine or bile. After oral administration, more than 87% of the cosalane dose was excreted in the feces as the parent compound. Also, cosalane was sequestered significantly in liver with quantifiable levels in all tissues tested, even 48 h after the dose was administered. Therefore it was concluded that the poor oral bioavailability of cosalane may be due to its poor enterocytic transport coupled with sequestration in liver parenchymal cell membrane layers.

Cosalane, (NSC 658586; Fig. 1A) a novel and unique inhibitor of HIV reproduction, was developed as a potential therapeutic agent for the treatment of AIDS (Cushman et al., 1994). It is a non-nucleoside agent with potent inhibitory action against laboratory strains of HIV isolates, and drug-resistant and clinical HIV isolates (Golebiewski et al., 1993, 1996; Cushman et al., 1994, 1995; Keyes et al., 1996). The primary mechanism of action of cosalane appears to involve inhibition of binding of gp120 to CD4 and inhibition of post attachment event(s) before reverse transcription (Cushman et al., 1994). The cholestane moiety of cosalane appears to be imbedded perpendicularly in the lipid bilayer of the cell and/or the viral envelope, causing the disacilylmethane (pharmacophore) moiety to protrude outward in an obstructive mode (Cushman et al., 1994).

Dihydrocosalane (Fig. 1B) is an analog of cosalane. The unsaturation at the three carbon linker chain of cosalane may impart some metabolic instability to the molecule. Oxidation at this site can lead to the formation of a diol. Saturation of the double bond yields dihydrocosalane (Golebiewski et al., 1996). However, dihydrocosalane is a less potent anti-HIV agent than cosalane (EC\textsubscript{50} of 20 versus 3 \mu M, respectively). The mechanism of action of dihydrocosalane is similar to that of cosalane.

Very little information is available on the pharmacokinetic disposition of cosalane and dihydrocosalane. In a previous study using mice, it was reported that cosalane showed poor renal excretion with a long plasma elimination half-life of 524 min (Bigelow et al., 1994). Because renal clearance of cosalane is a minor route of elimination, biliary excretion may play a major role in elimination of cosalane owing to its high molecular weight and considerable lipophilicity.
Therefore, this study was undertaken to characterize: 1) the pharmacokinetic disposition of both cosalane and dihydrocosalane in male Sprague-Dawley (SD) 1 rats, and 2) biliary excretion, enterohepatic circulation, and tissue distribution of cosalane after i.v. and/or oral administration to rats.

Materials and Methods

Chemicals. Cosalane disodium salt [1,1-di(3′-sodiumcarboxy-5′-chloro-4′-hydroxyphenyl)-4-(3β-cholestanyl-1-butene), dihydrocosalane sodium salt and (5α)-3-[di(3′-ammoniumcarboxy-5′-chloro-4′-hydroxy)phenylmethylene]cholestane (internal standard) were supplied by Professor Mark Cushman, Purdue University, West Lafayette, IN. HPLC-grade solvents used for HPLC analysis were obtained from Fisher Scientific Co. (St. Louis, MO). Nembutal sodium (sodium pentobarbital) was purchased from Abbott Laboratories (North Chicago, IL). Ketaset (ketamine HCl) was obtained from Fort Dodge Animal Health (Fort Dodge, IA). Rompun (xylazine) was purchased from Bayer Corporation (Agricultural Division, Animal Health, Shawnee Mission, KS). All other chemicals were of analytical grade and were used as received.

Animals. Jugular vein-cannulated male SD rats weighing 200 to 225 g were obtained from Charles River (Hartford, CT) and were used to obtain i.v. and oral pharmacokinetic parameters. A separate set of bile duct-cannulated (BC) male SD rats (200–225 g) were also obtained from Charles River and this set of animals was exclusively used for biliary excretion and enterohepatic circulation studies.

In Vivo Studies. Intravenous studies. Cosalane (1.0 to 10.0 mg/kg) solubilized in 10% ethanol was administered via the jugular vein to the rats. Dihydrocosalane (10.0 mg/kg) in 10% dimethyl sulfoxide was also dosed via the jugular vein. Blood samples (100 µl) were collected at predetermined time points over a period of 72 h. Heparinized saline (100 µl) was injected through the jugular vein to maintain a constant blood volume. Plasma was separated from blood samples and stored at −80°C until additional analyses.

Oral studies. A pilot study was carried out using 10% ethanol as a vehicle for oral dosing of cosalane. To improve the oral bioavailability a self-emulsifying lipid formulation system consisting of ethanol/cremophor-EL/caster oil (1:1:3 v/v/v) was used to solubilize cosalane and dihydrocosalane. The rats were fasted overnight but were allowed water ad libitum. Cosalane or dihydrocosalane was administered by oral gavage (maximum of 0.4 ml at 50 mg/kg) to jugular vein-cannulated rats. This was followed by the administration of 0.6 ml of water to enable the formation of the emulsion in situ. Blood samples (100 µl) were collected over a period of 24 h; plasma was obtained immediately and then stored at −80°C until additional analysis.

Enterohepatic circulation studies. BC and jugular vein-cannulated rats were used in the study. The animals were allowed an acclimatization period of at least 24 h in the Laboratory Animal Center (University of Missouri-Kansas City) before conducting the study. The rats were checked for overall general health, normal eating, defecation, urinary output (4–12 ml/100 g/24 h), and health, normal eating, defecation, urinary output (4–12 ml/100 g/24 h), (Tomlinson et al., 1981). The animals were housed individually in metabolic cages before the beginning of a study. The cannulas emerging from the bile duct and entering the duodenum were protected by a steel spring according to the method adopted from Tomlinson et al. (1981), allowing for free movement of rats in the cage. The modified spring was supported by clamping it in a suitable position above the metabolism cage. The rats were allowed water and food ad libitum. Cosalane was administered i.v. (10 mg/kg) via the jugular vein and blood samples were collected (100 µl) at predetermined time points for a period of 66 h. Bile was continuously collected into tared glass bottles cooled on ice. An aqueous solution of bile salts (sodium cholate/sodium deoxycholate, 1:1 w/w) was further diluted with 0.9% w/v NaCl and 0.05% w/v KCl to obtain a final concentration of 1% w/v of bile salts (Tomlinson et al., 1981). This aqueous bile salt solution was then infused into the duodenum using a slow infusion pump (CMA/102 micodialyzer pump; CMA/Microdialysis, Acton, MA) at a rate of 20 ml over a 24-h period. At the end of the experimental period(s), rats were first anesthetized (0.1 ml/100 g body weight of rat) with a mixture of ketamine (100 mg/ml) and xylazine (100 mg/ml) in the ratio of 9:1 (v/v) and then euthanized by an i.p. injection of an overdose of sodium pentobarbital (100 mg/ml).

Disposition Experiments. Biliary excretion. In a pilot study with two rats, the bile duct was cannulated under anesthesia (pentobarbital, 60 mg/kg, i.p.) and bile was collected for 2 h after administration of cosalane (10 mg/kg in 10% v/v solution) through the tail vein. In this period only about 0.1% of the administered dose was excreted in bile. This observation suggested that the duration of this experiment needed to be extended. Therefore, six BC rats were obtained from Charles River, four animals received cosalane disodium salt (10 mg/kg) dissolved in 10% v/v ethanol (10 mg/ml) through the tail vein and the other two rats received just 10% v/v ethanol (1 ml/kg) (placebo). Bile was collected every 15 min for the first 2 h and for 15 min at 3, 4, 6, 8, 12, 16, 24, 36, and 48 h post dose and stored immediately at −80°C until additional analysis. At the end of study, animals were sacrificed with an overdose of pentobarbital.

Elimination studies. Male SD rats were administered cosalane (10 mg/kg in 10% v/v ethanol) either by the tail vein or by gastric intubation and were individually restrained in a metabolic cage. Urine and feces were collected at predetermined intervals over a period of 120 h and were stored at −80°C until additional extraction and analysis.

Tissue distribution. Animals (four at each point) were randomly selected from the drug elimination studies at 2, 24, and 48 h post dose (i.v.) and were sacrificed by an overdose of pentobarbital. Various tissues such as blood, liver, spleen, lung, heart, adipose tissue, kidney, brain, muscle, and bone marrow were isolated and were immediately stored at −80°C until extraction for the analysis of drug and any possible metabolite(s).

Extraction of Cosalane. From plasma and rat tissues. Plasma samples or tissues (100 µl or 100 mg) were homogenized with a mixture of methanol and acetonitrile (5:4) (900 µl), vortexed, and centrifuged (GS-15R centrifuge; Beckman Instruments, Palo Alto, CA) at 16,000g for 15 min at 4°C. The supernatant was separated and 20 µl was injected onto the HPLC column. If the concentration was below the limit of detection (0.02 µg/ml), the supernatant was evaporated under N2 gas and the residue was reconstituted in 50 µl of methanol before HPLC analysis. The internal standard (5 µl of 1 mM) was added in bile and tissue analyte samples before homogenization.

From bile and urine. For extraction of cosalane from bile and urine, 100 µl of these tissue homogenate suspensions were treated in a similar manner to plasma as described above. Bile and urine samples were also incubated with mild and strong alkaline solutions, glucuronidase/sulfatase, before the above extraction process to identify any possible phase II metabolite(s).

From feces. The feces samples were freeze-dried overnight and pulverized with a mortar and pestle. To the pulverized feces (100 mg), a 900-µl mixture of methanol + tetrahydrofuran (75 + 25) was added and homogenized using a Tissue Tearer (Biospec, Bartlesville, OK), vortexed, and centrifuged at 16,000g for 15 min. The supernatant was diluted if necessary with the homogenizing mixture before injection onto a HPLC column.

HPLC Analysis. Plasma samples. A Varian star HPLC system (Varian Inc., Walnut Creek, CA) consisting of Varian 9012 solvent module, 9095 autosampler, 9050 UV-VIS detector (λmax 230 nm) connected in series with a Schoeffel 970 fluorescence detector (McPherson, Chelmsford, MA) was used to separate plasma samples. The excitation wavelength was 230 nm and emission wavelength was set at 450 nm. The mobile phase consisted of methanol/tetrahydrofuran/phosphoric acid (74:25:1, v/v). The plasma samples were diluted with a mixture of methanol and acetonitrile (5:4 v/v). The samples were then subjected to centrifugation at 13,375g for 15 min at room temperature. The supernatant was collected and was further diluted with 0.9% w/v NaCl and 0.05% w/v KCl to obtain a final concentration of 1% w/v of bile salts (Tomlinson et al., 1981). This aqueous bile salt solution was then infused into the duodenum using a slow infusion pump (CMA/102 micodialysis pump; CMA/Microdialysis, Acton, MA) at a rate of 20 ml over a 24-h period. At the end of the experimental period(s), rats were first anesthetized (0.1 ml/100 g body weight of rat) with a mixture of ketamine (100 mg/ml) and xylazine (100 mg/ml) in the ratio of 9:1 (v/v) and then euthanized by an i.p. injection of an overdose of sodium pentobarbital (100 mg/ml).

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Extraction Efficiency of Cosalane from Biological Samples. The extraction efficiencies of cosalane from biological samples were found to be 108.3 ± 9.8% from plasma, 98.2 ± 1.1% from bile, 95.6 ± 0.5% from liver tissue, and 78.5 ± 2.3% from feces.

Pharmacokinetic Modeling. The pharmacokinetic parameters for cosalane and dihydrocosalane were determined after i.v. administration using PCNONLIN program (Statistical Consultants, Lexington, KY). The initial data analysis was performed using RSTRIP program (MicroMath, Salt Lake City, UT). Preliminary estimates were fitted into two- and three-compartment models, using the PCNONLIN program. The model with the lowest Akaike Information Criteria value was selected. It was found that the data was best fit by a two-compartment model. The pharmacokinetic parameters of i.v. dosed cosalane and dihydrocosalane were obtained using a two-compartment open model with elimination from the central compartment.

Statistical Analysis. Statistical analyses of the experimental data were performed using Student’s t test and one-way ANOVA.

Results

Intravenous Cosalane Studies. The plasma cosalane concentration versus time profiles after i.v. administration of four doses (1, 2.5, 5.0, and 10.0 mg/kg) are shown in Fig. 2. Mean values for the pertinent pharmacokinetic parameters associated with a biexponential compartmental analysis are listed in Table 1. Using one-way ANOVA, statistical comparisons of individual pharmacokinetic parameters such as the AUC, α, β, β-half-life, clearance, and volume of distribution at steady state demonstrated no significant (P < .05) differences when each parameter was compared for the four test doses. The only significant difference detected was that between the Vdss calculated at a cosalane dose of 10 mg/kg (23.1 ± 4.4 ml/g) compared with the corresponding value resulting from the administration of 5 mg/kg dose (12.6 ± 1.9 ml/g). Moreover, secondary peaks were observed between 6 to 12 h in the plasma concentration versus time profiles (Fig. 2).

Cosalane versus Dihydrocosalane Pharmacokinetics after i.v. Administration. As illustrated in Fig. 3, the plasma cosalane and dihydrocosalane profile after an i.v. dose of 10 mg/kg displayed a biexponential disappearance. The relevant pharmacokinetic parameters obtained after mathematical modeling are listed in Table 1. Mean values for area under the curve, α, β, half-life, β half-life, clearance, and Vdss calculated after administration of cosalane were not significantly different from the corresponding mean values determined for dihydrocosalane.

Oral Studies. Oral bioavailability studies of cosalane using 10% ethanol and the self-emulsifying lipid emulsion showed no detectable plasma levels. However, dihydrocosalane showed detectable plasma levels on oral dosing.

Effect of Bile Duct Cannulation. Figure 4 illustrates the plasma concentration-time profiles for cosalane after i.v. administration to both normal and BC rats. Again, the disappearance of cosalane from the plasma for each treatment group followed a biexponential decline. After mathematical modeling and determination of pharmacokinetic parameters (Table 1), a statistical analysis (ANOVA) demonstrated no significant (P < .05) difference between mean values for area under the curve, α, β, α half-life, β half-life, clearance, and Vdss when each was compared with the corresponding mean values determined for BC rats.

Elimination of Cosalane. Figure 5 depicts the biliary excretion-time profile for cosalane after i.v. administration of 10 mg/kg in male SD rats. The cumulative percentage of unchanged cosalane excreted into bile was 1.121 ± 0.071 over 48 h. No detectable metabolites were found in the bile extract. Cosalane or any of its metabolite(s) were also not detected in urine after i.v. or oral administration. Figure 6 shows the cosalane excretion-time profile in feces over a period of 120 h after a 10 mg/kg oral dose. The cumulative percent of the dose excreted in feces as a parent compound was found to be 87.1 ± 9.9. Again, no detectable cosalane metabolite(s) were found in fecal extracts.

Tissue Distribution. Table 2 summarizes the distribution of cosalane in different tissues such as blood, liver, spleen, bone marrow, etc.
kidney, lung, heart, fat, muscle, and brain after 2, 24, and 48 h postadministration. Two hours after administration, blood had the highest cosalane concentration with significant levels in well perfused organs like the liver and kidney. Cosalane accumulated significantly in liver and spleen at all time points (Table 2). It was also detectable in almost all tissues analyzed at all time points. The compound was undetectable in urine samples over a period of 120 h after both i.v. and oral administration. Interestingly, more than 40% of the dose was found in the liver after 48 h as the parent compound, and no detectable metabolite(s) were found in the liver extract. Moreover, there was no statistical difference ($P < .05$) in the concentration of cosalane in liver extract at 24 and 48 h. Figure 7 shows the cosalane tissue/blood ratios at 2, 24, and 48 h post dose. The tissue/blood ratios of liver and spleen increased with time and were statistically different at all three time points.

**Discussion**

Data available in the literature on the disposition of cosalane and dihydrocosalane are scarce. A report shows that cosalane has poor renal excretion in mice (Bigelow et al., 1994). In a recent report from our laboratory, it was shown that cosalane is poorly metabolized by the liver and is highly accumulated in isolated perfused rat livers (Udata et al., 1999). This study was designed to obtain complete pharmacokinetics of cosalane and dihydrocosalane in male SD rats after i.v. and oral administration. In this study, biliary excretion, enterohepatic circulation, and tissue distribution results were also included to better understand the disposition of cosalane.
organ systems like the liver and the gastrointestinal tract to high
of enterohepatic circulation in the rat. Enterohepatic circulation pro-
which is a part of the hepatobiliary loop, are indicative of involvement
secondary peaks and a high accumulation of cosalane in the liver,
tissue distribution studies of cosalane after i.v. dosing revealed high
of increasing concentrations of cosalane around 6 to 12 h. In addition,
mg/kg), the plasma concentration-time profiles showed an incidence
Because renal clearance of cosalane is a minor route of elimination,
was set at 1 mg/kg for several reasons. First, this dose allowed assay
obtained by assessing pharmacokinetics over a wide dose range. No
water partition coefficient of 8.8
Cosalane, with a molecular weight 846, is extremely hydrophobic,
has an intrinsic aqueous solubility of 1 ng/ml, and has an octanol/
the AUC0–6; CI, and elimination half-life among normal and BC rats.
Cosalane and dihydrosalane appear to be widely distributed
throughout the body because volumes of distribution of each compo-
weight of biliary excretion was 325 ± 50 in rats (Hirom et al., 1972),
showed very low biliary excretion (1.12% of dose, Fig. 4). Incubations of bile and urine with β-glucuronidase or sulfatase, or
under mild or strong alkaline conditions, did not reveal the existence
of any cosalane conjugates (not shown). The poor biliary excretion of
cosalane may be explained by its limited metabolism and extreme
lipophilicity because it is known that for extensive biliary excretion
to occur, the molecule must have a large molecular weight and the
presence of a strong polar group (Smith, 1973). These results also
support the previously reported findings that cosalane accumulated
significantly in isolated perfused rat livers and failed to show any
detectable metabolites either in the effluent perfusate or liver homog-
enates (Udata et al., 1999).
Cosalane and dihydrosalane appear to be widely distributed
throughout the body because volumes of distribution of each compo-
was greater than the total body water (0.70 l/kg) of the rat
(Gerlowski and Jain, 1983). Protein binding studies of cosalane
(K.R.K., M.S. Ahmed, T.P.J., and A.K.M., data not published) dem-
proved that cosalane bound extensively to rat plasma (84.84
points tested clearly suggest a very high affinity of the compound for
the reticuloendothelial system with the highest concentration in the
liver even after 48 h. It is possible that cosalane may tend to self-
associate and favor a macromolecular structure because it is a deriv-
liver even after 48 h. It is possible that cosalane may tend to self-
associate and favor a macromolecular structure because it is a deriv-
endothelial system. The liver sinusoidal endothelial cells with their high endocytic
capacity, together with the fenestrae and the absence of basal lamina, makes them unique in the body (Wisse et al., 1985). Also, it has been proposed that distribution in the liver is a characteristic of the organ’s anatomical properties, such as the vascular architecture rather than the
physicochemical properties of the solute (Rowland and Evans, 1991;
Chou et al., 1995). The extreme hydrophobicity and membrane-
interactive nature of cosalane may also facilitate the accumulation of
the drug in the bilayer of the enterocytic cell membrane. Also, the
concentration of cosalane was significantly higher in the membrane
extract of the perfused liver cellular fractions (data not shown) further

\[ \text{Tissue/blood ratios of cosalane in rats after i.v. administration.} \]

A greater insight into the mechanisms of drug disposition can be
obtained by assessing pharmacokinetics over a wide dose range. No
obvious toxic effects due to cosalane administration were observed
over the dose range studied. The lowest dose investigated for cosalane
was set at 1 mg/kg for several reasons. First, this dose allowed assay
of plasma samples despite the small sample volumes (40–50 µl)
available over several (4–4.5) half-lives. Second, this dose yielded
reliable pharmacokinetic parameters. Third, evaluation of a dose
lower than 1.0 mg/kg was deemed unnecessary because the clearance
of cosalane is constant over the dose range studied (1.0–10.0 mg/kg).
Cosalane, with a molecular weight 846, is extremely hydrophobic,
has an intrinsic aqueous solubility of 1 ng/ml, and has an octanol/
water partition coefficient of 8.8 \( \times 10^6 \) (Venkatesh et al., 1996). Because renal clearance of cosalane is a minor route of elimination,
biliary excretion may play a major role in elimination of cosalane
(Bigelow et al., 1994). In some rats after i.v. administration (10
mg/kg), the plasma concentration-time profiles showed an increase
of concentrations of cosalane around 6 to 12 h. In addition,
tissue distribution studies of cosalane after i.v. dosing revealed high
concentration of the drug in the liver 48 h post dose. The presence
of secondary peaks and a high accumulation of cosalane in the liver,
which is a part of the hepatobiliary loop, are indicative of involvement
of enterohepatic circulation in the rat. Enterohepatic circulation pro-
longs the presence of cosalane in the systemic circulation and exposes
organ systems like the liver and the gastrointestinal tract to high

\[ \begin{array}{|c|c|c|c|}
\hline
\text{Tissue} & \text{Time} & \text{h} \\
\hline
\text{Blood} & 218.640 ± 11.190 & 5.637 ± 0.125 & 1.413 ± 0.162 \\
\text{Liver} & 157.015 ± 8.123 & 98.811 ± 3.468 & 96.474 ± 3.677 \\
\text{Spleen} & 100.401 ± 5.458 & 88.499 ± 14.011 & 72.143 ± 19.523 \\
\text{Bone marrow} & \text{nd} & \text{nd} & 26.938 ± 4.608 \\
\text{Kidney} & 10.490 ± 1.456 & 10.137 ± 2.567 & 3.397 ± 0.486 \\
\text{Lung} & \text{nd} & 10.393 ± 1.367 & 6.649 ± 5.519 \\
\text{Heart} & \text{nd} & 9.251 ± 2.117 & 3.596 ± 0.161 \\
\text{Fat} & 8.950 ± 2.004 & 4.032 ± 2.150 & 2.315 ± 1.088 \\
\text{Muscle} & \text{nd} & 1.509 ± 0.176 & 0.747 ± 0.109 \\
\text{Brain} & 1.862 ± 0.785 & 1.372 ± 0.765 & 0.000 \\
\text{Bile} & 3.800 ± 0.112 & \text{nd} & 0.382 ± 0.033 \\
\hline
\end{array} \]

\[ \text{nd: not determined} \]
supporting the hypothesis that cosalane is membrane-interactive and accumulates in the bilayer of the cell (Udata et al., 1999).

Martin (1981) has shown that the rate of disappearance from the intestine was positively correlated with the octanol-buffer distribution coefficient for more than 100 compounds of log octanol-buffer distribution coefficient values less than 3, stressing the fact that lipophilicity is a key factor in oral absorption. Although it is believed that the most lipophilic compounds will diffuse at a faster rate across the cellular membranes of the intestinal epithelium (Rowland and Tozer, 1989) which constitutes the main barrier for oral absorption, compounds that are extremely lipophilic (log octanol/buffer coefficient $>5$) have a diminished capacity to cross biological membranes (Wils et al., 1994). This finding may explain the undetectable plasma levels of cosalane as a result of poor intestinal absorption, and almost complete elimination in feces ($87.06 \pm 9.95\%$) after oral administration (Fig. 6). Furthermore, it was observed that the permeability of cosalane across Caco-2 monolayers is very poor and is limited by an equilibrium between the free drug, bound drug, and drug partitioning into the bilayer of the cell (Pal et al., 1998).

In conclusion, the poor oral bioavailability of cosalane is mainly due to its poor absorption through the intestinal membrane rather than its presystemic metabolism, and efforts are underway to improve the permeation of cosalane across enterocytes. Although cosalane may have a limited potential to treat intracellularly sequestered HIV, this permeability is a key factor in oral absorption.