Disposition of the Acyclic Nucleoside Phosphonate (S)-9-
(3-Hydroxy-2-Phosphonylmethoxypropyl)Adenine

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The acyclic nucleoside phosphonate (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA] has been shown to be active against pathogens, like hepatitis B viruses and Plasmodium parasites, that infect parenchymal liver cells. (S)-HPMPA is therefore an interesting candidate drug for the treatment of these infections. To establish effective therapeutic protocols for (S)-HPMPA, it is essential that the kinetics of its hepatic uptake be evaluated and that the role of the various liver cell types be examined. In the present study, we investigated the disposition of (S)-HPMPA and assessed its hepatic uptake. Rats were intravenously injected with [3H](S)-HPMPA, and after an initial rapid distribution phase (360 ± 53 mCi/kg of body weight), the radioactivity was cleared from the circulation with a half-life of 11.7 ± 1.4 min. The tissue distribution of [3H](S)-HPMPA was determined at 90 min after injection (when >99% of the dose cleared). Most (57.6% ± 1.1%) of the injected [3H](S)-HPMPA was excreted unchanged in the urine. The radioactivity that was retained in the body was almost completely recovered in the kidneys and the liver (68.4% ± 2.5% and 16.1% ± 0.4% of the radioactivity in the body, respectively). The uptake of [3H](S)-HPMPA by the liver occurred mainly by parenchymal cells (92.1% ± 3.4% of total uptake by the liver). Kupffer cells and endothelial cells accounted for only 6.1% ± 3.5% and 1.8% ± 0.8% of the total uptake by the liver, respectively. Preinjection with probenecid reduced the hepatic and renal uptake of [3H](S)-HPMPA by approximately 75%, which points to a major role of a probenecid-sensitive transporter in the uptake of (S)-HPMPA by both tissues. In conclusion, we show that inside the liver, (S)-HPMPA is mainly taken up by parenchymal liver cells. However, the level of uptake by the kidneys is much higher, which leads to nephrotoxicity. An approach in which (S)-HPMPA is coupled to carriers that are specifically taken up by parenchymal cells may increase the effectiveness of the drug in the liver and reduce its renal toxicity.

Many efforts in the search for effective antiviral agents have focused on the development of nucleoside analogs that selectively affect viral DNA synthesis (1, 11, 12, 15). These analogs need to be phosphorylated to their triphosphate derivatives to become biologically active. The triphosphates affect viral replication either by inhibiting viral transcriptase enzymes (e.g., reverse transcriptase and viral DNA polymerase) or by terminating viral DNA chain elongation. The phosphorylation of antiviral nucleoside analogs to their triphosphate derivatives is, in general, catalyzed by cellular kinases. To bypass the first critical phosphorylation step, monophosphorylated nucleoside analogs have been synthesized and tested for their antiviral activities. Acyclic nucleoside phosphate analogs have been found to be particularly promising. A key feature of these compounds is that their phosphonylmethyl ether group is resistant to the activities of the esterases that dephosphorylate regular monophosphorylated nucleosides. The compounds are, however, still readily phosphorylated to the active derivatives by cellular enzymes (27). Acyclic nucleoside phosphonates show broad-spectrum antiviral activity against several RNA and DNA viruses (10). The spectra of the antiviral activities of the nucleoside phosphonates vary largely and depend on the nature of the base and the nature of the acyclic side chains (11). The mechanisms of the antiviral actions of these analogs are, however, not entirely clarified. It has been shown that their active diphosphorylated derivatives can competitively inhibit the DNA polymerase- and reverse transcriptase-catalyzed incorporation of natural triphosphate nucleosides into DNA (16, 23). However, recent studies indicate that some of these analogs can also act as alternative substrates, which leads to DNA chain termination or a strongly reduced rate of DNA chain elongation (19, 37). Additional mechanisms, however, cannot be excluded. Studies with animals and patients indicate that acyclic nucleoside phosphonates are therapeutically effective in vivo but that renal toxicity is dose limiting (6, 27, 31).

The S enantiomer of 9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA] is of particular interest for the therapy of liver-associated disease. It has been shown that it is highly effective against human and duck hepatitis B viruses in cultured cells (38, 39). Furthermore, it has been shown in vivo with mice that (S)-HPMPA is active against the liver stage of the rodent parasite Plasmodium berghei (a murine model for human malaria). It prevents the development of a blood-stage infection and thus the severe symptoms of malaria (32). Both pathogens (i.e., hepatitis B viruses and Plasmodium parasites) infect parenchymal liver cells. To establish an effective therapeutic protocol for (S)-HPMPA for the treatment of these infections, it is essential that the kinetics of its uptake by the liver be evaluated and that the roles of the various types of liver cells be examined. Furthermore, these parameters need to be related to the dose-limiting renal uptake.

MATERIALS AND METHODS

Reagents. (S)-HPMPA was kindly provided by E. de Clercq (Rega Institute, Leuven, Belgium). [2,8-3H](S)-HPMPA (14 Ci/nmol) was purchased from...
The clearance of intravenously injected \(^{3}H\)(S)-HPMPA from plasma. Rats were intravenously injected with \(^{3}H\)(S)-HPMPA at a dose of 5 mg/kg of body weight. At the indicated times, blood samples were taken and the plasma was assayed for radioactivity. Values are means ± SEMs for six rats.

**RESULTS**

**Clearance of \(^{3}H\)(S)-HPMPA from plasma.** To study the clearance of (S)-HPMPA from plasma, rats received a bolus injection of \(^{3}H\)(S)-HPMPA and the radioactivity in blood plasma was monitored. The administered amount, 5 mg/kg of body weight, is in the range of doses of acyclic nucleoside phosphonates that have been found to be effective in vivo (14, 22, 28, 30). Figure 1 shows that after an initial rapid distribution phase (distribution volume, 360 ± 53 ml/kg of body weight), radioactivity was cleared from the circulation with a half-life of 11.7 ± 1.4 min (means ± standard errors of the means [SEMs] for six rats). The total body clearance was 20.9 ± 1.8 ml/min per kg of body weight. Analysis by reverse-phase HPLC and anion-exchange HPLC performed with plasma samples taken up to 60 min after injection indicated that >90% of the radioactivity in the plasma samples represented unchanged (S)-HPMPA.

**Disposition of \(^{3}H\)(S)-HPMPA.** To study the contribution of various tissues to the clearance of (S)-HPMPA from the circulation, rats were injected with \(^{3}H\)(S)-HPMPA, and at 90 min after injection the distribution of radioactivity over the tissues was examined. In addition, urine was collected. Table 1 shows that most of the radioactivity (57.0% ± 1.1% of the dose) was found in the urine. Analysis by reverse-phase HPLC and anion-exchange HPLC indicated that >95% of the radioactivity in the urine represented unchanged (S)-HPMPA. In the body, most of the radioactivity was recovered in the kidneys and liver (29.4% ± 0.8% and 6.9% ± 0.3% of the dose, respectively). The amounts of radioactivity in the tissues were also calculated as a percentage of the amount of radioactivity recovered from the body. Table 1 shows that kidneys and liver accounted for 68.4% ± 2.5% and 16.1% ± 0.4% of the radioactivity from the body, respectively. The small amount of remaining label was evenly distributed over the body. The relative specific radioactivity of the kidneys was by far the highest. It was more than 25-fold higher than the relative specific radioactivity of the liver and even more than 250-fold higher than that of any other tissue.

The kinetics of the renal and hepatic uptake of (S)-HPMPA...
and its urinary excretion was studied by injecting rats with $[^3H]$(S)-HPMPA and monitoring the accumulation of radioactivity in the liver, kidneys, and urine (Fig. 2). Radioactivity accumulated rapidly in the liver and kidneys, reaching maximal values after 60 min. The amount of radioactivity in the urine reached its maximum at 90 min after injection.

Role of liver cells in the uptake of (S)-HPMPA. The liver contains three highly metabolically active cell types: Kupffer cells, endothelial cells, and parenchymal cells. To identify the cell type(s) responsible for the hepatic uptake of (S)-HPMPA, rats were injected with $[^3H]$(S)-HPMPA. Sixty minutes later, parenchymal, endothelial, and Kupffer cells were isolated from the liver and assayed for radioactivity. The results are presented in Fig. 3. The parenchymal cells were found to be the main site of uptake. These cells accounted for 92.1% ± 3.4% of the total uptake by the liver, whereas endothelial cells and Kupffer cells accounted for only 1.8% ± 0.8% and 6.1% ± 3.5% of the total uptake by the liver, respectively.

Mechanism of hepatic and renal uptake of (S)-HPMPA. To study the mechanism of the renal and hepatic uptake of (S)-HPMPA, rats were preinjected with probenecid before injection of radiolabeled (S)-HPMPA. Probenecid is an inhibitor of organic anion transport, and it has been found that it protects against the nephrotoxicity induced by acyclic nucleoside analogs (28). Figure 4 indicates that pretreatment of rats with probenecid substantially reduced (by >75%) the accumulation of $[^3H]$(S)-HPMPA in both the kidneys and the liver. The amount of $[^3H]$(S)-HPMPA that was not taken up by the kidneys and the liver was quantitatively recovered in the urine. These findings indicate that a probenecid-sensitive transporter plays a major role in the uptake of (S)-HPMPA by both the kidneys and the liver.

**DISCUSSION**

We show in the present study that after intravenous injection into rats, the acyclic nucleoside phosphonate (S)-HPMPA is rapidly cleared from the circulation. Excretion into the urine was found to be the major route of elimination. Most of the (S)-HPMPA that was retained in the body was recovered in the kidneys. The renal clearance, calculated from the total body clearance and the contribution of the kidneys to the clearance,
was 18.1 ± 1.5 ml/min per kg of body weight. This value exceeds the values reported for the glomerular filtration rate in rats (6 to 8 ml/min per kg), but it is close to those reported for the renal blood plasma flow (20 to 28 ml/min per kg) (13, 20). The key role of the kidneys in the disposition of (S)-HPMPA was also observed for other acyclic nucleoside phosphonates. It has been found in earlier studies with 1-(2-phosphonomethoxyethyl)adenine (PMEA) and (S)-HPMPA that these analogs are extensively excreted in the urine and that significant amounts of these analogs accumulate in the kidneys (9, 24).

The high level of accumulation in the kidneys [in the case of (S)-HPMPA, 25 to 250 times higher levels in the kidneys than in other tissues] probably explains the nephrotoxicity of acyclic nucleoside phosphonates (6, 28, 31). The liver displayed the second highest level of accumulation of the acyclic nucleoside phosphonate, and it was found that virtually all liver-associated (S)-HPMPA was present in parenchymal cells. The accumulation of (S)-HPMPA in other tissues was negligible.

Acyclic nucleoside phosphonates need to be internalized to become pharmacologically active. The mechanisms of internalization of these compounds have so far not been fully clarified. Some studies have been performed with cells in culture. Depending on the cell line and the compound investigated, different mechanisms have been found. A study with Vero cells indicated that the uptake of (S)-HPMPA by these cells proceeds via fluid-phase endocytosis (8). Examination of the uptake of PMEA by HeLa cells, on the other hand, provided evidence for the involvement of a specific cellular protein (7).

The uptake of PMEA by these cells appeared to be strictly structure specific, because it could be inhibited only by itself but not by closely related side chain-substituted compounds, including (S)-HPMPA. The present in vivo results indicate that uptake of (S)-HPMPA by the two organs mainly involved in clearance of (S)-HPMPA, the kidneys and the liver, occurs via a probenecid-sensitive transporter. Both renal proximal tubular cells and parenchymal liver cells are equipped with multiple transport systems that are capable of taking up organic anions, some of which are susceptible to inhibition by probenecid (29, 33, 34, 36). These systems mediate the transport of a wide variety of extracellular organic anions into the cytosol. The molecular nature of the renal probenecid-sensitive transporter(s) has not yet been clarified. Recently, a hepatic organic anion-transporting polypeptide has been cloned and characterized (17, 21, 36). Transport of organic anions by this protein is inhibited by probenecid (21), and it may therefore be implicated in the uptake of (S)-HPMPA by parenchymal liver cells. However, the involvement of other transport systems in the hepatic uptake of (S)-HPMPA cannot be excluded. (S)-HPMPA displays broad-spectrum antiviral activity against several viruses, including hepadnaviruses that cause hepatitis (10). In mice (S)-HPMPA is also active against the liver stage of the rodent parasite P. berghei (32), an established murine model for the development of human malaria. Because both the hepatitis viruses and the Plasmodium parasites replicate in parenchymal liver cells, this cell type is a highly relevant target cell for (S)-HPMPA. Unfortunately, we found that only a limited amount of the (S)-HPMPA administered is taken up by the liver. The level of uptake by the kidneys was more than 25 times higher than that by the liver, and the resulting nephrotoxicity precludes the administration of a therapeutically effective dose to the liver. Probenecid has been proposed as a drug that can be used to reduce the nephrotoxicity of acyclic nucleosides (28). Indeed, we found that probenecid decreases the level of uptake of (S)-HPMPA by the kidneys (and consequently nephrotoxicity) by >75%. However, uptake by the liver is reduced to the same extent by probenecid, so there is no benefit for the treatment of hepatic disease. A much more effective therapy of diseases associated with parenchymal liver cells can be achieved if the uptake of (S)-HPMPA by these cells is increased, with a concomitant reduction of uptake by the kidneys. This therapeutic goal may be accomplished by associating (S)-HPMPA with carriers that are specifically taken up by the parenchymal liver cell. Carriers that can be used for this approach include several galactose-terminated targeting devices that are taken up via the asialoglycoprotein receptor (2, 3, 5, 18) and the recently developed artificial chylomimics, which are taken up via the remnant receptor (30).

REFERENCES


