High-Dose Treatment with $^{186}$Re-HEDP or $^{153}$Sm-EDTMP Combined with Amifostine in a Rabbit Model

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The aim of this experimental study was to investigate the myeloprotective potential of amifostine in rabbits receiving high-dose treatment with either $^{153}$Sm-ethylenediaminetetramethylene phosphonate (EDTMP) or $^{186}$Re-hydroxyethylidene diphosphonate (HEDP) and to check for drug interactions impairing the skeletal uptake of these radiopharmaceuticals by amifostine.

**Methods:** To a total of 24 rabbits, we administered 1,000 MBq of either $^{153}$Sm-EDTMP ($n = 12$) or $^{186}$Re-HEDP ($n = 12$). Six animals of each group received 500 mg amifostine intravenously 10–15 min before injection of the radiopharmaceutical, whereas the other 6 animals served as controls. Up to 8 wk after treatment, blood samples were collected every 3–5 d to measure platelet and leukocyte counts. Furthermore, whole-body images were acquired at 3 min, 3 h, and 24 h after injection of the radiopharmaceutical to quantify the skeletal uptake. **Results:** For $^{186}$Re-HEDP, the mean decrease in platelets was significantly less in the amifostine group (35.5% ± 2.4%) than in the control group (61.3% ± 5.4%, $P < 0.001$). Similar results were found for $^{153}$Sm-EDTMP (36.5% ± 8.3% vs. 52.3% ± 14.0%, $P < 0.05$). No significant differences in leukocyte counts were found for $^{186}$Re-HEDP (75.3% ± 12.3% in the amifostine group and 72.5% ± 4.1% in the control group, $P > 0.05$), whereas rabbits treated with $^{153}$Sm-EDTMP plus amifostine showed a significantly greater decrease in leukocytes (69.2% ± 10.8%) than did the control group (56.6% ± 4.0%, $P < 0.05$). Bone uptake in percentage of initial total whole-body activity was significantly decreased in animals treated with amifostine compared with the control groups for both $^{186}$Re-HEDP (15.8% ± 3.1% vs. 30.9% ± 1.9%, $P < 0.001$) and $^{153}$Sm-EDTMP (31.7% ± 8.9% vs. 44.0% ± 6.5%, $P < 0.05$). **Conclusion:** For amifostine, we found a highly significant cytoprotective effect on platelets but no leukoprotective effect. The latter probably relies on the intrinsic myelotoxicity of high-dose amifostine, which seemed to potentiate the leukodepression of the radiopharmaceuticals. The lower bone uptake in amifostine-treated animals may be caused by the chemical structure of amifostine, which is a potentially complex-forming compound that may be able to displace bisphosphonates from the rhenium– and samarium–bisphosphonate complexes, resulting in altered biodistribution patterns.

**Key Words:** amifostine; myeloprotection; $^{186}$Re-hydroxyethylidenephosphonate; $^{153}$Sm-ethylenediaminetetramethylene phosphonate; skeletal uptake
pair bone uptake of these radiopharmaceuticals would be of particular interest because of the possibility that amifostine may alter renal drug clearance (14) and bone metabolism (15,16).

The aim of this experimental study, therefore, was to investigate the myeloprotective potential of amifostine in rabbits receiving high-dose treatment with either 153Sm-EDTMP or 186Re-HEDP and to determine whether any drug interactions with amifostine were impairing skeletal uptake of the radiopharmaceuticals.

MATERIALS AND METHODS

Animals and Study Design

The studies were performed on 24 female New Zealand White rabbits (age range, 10–12 wk; mean weight ± SD, 2.5 ± 0.2 kg; Charles River, Kissleg, Germany). For radionuclide bone therapy, we used 186Re-HEDP (Mallinckrodt, Petten, The Netherlands) and 153Sm-EDTMP (CIS, GIF-Sur-Yvette, France). A standard activity of 1,000 MBq of either 153Sm-EDTMP (n = 12) or 186Re-HEDP (n = 12) was administered intravenously according to a dose of 400 MBq/kg of body weight. As suggested from patient studies with amifostine, all animals received 4 mg dexamethasone (Forteccortin; Merck, Darmstadt, Germany) as an antiemetic treatment to 4 additional animals, which served as negative controls with regard to the radiopharmaceuticals. The animal studies further treatment to 4 additional animals, which served as negative amifostine with 4 mg dexamethasone was administered without any procedure was found effective, with no major side effects, for radio-

Animals were divided into 2 subgroups: 6 animals from each group received 500 mg (200 mg/kg of body weight) of amifostine (i.e., ethanethiol, or 2-[(3-aminopropyl)amino]-, dihydrogen phosphate [ester]) (Ethyol; Essex, Munich, Germany) as a 5-min intravenous infusion 10–15 min before injection of the radiopharmaceutical, and 6 rabbits from each group served as controls. This administration of ROI positions is shown in Figure 1. From these data, the geometric mean for each ROI was calculated after correction for different acquisition times, radioactive decay, and bremsstrahlung.

The myeloprotective effects of amifostine were investigated by collecting blood samples before therapy and then every 3–5 d until ±8 wk after radionuclide treatment to measure platelet and leukocyte counts. Furthermore, quantitative bone scanning was performed to assess whether amifostine was interfering with 153Sm-EDTMP or 186Re-HEDP.

Scintigraphic Skeletal Uptake Measurement

Whole-body images from anterior and posterior views were acquired simultaneously at 3 min, 3 h, and 24 h after a bolus injection of the respective radiopharmaceutical. The acquisition time was 1 min during the blood-pool phase and 10 min for the later images. Images were obtained under the same conditions for each rabbit, using a double-head gamma camera (Bodyscan; Siemens, Erlangen, Germany) equipped with high-resolution low-energy collimators. The energy window used was 137 keV ± 15% for 186Re and 103 keV ± 15% for 153Sm.

For each image, activity in the whole body, urinary bladder, and soft tissue of the flank was measured by region-of-interest (ROI) technique using both anterior and posterior views. Additionally, a rectangular ROI adjacent to the body was used to measure the bremsstrahlung of the β-particles of the radionuclide. An example

of ROI positions was shown in Figure 1. From these data, the geometric mean for each ROI was calculated after correction for different acquisition times, radioactive decay, and bremsstrahlung. The initial whole-body activity of each rabbit was used as the reference value for further calculations of activity data, which are all given as a percentage of the whole-body activity. According to a simplified 3-compartment model, soft-tissue activity compared with initial whole-body activity equals activity in muscles compared with initial muscle activity at 3 min after injection. Urinary excretion was determined by the difference in whole-body activity between the reference image at 3 min and the image of interest plus urinary bladder activity. From these data, bone uptake at 24 h after injection was calculated as 100% of initial whole-body activity minus both urinary excretion and soft-tissue retention, as has been described in detail elsewhere (8).

Statistics

Data are given as mean ± 1 SD. A 2-tailed Student t test for unpaired data (including both the F test and the test of David, Pearson, and Stephens to prove normal distribution of the data) was used to evaluate the significance of differences between rabbit subgroups, with P < 0.05 considered to be statistically significant (18).

RESULTS

Platelets and Leukocytes

In rabbits treated with 1,000 MBq 186Re-HEDP plus amifostine (ReA; n = 6), the mean pretherapeutic platelet counts were 316 ± 14/nL. Platelet counts reached a mean nadir of 201 ± 11/nL after 11 ± 3 d, corresponding to a mean decrease of 35.5% ± 2.4%. In the control group, which was treated with 1,000 MBq 186Re-HEDP alone (ReC; n = 6), pretherapeutic platelet counts of 342 ± 28/nL decreased to 134 ± 29/nL after 13 ± 2 d, corresponding to a decrease of 61.3% ± 5.4% (Table 1). Thus, the mean decrease in platelets was significantly greater in ReC than in
ReA (P < 0.001), whereas the intervals of the nadir were not different (P > 0.05). The corresponding data for the pretherapeutic leukocyte counts were 5.2 ± 1.4/nL for ReA and 5.4 ± 1.4/nL for ReC (Table 1). The respective nadirs were reached after 7 ± 1 d and 6 ± 0 d (P > 0.05), with a mean decrease in leukocytes to 1.3 ± 0.6/nL for ReA (75.3% ± 12.3%) and 1.5 ± 0.2/nL for ReC (72.5% ± 4.1%). Thus, the decrease in leukocytes did not significantly differ between the 2 subgroups (P > 0.05).

In rabbits treated with 1,000 MBq $^{153}$Sm-EDTMP plus amifostine (SmA; n = 6), the mean pretherapeutic platelet counts were 269 ± 33/nL. A mean nadir of 169 ± 17/nL was reached after 24 ± 2 d, corresponding to a mean decrease of 36.5% ± 8.3%. In the control group, which was treated with 1,000 MBq $^{153}$Sm-EDTMP alone (SmC; n = 6), pretherapeutic platelet counts of 270 ± 36/nL decreased to 125 ± 21/nL after 27 ± 3 d, corresponding to a decrease of 52.3% ± 14.0%, which was significantly greater than the decrease for the SmA group (P < 0.05) (Table 2), with no difference between the intervals of the nadir. The corresponding data for the pretherapeutic leukocyte counts were 5.4 ± 1.0/nL for SmA and 6.1 ± 1.3/nL for SmC (Table 2). The respective nadirs were reached after 7 ± 3 d and 10 ± 4 d (P > 0.05): 1.6 ± 0.3/nL for SmA (69.2% ± 10.8%) and 2.6 ± 0.6/nL for SmC (56.6% ± 4.0%). Thus, the decrease was significantly greater (P < 0.05) for the amifostine group.

Comparing the intervals between administration of the radiopharmaceutical and the nadir, we found no significant differences in leukocyte counts between $^{186}$Re-HEDP and $^{153}$Sm-EDTMP (6 ± 0 d for ReC and 10 ± 4 d for SmC, P > 0.05), whereas the nadir of the platelet counts was reached significantly earlier in rabbits treated with $^{186}$Re-HEDP (13 ± 2 d for ReC and 27 ± 3 d for SmC, P < 0.001). However, both the leukocyte counts and the platelet counts recovered completely, reaching the initial values 6 wk after treatment with $^{186}$Re-HEDP either with or without amifostine. The same recovery time, 6 wk, was observed for both the leukocyte counts and the platelet counts of SmA and SmC after treatment with $^{153}$Sm-EDTMP. Thus, for both radiopharmaceuticals, the recovery times of rabbits treated with amifostine did not differ from those of rabbits treated without it.

In 4 animals that served as negative controls with regard to radiopharmaceuticals, that is, animals treated with amifostine and dexamethasone only, both the leukocyte counts and the platelet counts showed no changes up to 5 wk after injection. Thus, no myelotoxic effects from use of amifostine in combination with dexamethasone were found by simple blood cell counting.

**Skeletal Uptake of $^{186}$Re-HEDP and $^{153}$Sm-EDTMP**

In rabbits receiving ReA (n = 6), the mean bone uptake at 24 h after injection was 15.8% ± 3.1%. For the control group (ReC; n = 6), uptake of 30.9% ± 1.9% was obtained at 24 h after injection, and this value was significantly higher (P < 0.001) than that for the amifostine group. Although the remainder soft-tissue activity at 24 h after injection was not significantly different between the groups (ReA, 14.8% ± 3.2%; ReC, 14.0% ± 2.7%), urinary excretion was significantly increased in rabbits treated with amifostine (ReA, 69.4% ± 2.5%; ReC, 55.1% ± 3.8%; P < 0.001).

For $^{153}$Sm-EDTMP in combination with amifostine (SmA; n = 6), the mean bone uptake at 24 h after injection was 31.7% ± 8.9%, whereas in the control group a mean uptake value of 44.0% ± 6.5% at 24 h after injection was found, showing a significant difference from the amifostine group (P < 0.05). On the other hand, the 18.4% ± 7.0% remainder soft-tissue activity found in animals treated with amifostine was significantly higher (P < 0.05) than the 9.8% ± 4.4% found in control animals. The mean urinary excretion rate was similar for both groups: 49.9% ± 11.8% and 46.3% ± 7.5% in rabbits treated with amifostine and without amifostine, respectively.

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**TABLE 1**

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<th>Platelets</th>
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*Significant difference (P < 0.001).
†No significant difference (P > 0.05).

Data are percentage of initial values. Final row is mean ± SD for 6 rabbits.

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**TABLE 2**

<table>
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<td>36.5 ± 8.3*</td>
<td>52.3 ± 14.0*</td>
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</table>

*Significant difference (P < 0.05).
†Significant difference (P < 0.05).

Data are percentage of initial values. Final row is mean ± SD for 6 rabbits.
Significantly higher bone uptake at 24 h after injection was found for \( ^{153} \text{Sm}-\text{EDTMP} \) than for \( ^{186} \text{Re}-\text{HEDP} \) for both the animals treated with amifostine (\( P < 0.01 \)) and the control animals (\( P < 0.001 \)), whereas the remainder soft-tissue activity was in the same range for SmA and ReA and for SmC and ReC (\( P > 0.05 \) for each).

**DISCUSSION**

The cytoprotective potential of thiol-containing compounds against radiation damage has been known for many years (19). Amifostine was selected from >4,000 of such compounds as the agent with the best clinical profile. In animal studies, amifostine protected normal tissues such as bone marrow, salivary glands, skin, and oral mucosa from the toxic effects of lethal doses of ionizing radiation, with no impact on malignant transformed cells (20,21). The mechanism of this selective radioprotection of amifostine has been shown to be related to its preferential rapid uptake into normal tissues and no or only slow uptake into tumor cells. This selectivity results mainly from differences in the presence of alkaline phosphatase at capillary endothelial cells and the pH of normal tissues compared with the pH of tumor tissue. These conditions favor the dephosphorylation of amifostine by the membrane-bound alkaline phosphatase to its active metabolite, the free thiol WR-1065 (Fig. 2), which immediately enters normal tissues (22). Once inside the cell, WR-1065 acts as a scavenger of oxygen free radicals by detoxifying these radicals before they can damage the DNA or RNA (23). After intravenous injection, amifostine is rapidly cleared from the plasma, with half-lives of <1 min and <8 min (23). In contrast to the brief systemic half-life, retention of amifostine and its metabolites in normal tissue is prolonged. During the first 30 min after administration, uptake into normal tissues such as salivary glands, kidneys, liver, and bone marrow has been shown to be >100-fold higher than uptake into tumor tissue, with intracellular retention up to 24 h after injection (24). As a result of these experimental findings, numerous clinical studies have been performed and have shown amifostine to be a potent cyto- and myeloprotective agent in patients undergoing chemotherapy or radiation therapy (16,25,26). Consequently, amifostine has gained drug approval in the United States and in Europe (13,27).

![FIGURE 2. Chemical structure of amifostine and its active metabolite, the free thiol WR-1065.](image-url)
to Klutmann et al., we found such an additive leukotoxic effect only for $^{153}$Sm-EDTMP, not for $^{186}$Re-HEDP, possibly because of a statistical problem caused by the small number of animals. Summarizing all these observations, we conclude that in rabbits, a dosage of 200 mg amifostine per kilogram of body weight is close to but below the threshold of measurable leukotoxic effects, which may become detectable during combined treatment with amifostine and myelotoxic drugs such as $^{153}$Sm-EDTMP or $^{186}$Re-HEDP. Therefore, for further studies in rabbits, the dosage of amifostine should be reduced if white blood cell counts are part of the study design. To our knowledge, however, there are no other reports on the use of amifostine in radionuclide bone therapy that might support our conclusions or explain the underlying mechanisms in more detail.

Our study used a recently introduced quantification method based on whole-body bone scanning to measure skeletal uptake, soft-tissue retention, and urinary excretion of radiolabeled bisphosphonates ($8,31$) and, thus, document drug interactions with amifostine. This step seemed necessary because of the known possibility that amifostine may alter renal drug clearance ($14$) and bone metabolism ($15,16$). For $^{186}$Re-HEDP, bone uptake at 24 h after injection was significantly lower in animals treated with amifostine than in control animals. The remainder soft-tissue activity at 24 h after injection was the same in amifostine-treated rabbits as in control animals, suggesting that the higher amount of nonskeletal bound activity was completely excreted by the kidneys. For $^{153}$Sm-EDTMP also, significantly reduced bone uptake was found in amifostine-treated animals. However, in these rabbits the main observation was a shift in activity toward the soft-tissue compartment, whereas urinary excretion was nearly the same for both amifostine-treated animals and untreated animals. Because amifostine can alter bone metabolism through both parathyroid hormone–independent and parathyroid hormone–dependent mechanisms, resulting in hypocalcemia and an inhibition of osteoclastic bone resorption ($15,16,32$), the reduced bone uptake of $^{186}$Re-HEDP and $^{153}$Sm-EDTMP after administration of amifostine might be explained by subsequent reduction of osteoblastic activity in response to transient osteoclastic inhibition. However, this hypothesis would not explain the increased remainder soft-tissue activity of $^{153}$Sm-EDTMP, because excretion of nonskeletal-bound $^{153}$Sm-EDTMP by the kidneys is expected, as shown in patients with bone metastases: the lower the number and extent of bone metastases, the lower was bone uptake and an altered biodistribution pattern.

**CONCLUSION**

In rabbits undergoing high-dose treatment with 1,000 MBq of either $^{186}$Re-HEDP or $^{153}$Sm-EDTMP, we found amifostine to have a highly significant cytoprotective effect on platelets. However, no leukoprotection was found, and even leukotoxicity was shown for the combination of amifostine and $^{153}$Sm-EDTMP. This effect most probably relies on the intrinsic myelotoxicity of high-dose amifostine, which seemed to potentiate radiopharmaceutical-induced myelodepression. These findings suggest the use of lower doses of amifostine in rabbits. Furthermore, significantly reduced bone uptake of $^{186}$Re-HEDP and $^{153}$Sm-EDTMP was found in amifostine-treated animals. Amifostine, because of its potential to form complexes, may be able to displace bisphosphonates from the rhenium– and samarium–bisphosphonate complexes, resulting in reduced bone uptake and an altered biodistribution pattern.

**ACKNOWLEDGMENTS**

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**REFERENCES**


