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Bone marrow protection by amifostine in Re-186-HEDP treatment: first results obtained in a rabbit animal model

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Background. *In the last few years various reports dealt with radioprotective effects of amifostine (Ethyol®, USB, Philadelphia, PA). Since amifostine is markedly accumulated in bone marrow it looks worthwhile to study the radioprotective effects of amifostine on bone marrow in patients treated with Re-186-HEDP. As a first step animal studies were performed using New Zealand White rabbits.*

Materials and methods. *A total of 18 rabbits received 300 MBq Tc-99m-HDP for whole-body scintigraphy. Thereafter, 9 animals were treated with 200 mg/kg body weight amifostine, and 9 rabbits served as controls receiving physiological saline solution. Then, these 18 rabbits received 400 MBq Re-186-HEDP i.v. Two rabbits served as untreated controls and received neither Tc-99m-HDP nor Re-186-HEDP. Blood samples were taken at the beginning and in two-week-intervals for the duration of two months in all 20 animals. Laboratory findings were determined for white and red blood cells, for platelet count and for hemoglobin. Two months after therapy all animals were sacrificed, and both femora were removed surgically for histopathological examination of bone marrow.*

Results. *In controls as well as in amifostine-treated animals, the red blood cell count and the hemoglobin were almost constant at all times of observation. In 9 control rabbits the mean platelet count was 265.22±127.41 Mrd/l prior to Re-186-HEDP-therapy. Two weeks after therapy the mean platelet count was reduced to 211.22±52.8 Mrd/l. Prior to Re-186-HEDP-therapy the mean platelet count of amifostine-treated rabbits was not significantly different ($p>0.05$) from control rabbits. Two weeks after injection of the radionuclide the mean platelet count decreased to 180.67±37.43 Mrd/l in the amifostine group. There was no significant difference between rabbits of the control group and amifostine-treated animals ($p>0.05$). Thus, amifostine was not able to prevent a transient thrombocytopenia. Two weeks after therapy only a slight decrease of the white blood cell count was recognized in controls. In contrast, amifostine-treated rabbits showed a considerable decrease of the white blood cell count two weeks after therapy with a mean value of 3.39±0.91 Mrd/l. This difference was statistically significant ($p<0.0002$).*

Discussion. *The insufficient radioprotection of amifostine concerning the platelet count was probably due to pharmacocinetics of amifostine. Since the generation of free radicals in the bone marrow caused by Re-186-HEDP lasts several times longer than the radioprotective effect of amifostine given as one single dose prior to the application of Re-186-HEDP. However, the observed decline of white blood cells due to amifostine application is yet unknown.*

Conclusion. *In order to use amifostine as a suitable agent for radioprotection multiple doses of amifostine might be applied, i.e. two shots per day for the duration of 3 to 4 days after the application of Re-186-HEDP. The observed leucopenia should be studied in further animal studies.*

Key words: Radiation-protection agents; bone marrow; amifostine; erythrocyte count; platelet count, hemoglobin; Re-186-HEDP; bone metastases

Introduction

Patients with prostate cancer will develop bone metastases in nearly 70 %,[1] which may result in pathological fractures as well as in severe bone pain.[2,3] The application of β⁻-emitting osteotropic radionuclides, *i.e.* Sr-89-chloride, Sm-153-EDTMP, and Re-186-HEDP is one therapeutic approach in palliative treatment of painful, multilocular bone metastases.[4-10] Since bone metastases show a preferential uptake of bone-seeking radionuclides, *i.e.* Re-186-HEDP, this therapy is rather specific, while non-affected tissue is spared from the effects of the β⁻-irradiation.[11] In 75% of the patients pain relief occurs within one to two weeks after the application of Re-186-HEDP and lasts for about 1-6 months.[3,10,12] However, Re-186-HEDP delivers a substantial dose to the bone marrow, thus, a potential bone marrow suppression is still the most important dose-limiting factor.[3,10-13] This radiobiological side-effect is mainly confined to a decline of the platelet count, called thrombocytopenia.[14,15] Therefore, one inclusion criteria for patients undergoing rhenium-therapy is a platelet count of at least 150 Mrd/l prior to therapy, and in clinical routine a blood count is performed directly prior to the application of the bone-seeking radionuclide.[16] However, some patients do not fulfill this inclusion criteria at the day of admission and therefore, rhenium-therapy can not be performed as planned. Thus, the reduction of radiobiological side-effects is of major interest in patients treated for painful bone metastases. On the other hand, therapy with standard activities of 1200 MBq Re-186-HEDP is still a palliative treatment which does not influence the prognosis of the underlying disease at all. However, a reduction of side-effects of Re-186-HEDP might improve tolerability of rhenium-therapy. Thus, in the future, Re-186-HEDP might be applied not only as palliative but also as curative means in patients with multilocular bone metastases.

In the last few years various reports dealt with radioprotective effects of the phosphorylated aminothiol amifostine (Ethyol®, USB, Philadelphia, PA).[17-29] Since amifostine markedly accumulates in salivary glands the application of amifostine has been successfully used both in external radiotherapy in patients with head and neck tumors[30] and high-dose radioiodine therapy in patients with differentiated thyroid cancer.[26-29,31-34] Since amifostine is also markedly accumulated in bone marrow[25,35-38] it looks worthwhile to study the radioprotection of bone marrow in order to avoid/reduce bone marrow toxicity in patients treated with Re-186-HEDP, and thus, to increase the tolerability of rhenium-therapy. As a first step animal studies were performed.

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Material and method

Animal studies

In order to investigate the radioprotective effects of amifostine an animal model was established using New Zealand White rabbits. A total of 20 animals aged between two and three months and weighing 2.1 to 3.1 kg with a mean weight of 2.86±0.11 kg were used. As a first step, 18 rabbits received 300 MBq Tc-99m-HDP as intravenous injection by a vein flow placed in the ear. For bone scintigraphy 2 hrs p.i., all rabbits were positioned in prone position directly onto a low-energy high-resolution collimator of a large field of view gamma camera (Diacam, Siemens, Erlangen, Germany). For anesthesia, 50 mg/kg Ketanest® (Ketaminhydrochlorid,

Parke-Davis, Berlin, Germany) and 4 mg/kg Rompun® (Xylazinhydrochlorid, BayerVital, Leverkusen, Germany) were administered in a mixed syringe as intramuscular injection in the upper leg directly prior to image acquisition. Images were acquired over 20 min each and stored digitally in a 256x256 matrix. Directly after bone scintigraphy 9 animals were treated with 200 mg/kg Amifostine® (Amifostine, USB, Philadelphia,PA) as slow intravenous infusion over 5 min. Nine rabbits served as controls receiving a corresponding volume of physiological saline solution. Then, these 18 rabbits received 400 MBq Re-186-HEDP dissolved in less than 0.6 ml. Whole-body scintigraphy was performed at 24 hrs or 48 hrs p.i. in order to evaluate the distribution of the Re-186-HEDP applied. Again, 50 mg/kg Ketanest® (Ketaminhydrochlorid, Parke-Davis, Berlin, Germany) and 4 mg/kg Rompun® (Xylazinhydrochlorid, BayerVital, Leverkusen, Germany) were administered for anesthesia in a mixed syringe as intramuscular injection in the upper leg. Rabbits were positioned as described above directly onto a low-energy high-resolution collimator of a large field of view gamma camera (Diacam, Siemens, Erlangen, Germany). Images were acquired over 20 min each and were stored digitally in a 256x256 matrix.

Two rabbits served as untreated controls and received neither Tc-99m-HDP nor Re-186-HEDP. Blood samples were taken at the beginning and in two-week-intervals for the duration of two months in all 20 animals. Laboratory findings were determined for white and red blood cells, for platelet count and for hemoglobin. Two months after therapy all animals were sacrificed, and both femora were removed surgically for histopathological examination of bone marrow.

Histopathological examination

The bone marrow was stained in conventional manner with Hematoxilin/Eosin, Giemsa, and Berlin blue. An experienced pathologist performed histopathological examinations in a blind manner. Evaluation criteria were the cellularity of the bone marrow, the number and differentiation of the erthropoesis, granulopoesis and thrombocytopoesis, the evaluation of bone marrow vessels, and the quantity of bone marrow's iron content.

Evaluation

Blood samples were directly transferred to the central laboratory and determined the same day in order to avoid an artificial decline of platelets. Hemoglobin was measured in g/dl. White blood cell count, red blood cell count, and platelet count were measured in Mrd/l, respectively.

Whole-body scintigramms acquired after injection of Tc-99m-HDP or Re-186-HEDP were.evaluated by visual inspection.

Statistics

Data are given as mean ± one standard deviation. Two-tailed Student's t-test for paired data was used to evaluate statistical differences between animal subsets. $P<0.05$ was considered to be significant.[39]

Results

Animal model

The animal model chosen was easy to handle. Due to the size of the ear veins both the injection of the radiopharmaceuticals and the drawing of blood samples were easy to perform. Moreover, the chosen anesthesia consisting of a mixture of Ketanest® and Rompun® was safe and allowed image acquisition without any movement artifacts.

Red blood cells

Details of the red blood cell count for all groups of rabbits are given in Table 1. In con-

trols, the red blood cell count was almost constant at all times of observation. Prior to therapy a mean red blood cell count of 6.07(0.84 Mrd/l was measured in this group. At two, four, six and eight weeks after therapy the red blood cell count was 6.07±0.39, 6.61±0.42, 6.59±0.63 and 6.18±0.58 Mrd/l, respectively. Corresponding results were observed for animals of the amifostine-treated group. Prior to therapy a mean red blood cell count of 6.29±0.51 Mrd/l was measured in these animals. Two, four, six and eight weeks after therapy the mean red blood cell count amounted to 6.25±0.46, 6.12±0.41, 5.82±0.34 and 6.19±0.4 Mrd/l, respectively. Thus, amifostine-treated animals showed almost unchanged red blood cell counts during Re-186-HEDP-therapy.

Two completely untreated animals showed an almost constant red blood cell count during all times of observation. In these two animals a mean red blood cell count of 6.0±0.13 Mrd/l was measured at first time of observation and amounted to 6.25±0.13, 6.7±0.04, 6.07±0.86 and 5.98±0.23 in two-week-intervals up to eight weeks after beginning of the study.

Hemoglobin

Details of values for hemoglobin are given in Table 1. In animals of the control group mean hemoglobin was 12.53±1.14 g/dl prior to therapy and amounted to 12.84±0.9, 14.32±0.75, 14.09±1.01 and 13.71±0.72 g/dl two, four, six and eight weeks after therapy, respectively. Thus, animals pretreated with physiological saline solution only showed near unchanged hemoglobin at all times of observation. Laboratory findings of hemoglobin determined prior and in two-week intervals showed corresponding results for amifostine-treated animals. In these rabbits mean hemoglobin amounted to 13.41±0.96 g/dl prior to therapy and was 13.23±0.94, 13.64±0.52, 12.74±0.54 and 13.87±0.86 g/dl at two, four, six and eight weeks after therapy, respectively.

Moreover, unchanged values for hemoglobin were observed in both untreated animals with hemoglobin amounting to 12.75±0.49 g/dl at the beginning and amounting to 13.4±0.99, 14.6±0.99, 13.25±0.92 and 13.2±0.57 g/dl at two-week-intervals.

Table 1. Mean values and standard deviation of the red and white blood cell count, the platelet count and the hemoglobin prior to and 2, 4, 6 and 8 weeks after Re-186-HEDP treatment in the control group, the amifostine-treated group and in the untreated rabbits

		Time with respect to Re-186-HEDP i.v				
		Before	2 weeks after	4 weeks after	6 weeks after	8 weeks after
RBC [Mrd/l]	Controls	6.07±0.53	6.07±0.39	6.61±0.42	6.59±0.63	6.18±0.6
	Amifostine	6.29±0.51	6.25±0.46	6.12±0.41	5.82±0.34	6.19±0.4
	Untreated	6.00±0.13	6.25±0.13	6.70±0.04	6.07±0.86	5.98±0.2
Hgb [g/dl]	Controls	12.50±1.11	12.84±0.90	14.32±0.75	14.09±1.01	13.71±0.72
	Amifostine	13.40±1.12	13.23±0.94	13.64±0.52	12.74±0.54	13.87±0.86
	Untreated	12.80±0.54	13.40±0.99	14.60±0.99	13.25±0.92	13.20±0.57
Platelets [Mrd/l]	Controls	265.2±127.4	211.2±52.8	359.7±93.3	338.8±90.5	359.0±86.7
	Amifostine	286.2±73.2	180.7±37.43	214.8±122.0	267±89.6	251.4±102.9
	Untreated	378.0±161.2	315.5±129.4	379.5±112.4	275.5±105.4	327.0±70.7
WBC [Mrd/l]	Controls	6.64±1.27	5.43±0.88	6.96±1.41	8.1±1.46	7.79±0.84
	Amifostine	5.73±2.1	3.39±0.91	4.61±1.48	5.29±1.24	5.76±1.34
	Untreated	6.6±1.41	6.15±0.21	6.75±1.63	9.2±4.53	9±2.55

Platelet count

Details of platelet count are given for all rabbits in Table 1. In 9 control rabbits mean platelet count was 265.22±127.41 Mrd/l prior to Re-186-HEDP-therapy. Two weeks after injection of the radionuclide mean platelet count was reduced to 211.22±52.8 Mrd/l. Four and six weeks after therapy mean platelet count increased to 359.67±93.26 and 338.78±90.46 Mrd/l, respectively. Prior to the sacrification mean platelet count of 359±86.72 Mrd/l was measured for all animals pre-treated with physiological saline solution only.

Prior to Re-186-HEDP-therapy the mean platelet count of amifostine-treated rabbits was not significantly different (p>0.05) from control rabbits amounting to 286.22±73.2 Mrd/l. Two weeks after injection of the radionuclide the mean platelet count of the amifostine group decreased to 180.67±37.43 Mrd/l. There was no significant difference between rabbits of the control group and amifostine-treated animals (p>0.05). Four, six and eight weeks after Re-186-HEDP-therapy the platelet count increased to 214.78±121.97, 267.67±89.6 and 251.44±102.89 Mrd/l, respectively. Moreover, there was no significant difference between animals treated with either physiological saline solution or amifostine.

Laboratory findings of two untreated animals revealed platelet count of 378±161.22 Mrd/l at the beginning and 315.5±129.4, 379.5±112.43, 275.5±105.36 and 237±70.71 Mrd/l at two-week-intervals follow-up.

White blood cells

Values of the white blood cell count are given in detail in Table 1. Prior to Re-186-HEDP-therapy mean white blood cell count of

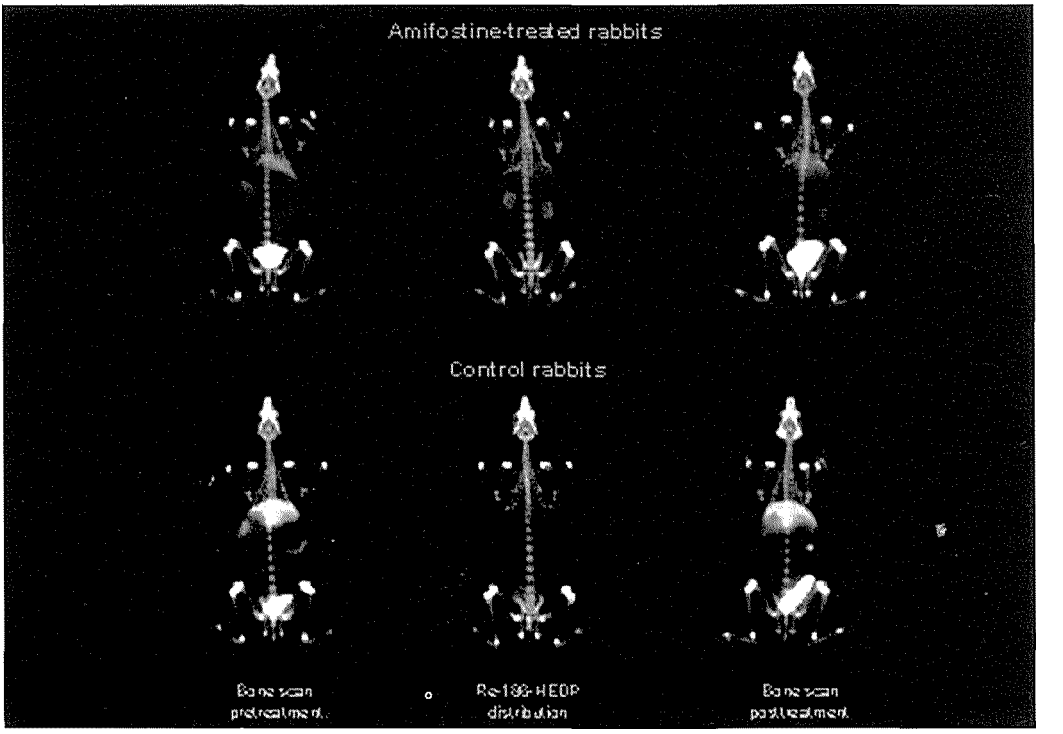

Figure 1. Whole body scintigraphy after injection of 300 MBq Tc-99m-HDP prior to (left column) and 2 months after Re-186-HEDP-therapy (right column) in a rabbit of the control group (lower row) and in an amifostine-treated animal (upper row). Distribution of Re-186-HEDP 48 hrs after i.v. application is displayed in the middle column.

6.64±1.27 Mrd/l was measured for rabbits of the control group. Two weeks after therapy a slight decrease of white blood cell count was recognized in this animal group with a mean value of 5.43±0.88 Mrd/l. Four, six, and eight weeks after therapy white blood cell count increased to mean values of 6.96±1.41, 8.1±1.46 and 7.97±0.84 Mrd/l, respectively. Amifostine-treated animals revealed mean white blood cell count of 5.73±2.1 Mrd/l prior to the injection of the radionuclide. In contrast to the control group, amifostine-treated rabbits showed a considerable decrease of white blood cell count two weeks after therapy with a mean value of 3.39±0.91 Mrd/l. This difference was statistically significant ($p<0.0002$). In follow-up studies the white blood cell count removed to 4.61±1.48, 5.29±1.24 and 5.76±1.34 Mrd/l at four, six and eight weeks after therapy, respectively.

In two completely untreated animals white blood cell count remained almost unchanged with values of 6.6±1.41, 6.15±0.21, 6.75±1.63, 9.2±4.53 and 9±2.55 Mrd/l at the beginning and in two-week intervals, respectively.

Scintigraphic findings

Left column of Figure 1 shows examples of whole-body scintigraphy 2 hrs after injection of Tc-99m-HDP of one animal of the control group (lower row) and one animal of the amifostine-treated group (upper row). Prior to Re-186-HEDP-therapy there was no difference between the control animals and the amifostine-treated animals concerning the distribution of Tc-99m-HDP. Examples of whole-body scintigraphy 48 hrs after injection of Re-186-HEDP are displayed in the middle column of Figure 1. The visual evaluation of the scintigramms showed a distribution of Re-186-HEDP comparable to bone scans in both groups of rabbits. Examples of bone scintigraphy at 8 weeks after Re-186-HEDP-therapy are shown in the right column of Figure 1. There was no visual difference of the distribution of Tc-99m-HDP in either control animals or amifostine-treated animals. Moreover, there was no visual difference between the distribution of the Tc-99m-HDP prior to and eight weeks after therapy with Re-186-HEDP.

Histopathological findings

Eight weeks after Re-186-HEDP-therapy all 18 rabbits showed hyperemic bone marrow vessels as compared to untreated animals (Figure 2).

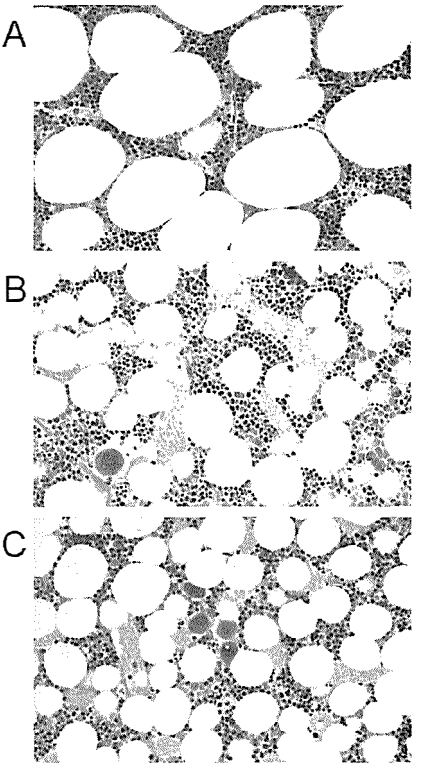

Figure 2. Histological examination of bone marrow in conventional Giemsa staining, magnification: 200fold. A: animal which received neither Tc-99m-HEDP nor Re-186-HEDP. B: animal of the control group. C: animal of the amifostine group. Note the comparable cellularity and differentiation of the different cell lines of B and C as compared to the bone marrow of the completely untreated animal (A). However, in contrast to the untreated animal the blood vessels of B and C are filled with red blood cells, and thus, appear hyperemic.

There was no difference between animals treated with or without amifostine concerning the cellularity of bone marrow, the number and differentiation of erythropoesis, granulopoesis and thrombocytopoesis, and the quantity of iron. Moreover, histopathological examination revealed no difference between animals treated with amifostine and those rabbits, which received physiological saline solution only.

Discussion

Prostate cancer is the second most common malignancy in men in Western Europe.[1] The incidence is 15-16 per 100.000 habitants per year with increasing tendency. As much as 80% of patients with prostate cancer will develop bone metastases.[1] In about one third of all patients osseous metastases are detected at primary staging. Moreover, the skeleton is the only single site of metastases in a reasonable amount of patients.[3] In case of multilocular, osseous metastases a complete remission of prostate cancer is nearly impossible.

Since osseous metastases are often associated with bone pain, effective pain relief is the primary goal when caring for patients with prostate cancer and multiple osseous metastases. Traditional therapeutic approach is the application of central or peripheral analgesics in combination with neuroleptics.[6] Moreover, a steroid medication, diphosphonates and hormonal drugs may complete analgesic effects. However, the therapy with opioids is limited in many patients due to side-effects, *i.e.* nausea, vomits and gastrointestinal symptoms and thus, often associated with a loss of patient's quality of life.[7]

Skeletal pain confined to a single site metastasis usually responds to external beam radiotherapy in 70-80 %.[6,40,41] In case of multilocular, osseous metastases external beam radiation is helpful to avoid pathologic fractures or compression of the spinal cord.[42] However, hemibody or whole-body irradiation for pain relief is often limited by bone marrow suppression, gastrointestinal symptoms and a radiation pneumonitis.[14,43] Therefore, an effective relief of pain with low side-effects and an improvement in patient's quality of life is warranted in these patients.

The application of β^-–emitting osteotropic radionuclides is a promising method to selectively irradiate osseous metastases by sparing normal tissues from short-range irradiation.[11,13] Due to the osteoblastic character of osseous metastases the radiopharmaceutical is predominantly accumulated in malignant transformed cells, which leads to a rather selective irradiation of bone metastases. Re-186-hydroxyethylidendiphosphonate (Re-186-HEDP) has recently been developed for palliative treatment of painful osseous metastases.[5] Re-186 has a therapeutic β^-–emission of 1,07 MeV associated with a γ^-–emission of 137 keV. Moreover, Re-186-HEDP and Tc-99m-HDP, which is commonly used for diagnostic bone scintigraphy, have an almost exactly similar bone distribution since both sorts of diphosphonates bridge to the hydroxyapatite of bone substance. Therefore, pretherapeutic and posttherapeutic scintigraphic imaging is possible which allows a control of Re-186-HEDP distribution as shown in Figure 1 (middle column). Re-186 has a short physical half-life of 3,8 days that allows a single application of activities of 1110 to 1850 MBq with high tumor doses as well as an easy handling of radioactive waste, *i.e.* urine. About 50% and70 % of the activity injected are excreted via the kidneys into the urine within the first 6 hours and 24 hours after application, respectively. Apart from the distribution in osseous structures Re-186-HEDP is not accumulated in any other structures of the body.

Pain relief is attained within two weeks after application of Re-186-HEDP and lasts for about 1-6 months. Response rates of Re-186-HEDP-therapy of 70-80 % have been

reported.[3,10,12] Especially in patients with oral medication of non-opioid analgesics, Re-186-HEDP-therapy led either to a reduction or to a stop of oral drug medication. Due to the short physical half-life, Re-186-HEDP treatment can be repeated after 4-6 months.

The main radiobiological side-effect of bone seeking radiotherapeuticals is their potential bone marrow toxicity.[11,13-15] Thrombocytopenia plays the major role in this bone marrow suppression. The decline of platelets presents with a nadir about two to three weeks after its application. Since patients with prostate cancer often show a bleeding tendency caused by the additional use of non-steroid anti-inflammatory drugs and by a tumor infiltration of the bladder frequent controls of platelet count are necessary in posttherapeutic follow-up. In clinical practice platelet counts are regularly defined in two-week intervals for the duration of two months after Re-186-HEDP-therapy. Since thrombocytopenia was proven to be the main side-effect a reduction of the platelet counts' decline would improve tolerability of Re-186-therapy. On the other hand, thrombocytopenia is the dose-limiting factor. Thus, by reducing the bone marrow toxicity of Re-186-HEDP higher activities of more than 1200 MBq might be injected as one single dose in the future. Thus, Re-186-HEDP might be applied not only as a palliative but also as curative means in patients with prostate cancer.

The pro-drug amifostine emerged as the most promising radioprotector synthesized and tested in a large study funded by the US army. In clinical trials amifostine was shown to protect salivary glands from irradiation damage in patients with thyroid cancer under high-dose radioiodine therapy.[26-29,31,32,44,45] Moreover, amifostine was proven helpful in patients with cancer of the head and neck treated with chemo-irradiation.[30,45,46] In these patients amifostine was able to significantly reduce side-effects, *i.e.* mucositis, xerostomia and thrombocytopenia as compared to a non-amifostine treated patient group. Moreover, either in patients with thyroid cancer nor in patients with head and neck cancer amifostine was shown not to protect tumor tissue, which is a prerequisite for its potential use in tumor therapy.[25,33,47]

When administered intravenously, amifostine is rapidly cleared from the plasma with an alpha half-life of as less than one minute and a beta half-life of less than 10 minutes.[48] In contrast to its brief systemic half-life, there is a prolonged retention of the drug and its metabolites in normal tissues.[36] In the first 30 minutes after amifostine administration, the drug uptake into normal tissues such as salivary glands, liver, kidney, heart and bone-marrow demonstrated an up to 100-fold greater difference as compared to tumor tissue.[36]

In this preclinical study the radioprotective effect of amifostine on bone marrow suppression under Re-186-HEDP-therapy was studied using a rabbit bone marrow model. Therefore, in a total of 18 rabbits, 400 MBq Re-186-HEDP were applied intravenously in order to evaluate the bone marrow suppression in rabbits pretreated either with amifostine or with physiological saline solution only. Neither red blood cell count nor hemoglobin was changed by Re-186-HEDP in controls and in rabbits pretreated with amifostine. Moreover, by histopathological examination there was no difference concerning the red blood cell line in all three groups of animals. This is in accordance with the observations of other investigators who reported no influence of Re-186-HEDP on hemoglobin and red blood cells.[16]

In contrast, a marked decline of the platelet count of about 20 % was observed two weeks after application of Re-186-HEDP in controls. This is again in accordance with the observation of other investigators.[7,11,13-16] Following whole-body exposure by external radiation, thrombocytopenia develops slowly over a period of approximately 30 days after doses of 200-400 cGy. After the application of a dose of 600-1000 cGy the production of

platelets is completely stopped, which leads to a decrease of the platelet count reflecting the life range of the platelets of approximately 9 days.[15] It was estimated that standard activities of 1200 MBq Re-186-HEDP deliver a radiation dose of about 75 cGy to the bone marrow leading to the marked platelet suppression.[8] In contrast to the investigations of de Klerk[15] who reported a nadir of decline of platelet count at week 4 after therapy in this rabbit animal model a marked reversible decline of platelets occurred as early as two weeks after therapy. Thus, in amifostine-treated animals peripheral platelet count showed a comparable decrease of about 37.1%. Thus, amifostine could **not** reduce the transient thrombocytopenia in animals treated with Re-186-HEDP. This is probably due to the pharmacocinetic properties of amifostine. First, amifostine was applied as a single dose prior to Re-186-HEDP. Second, while Re-186-HEDP has both a biological and a physical half-life of about three days, the biological half-life of amifostine within the bone marrow is probably less than 24 hrs. Thus, the generation of free radicals within the bone marrow caused by Re-186-HEDP lasts several times longer than the radioprotective effect of amifostine. This leads to the conclusion that in order to use amifostine as a suitable agent for radioprotection, multiple doses of amifostine might be applied, *e.g.* two single shots per day for the duration of 3 to 4 days after the application of Re-186-HEDP. However, concerning the thrombocytopoesis 8 weeks after therapy the pathologist described no difference between animals treated with Re-186-HEDP and totally untreated animals. Thus, in order to investigate the myelotoxic effect of Re-186-HEDP the sacrification of the animals might be performed earlier than 8 weeks after therapy, *e.g.* 2 weeks after therapy while the platelet count decrease exhibits its nadir.

As far as white blood cells are concerned amifostine-treated animals showed a significant reduction of leucocytes as compared to animals of the control group. There was no explanation for this side-effect of amifostine while nausea, vomiting and potentially hypotension are well-described side-effects after the administration of amifostine. However, a reduction of white blood cells is yet unknown. Moreover, there was no difference in the granulopoesis between all three groups of rabbits investigated as demonstrated by histopathology. Thus, in order to evaluate cytotoxic effects of amifostine or Re-186-HEDP on the genesis of white blood cells the sacrification of the rabbits might be performed earlier. However, the hyperemic blood vessels of all animals treated with RE-186-HEDP might be interpreted as a late reactive sign of bone marrow damage.

Conclusions

In this animal model amifostine given in one single dose was not able to avoid the transient thrombocytopenia in rabbits treated with Re-186-HEDP. However, in order to use amifostine as a suitable agent for radioprotection multiple doses of amifostine might be applied, *e.g.* two single shots per day for the duration of 3 to 4 days after the application of Re-186-HEDP. The observed leucopenia should be studied in further animal studies.

References

1. Jacobs SC. Spread of prostatic cancer to bone. *Urology* 1983; **21:** 337-44.

2. Quirijnen JMSP, Han SH, Zonnenberg BA, de Klerk JM, van het Schip AD, van Dijk A, et al. Efficacy of Rhenium-186-etidronate in prostate cancer patients with metastatic bone pain. *J Nucl Med* 1996; **37:** 1511-5.

3. Palmedo H, Bender H, Schomburg A, Grunwald F, Schoneich G, Zamorra P, et al. Schmerztherapie mit Rhenium-186 HEDP bei multiplen Knochenmetastasen. [Pain therapy with rhenium-186 HEDP in multiple bone metastases]. *Nuklearmedizin* 1996; **35:** 63-7.

4. Blake GM, Zivanovic MA, McEwan AJ, Ackery DM. Strontium-89 therapy: strontium kinetics in disseminated carcinoma of the prostate. *Eur J Nucl Med* 1986; **12:** 447-54.

5. Collins C, Eary JF, Donaldson G, Vernon C, Bush NE, Petersdorf S, et al. Samarium-53-EDTMP in bone metastases of hormone refractory prostate carcinoma: A phase I/II trial. *J Nucl Med* 1993; **34:** 1839-44.

6. Schoeneich G, Palmedo H, Heimbach D, Biersack HJ, Müller SC. Advanced prostate cancer; systemic radiopharmaceutical therapy of metastatic prostate cancer with Rhenium-186 hydroxyethylidene diphosphonate. *Onkologie* 1997; **20:** 316-9.

7. Schoeneich G, Palmedo H, Dierke-Dzierzon C, Müller SC, Biersack HJ. Rhenium-186 HEDP: palliative radionuclide therapy of painful metastases. Preliminary results. *Scand J Urol Nephrol* 1997; **31:** 445-8.

8. Maxon HR, Deutsch EA, Thomas SR. Rhenium-186 (sn) HEDP for treatment of multiple metastatic foci in bone: human biodistribution and dosimetric studies. *Radiology* 1988; **166:** 501-7.

9. Maxon HR, Schroder LE, Hertzberg VS. Rhenium-186 HEDP for treatment of painful osseous metastases. Results of a double blind cross over comparison with placebo. *J Nucl Med* 1991; **19:** 1877-81.

10. Maxon HR, Thomas SR, Hertzberg VS. Rhenium-186 HEDP for treatment of painful osseous metastases. *Semin Nucl Med* 1992; **22:** 33-40.

11. de Klerk JM, van het Schip AD, Zonnenberg BA, van Dijk A, Quirijnen JM, Blijham GH, et al. Phase I study of Rhenium-186-HEDP in patients with bone metastases originating from breast cancer. *J Nucl Med* 1996; **37:** 224-49.

12. Zonnenberg BA, de Klerk JM, van Rijk PP. Re-186 HEDP for treatment of painful bone metastases in patients with metastatic prostate or bone cancer. *J Nucl Med* 1991; **32:** 1082-91.

13. de Klerk JM, van Dieren EB, van het Schip AD, Hoekstra A, Zonnenberg BA, van Dijk A, et al. Bone marrow absorbed dose of Rheium-186-HEDP and the relationship with decreased platelet counts. *J Nucl Med* 1996; **37:** 38-41.

14. de Klerk JM, Zonnenberg BA, van het Schip AD, van Dijk A, Han SH, Quirijnen JM, et al. Dose escalation study of rhenium-186 hydroxyethylidene diphosphonate in patients with metastatic prostate cancer. *Eur J Nucl Med* 1994; **21:** 1114-20.

15. de Klerk JM, van het Schip AD, Zonnenberg BA, van Dijk A, Stokkel MP, Han SH, et al. Evaluation of thrombocytopenia in patients treated with Rhenium-186 HEDP: Guidelines for individual dosage recommendations. *J Nucl Med* 1994; **35:** 1423-8.

16. Holle L-H, Humke U, Trampert L, Ziegler M, Kirsch CM, Oberhausen E. Palliative Schmerztherapie beim ossšre metastasierten Prostatakarzinom mit dem osteotropen B-Strahler 186 Rhenium-Hydroxyethylidendiphosphonat (HEDP). *Urologe* 1997; **36:** 540-7.

17. Utley JF, Marlowe C, Waddell WJ. Distribution of 35S-labeled WR-2721 in normal and malignant tissues of the mouse. *Radiat Res* 1976; **68 :** 284-91.

18. Utley JF, Phillips TL, Kane LJ. Protection of normal tissues by WR-2721 during fractionated irradiation. *Int J Radiat Oncol Biol Phys* 1976; **1:** 699-703.

19. Utley JF, King R, Giansanti JS. Radioprotection of oral cavity structures by WR-2721. *Int J Radiat Oncol Biol Phys* 1978; **14:** 643-7.

20. Utley JF, Quinn CA, White FC, Seaver NA, Bloor CM. Protection of normal tissue against late radiation injury by WR-2721. *Radiat Res* 1981; **85:** 408-15.

21. Utley JF, Seaver N, Newton GL, Fahey RC. Pharmacokinetics of WR-1065 in mouse tissue following treatment with WR-2721. *Int J Radiat Oncol Biol Phys* 1984; **10:** 1525-8.

22. Pratt NE, Sodicoff M, Liss J, Davis M, Sinesi M. Radioprotection of the rat parotid gland by WR-2721 S-2-3 aminopropylaminoethyl phosphorothioate morphology at 60 days post irradiation. *Int J Radiat Oncol Biol Phys* 1980; **6:** 431-6.

23. Niibe H, Takahashi I, Mitsuhashi N, Miyaishi K, Itoh J, Maehara Y, et al. [An evaluation of the clinical usefulness of amifostine (YM-08310), radioprotective agent. A double-blind placebo-controlled study. 1. Head and neck tumors]. *Nippon Gan Chiryo Gakkai Shi* 1985; **20:** 984-93.

24. Takahashi I, Nagai T, Miyaishi K, Maehara Y, Niibe H. Clinical study of the radioprotective effects of amifostine (YM-08310, WR-2721) on chronic radiation injury. *Int J Radiat Oncol Biol Phys* 1986; **12:** 935-8.

25. Yuhas JM, Spellman JM, Culo F. The role of WR-2721 in radiotherapy and/or chemotherapy. *Cancer Clin Trials* 1980; **3:** 211-6.

26. Bohuslavizki KH, Klutmann S, Jenicke L, Brenner W, Lüttges J, Feyerabend B, et al. Radioprotection of salivary glands by S-2-(3-aminopropylamino)-ethylphophorothioic acid (amifostine) obtained in a rabbit animal model. *J Nucl Med* 1999; **40:**. 232P.

27. Bohuslavizki KH, Klutmann S, Brenner W, Henze E, Mester J, Clausen M. Salivary gland protection by amifostine: results of a double-blind multi-arm trial. *J Nucl Med* 1999; **40:** P18.

28. Bohuslavizki KH, Klutmann S, Jaenicke L, Brenner W, Feyerabend B, Henze E, et al. Radioprotection of salivary glands by S-2-(3-aminopropylamino)-ethylphosphorothioic (amifostine) obtained in a rabbit animal model. *Int J Rad Oncol Biol Phys* 1999; **45(1):**181-6.

29. Bohuslavizki KH, Klutmann S, Kröger S, Kampen WU, Tinnemeyer S, Brenner W, et al. Salivary gland protection by amifostine in high-dose radioiodine therapy: results of a double-blind placebo-controlled study. *Nucl Med Commun* 1998; **19:** 359.

30. Buntzel J, Schuth J, Kuttner K, Glatzel M. Radiochemotherapy with amifostine cytoprotection for head and neck cancer. *Support Care Cancer* 1998; **6:** 155-60.

31. Bohuslavizki KH, Klutmann S, Brenner W, Kröger S, Kampen WU, Tinnemeyer S, et al. Protection of salivary glands by amifostine in high-dose radioiodine therapy: results of a double-blind placebo-controlled study. *Eur J Nucl Med* 1998; **25:** 905.

32. Bohuslavizki KH, Klutmann S, Brenner W, Jenicke L, Kampen WU, Kröger S, et al. Radioprotection of salivary glands by amifostine in high-dose radioiodine treatment (HD-RIT): results of a double-blind, placebo-controlled study. *Proc Amer Assoc Cancer Res* 1998; **39:** 506.

33. Bohuslavizki KH, Klutmann S, Brenner W, Mester J, Henze E, Clausen M. Salivary gland protection by amifostine in high-dose radioiodine therapy: results of a double-blind placebo-controlled study. *J Clin Oncol* 1998; **16:** 3542-9.

34. Bohuslavizki KH, Brenner W, Klutmann S, Hubner RH, Lassmann S, Feyerabend B, et al. Radioprotection of salivary glands by amifostine in high-dose radioiodine therapy. *J Nucl Med* 1998; **39:** 1237-42.

35. Davidson DE, Grenan MM, Sweeney TR. Biological characteristics of some improved radioprotectors. In: Brady LW, editor. *Radiation sensitizers. Their use in the clinical management of cancer.* New York, NY: Masson; 1980. p. 309-20.

36. Yuhas JM. Active versus passive absorption kinetics as the basis for selective protection of normal tissues by S-2-(3-aminopropylamino)-ethylphosphorothioic acid. *Cancer Res* 1980; **40:** 1519-24.

37. Washburn LC, Carlton JE, Hayes RL, Yuhas JM. Distribution of WR-2721 in normal malignant tissues of mice and rats bearing solid, tumors: dependence on tumor type, drug dose and species. *Radiat Res* 1974; **59:** 475-83.

38. Phillips TL, Kane LJ, Utley JF. Radioprotection of tumor and normal tissues by thiophosphonate compounds. *Cancer* 1973; **32:** 528-35.

39. Sachs L. *Applied statistics - a handbook of techniques.* New York, NY: Springer; 1984.

40. Gilbert HA, Kagan AR, Nussbaum H, Rao AR, Satzman J, Chan P, et al. Evaluation of radiation therapy for bone metastases: pain relief and quality of life. *Am J Roentgenol* 1977; **129:** 1095-6.

41. Hendrickson FR, Shehata WM, Kirchner AB. Radiation therapy for osseous metastasis. *Int J Radiat Oncol Biol Phys* 1976; **1:** 275-8.

42. Poulsen HS, Nielsen OS, Klee M, Rort M. Palliative irradiation of bone metastases. *Cancer Treat Rev* 1989; **16:** 41-8.

43. Perez CA, Cosmatos D, Carcia DM, Eisbruch AE, Poulter CA. Irradiation in relapsing carcinoma of the prostate. *Cancer* 1993; **71:** 1110-22.

44. Hübner RH, Bohuslavizki KH, Brenner W, Klutmann S, Feyerabend B, Lüttges J, et al. Radioprotection of salivary glands by amifostine in high-dose radioiodine therapy investigated in a new rabbit animal model. *Radiol Oncol* 1997; **31(3):** 279-85.

45. Rasey JS. Amifostine. *Cancer Biotherapy & Radiopharmaceuticals* 1999; **14:** 331-5.

46. Peters GJ, van der Vijgh WJF. Protection of normal tissue from cytotoxic effects of chemotherapy radiation by, amifostine: preclinical experiences. *Eur J Cancer* 1995; **31A:** 1-7.

47. Wasserman TH, Phillips TL, Ross G, Kane LJ. Differential protection against cytotoxic chemotherapeutic effects on bone marrow CFUs by WR-2721. *Cancer Clin Trials* 1981; **4:** 3-6.

48. Shaw LM, Glover DJ, Turrisi A, Brown DQ, Bonner HS, Norfleet AL, et al. Pharmacokinetics of WR-2721. *Pharmac Ther* 1988; **39:** 195-201.