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Radioprotection of salivary glands by amifostine in high-dose radioiodine therapy investigated in a new rabbit animal model

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Salivary gland damage following high-dose radioiodine treatment (HD-RIT) is a well known side effect. Since differentiated thyroid cancer (DTC) has a very good prognosis, the reduction of long-term side effect is of major interest. Therefore, the radioprotective effect of amifostine was investigated in a rabbit animal model. Quantitative salivary gland scintigraphy was performed on 5 rabbits prior to and up to 3 months after HD-RIT applying 1 GBq I-131. The uptake of Tc-99m-pertechnetate was calculated as a measure of parenchymal function. Three animals received 200 mg/kg amifostine prior to HD-RIT, and two served as controls. Salivary glands were examined histopathologically. In two control rabbits HD-RIT significantly ($p<0.001$) reduced pertechnetate uptake by 63 % and 46 % in parotid and submandibular glands, respectively, and lipomatosis was found histopathologically. In contrast, in three rabbits treated with amifostine parenchymal function was not decreased significantly ($p = 0.953$), and lipomatosis was negligible. In conclusion, salivary gland impairment induced by HD-RIT can be evaluated quantitatively by salivary gland scintigraphy in rabbits, and amifostine significantly reduced salivary gland damage induced by HD-RIT. These encouraging results need further evaluation in patients since it may help to increase the quality of life of patients with diffentiated thyroid cancer.

Key words: Salivary glands – radiation effects; radiation-protective agents; – amifostine; radiotherapy-adverse effects; rabbits

Introduction

A standard therapy in differentiated thyroid cancer requires a total thyroidectomy and a high-dose radioiodine therapy in order to completely ablate thyroid remnants.[1] Apart from thyroid tissue the β-emitting iodine isotope I-131 used for radioiodine therapy is accumulated actively by an ATP dependent $Na^+/K^+/2Cl^-$-cotransport due to its similar atomic diameter and its comparable electric charge.[2-6] This causes an undesired accumulation of I-131 in parietal cells of the stomach as well as in acinar cells of salivary glands.[6-9] Consequently, well recognized side effects of high-dose radioiodine therapy are transient gastritis and long-lasting xerostomia.[10-16] Therefore, a radioiodine therapy is performed under salivary gland stimulation in order to decrease the impairment of salivary gland function.[17-23] However, even under salivary gland stimulating conditions, a parenchymal damage could be shown after high-dose radiodiodine therapy using quantitative salivary gland scintigraphy.[13, 24-27] Since

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differentiated thyroid cancer has very good prognosis, reduction of long-term side effects following high-dose radioiodine therapy is important for the patients' quality of life.[1]

In the last few years various reports dealt with radioprotective effects of amifostine,[28–33] a phosphorylated aminothiol chemically described as S-2-[3-aminopropylamino]-ethylphosphorotioic acid (Figure 1). Since amifostine accumulates markedly in salivary glands,[34] it has been used successfully in external radiotherapy in patients with head and neck tumors in order to prevent xerostomia.[35–40]

$$H_2N–CH_2–CH_2–CH_2–NH–CH_2–CH_2–S–PO(OH)_2$$
$$\downarrow \text{[Alkaline Phosphatase]}$$
$$H_2N–CH_2–CH_2–CH_2–NH–CH_2–CH_2–SH$$

Figure 1. Chemical structure of amifostin (above) and its active metabolite WR–1065 (below).

Therefore, it looked worthwhile to transfer the radioprotection of salivary glands by amifostine to high-dose radioiodine therapy in order to prevent patients from xerostomia, and, thus, to increase the tolerance of high-dose radioiodine therapy.

As a first step we established a rabbit animal model and report on first results.

Materials and methods

In order to investigate the cytoprotective effect of amifostine an animal model was established. Five male New Zealand white rabbits aged three months, weighing 2.5±0.1 kg, were treated with 1 GBq I-131 intravenously in order to ablate the thyroid and to destruct salivary gland parenchyma. Prior to the application of radioiodine all animals received 4 mg Dexamethason (Fortecortin®, Merck, Darmstadt) and 0.5 mg Tropisetron (Navoban®, Sandoz, Nürnberg) as antiemetic treatment. In addition, three out of five rabbits received 200 mg/kg amifostine (Ethyol®, Essex, München), and two rabbits served as controls, receiving physiological saline solution.

To quantify parenchymal function, salivary gland scintigraphy was performed prior to as well as four weeks, eight weeks and twelve weeks after the application of I-131. Rabbits were put in prone position directly onto a low energy high resolution collimator of a large field – of – view gamma camera (Bodyscan, Siemens, Erlangen). After injection of 100–140 MBq Tc-99m-pertechnetate sequential images of one minute each were acquired up to 25 minutes. Images were stored digitally in a 256 × 256 matrix. For quantification one rectangular background ROI was positioned caudally to the left parotid gland, and five oval ROIs were drawn over both parotid and submanidbular glands and the thyroid gland, respectively. ROIs were copied from the study performed prior to radioiodine treatment to the studies obtained after radioiodine treatment. As a measure for parenchymal function the uptake of Tc-99m-pertechnetate was calculated in percent of the injected activity. For compensation of noise and, thus for stabilisation of data, uptake was averaged from 21.–23. minute post injection. Whole body distribution of Tc-99m-pertechnetate in a rabbit is shown in Figure 2A and ROIs used for quantification are depicted in Figure 2B.

Twelve weeks after radioiodine therapy all animals were sacrificed to remove salivary glands for histopathological examination. Salivary glands were stained with Hematoxilin/Eosin in conventional manner.

Animal studies were approved by the local government (XI 330a 72241.11-17).

Data are given as mean ± one standard deviation. Two-tailed U-test according to Wilcoxon, Mann and Whitney was used to evaluate statistical differences between animal subsets.[41] For $p<0.05$ data were considered to be statistically significant.

Results

Controls

Details of Tc-99m-pertechnetate uptake in salivary glands of controls and amifostine rabbits are given in Table 1. Salivary gland scintigrams of a control rabbit are given in Figure 3 (upper row). In controls thyroid uptake declined to almost zero as early as four weeks after radioiodine treatment, thus documenting a thyroid ablative dose of radioiodine. In parallel, parenchymal function of salivary glands decreased. Twelve weeks after the injection of I-131 Tc-99m-pertechnetate uptake was reduced by 63 % and 46 % in parotid and submandibular glands, respectively, (Figure 4, open symbols).

Amifostine group

Rabbits treated with amifostine exhibited complete ablation of the thyroid four weeks after the application of I-131 as well. This is shown in Figure 3 (lower row). In contrast, in these animals parenchy-

Table 1. Uptake of Tc-99m-pertechnetate in percent of injected activity prior to, 4, 8, and 12 weeks after the application of 1 GBq Iod–131 in control rabbits and in rabbits treated with amifostine 200 mg/kg body weight. Numbers represent mean of right and left parotid and submandibular glands, respectively.

	Controls		Amifostine	
	Parotid glands	Submandibular glands	Parotid glands	Submandibular glands
prior to I-131	0.226 ± 0.042	0.295 ± 0.070	0.241 ± 0.030	0.230 ± 0.074
4 weeks after	0.140 ± 0.018	0.199 ± 0.046	0.215 ± 0.038	0.215 ± 0.060
8 weeks after	0.106 ± 0.019	0.187 ± 0.067	0.209 ± 0.032	0.210 ± 0.065
12 weeks after	0.080 ± 0.011	0.154 ± 0.057	0.208 ± 0.023	0.212 ± 0.057

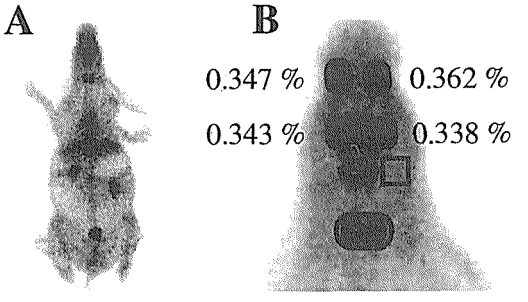

Figure 2. Whole body distribution of Tc-99m-pertechnetate (A) and the magnification of the head (B) visualizing the ROIs used for quantification. Numbers represent uptake of Tc-99m-pertechnetate in percent of the injected activity in parotid, submandibular glands, and thyroid gland, respectively.

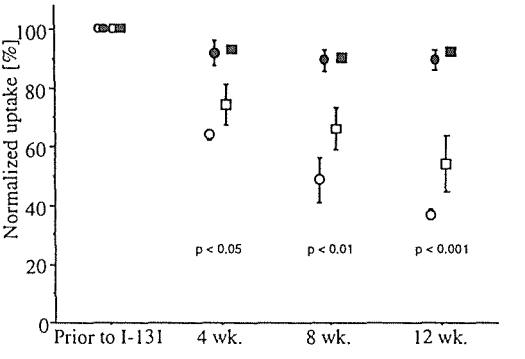

Figure 4. Normalized uptake of Tc-99m-pertechnetate in parotid (circles) and submandibular (squares) glands of control rabbits (open symbols) and of trabbits treated with amifostine (filled symbols) prior to, 4, 8 and 12 weeks after application of 1 GBq I-131.

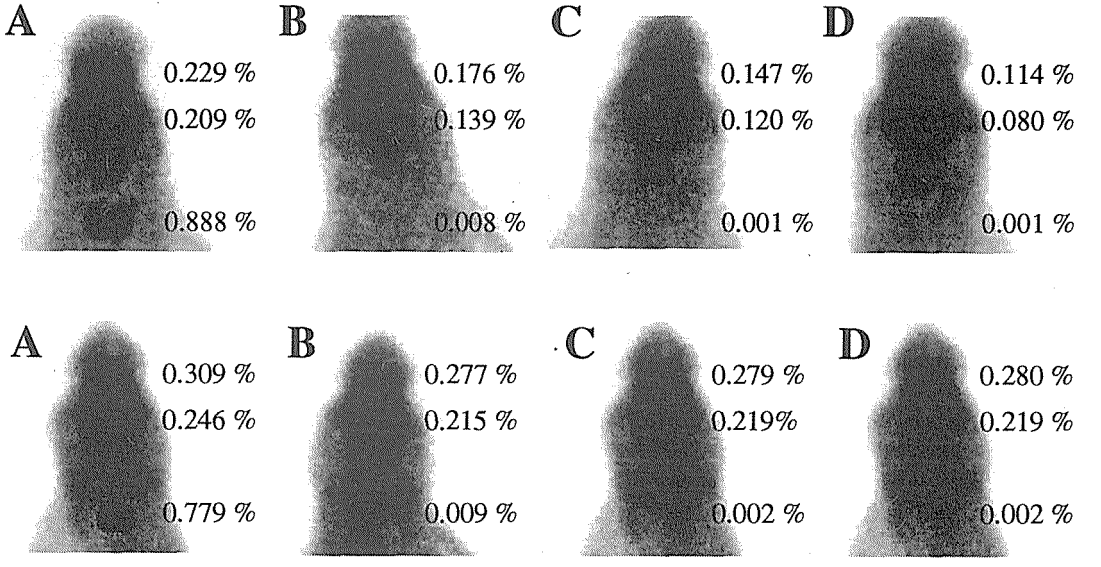

Figure 3. Salivary gland scintigraphy in the control group (upper row) and in the amifostine group (lower row) prior to (A), 4 (B), 8 (C), and 12 weeks (D) after the application of 1 GBq I-131. Numbers represent uptake of Tc-99m-pertechnetate in percent of the injected activity in parotid, submandibular glands, and thyroid gland, respectively.

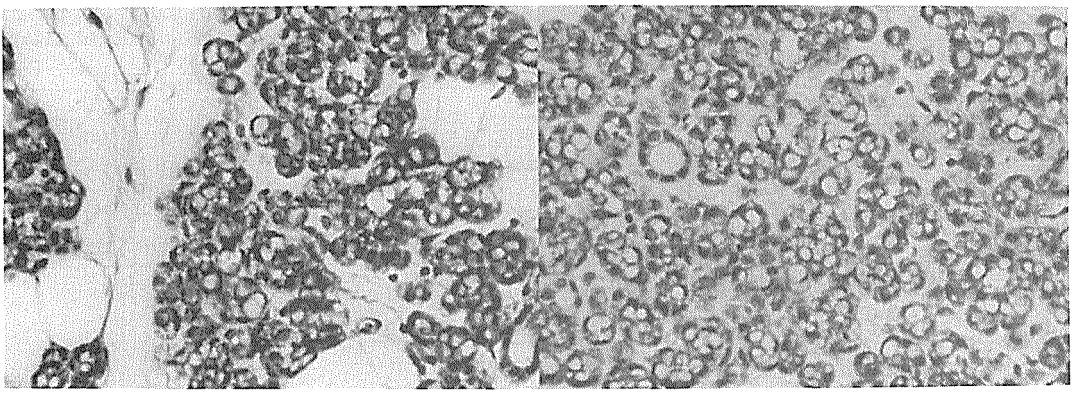

Figure 5. Hematoxilin/Eosin-stained slices of parotid glands of the control group (left), and of the amifostine group (right) twelve weeks after the application of 1 GBq I-131. Note a significantly more pronounced lipomatosis in the control animal. Magnification: 500 times.

mal function of salivary gland was decreased not significantly (p=0.953) by only 10 % and 7 % in parotid and submandibular glands, respectively (Figure 4, filled symbols).

Histopathology

Results of histopathological examinations are given in Figure 5. Salivary glands of control rabbits exhibited a marked lipomatosis as a typical sign of radiogenic damage, but no signs of inflammation (Figure 5, left), whereas lipomatosis was much less pronounced in animals treated with amifostine (Figure 5, right).

Discussion

Quantification of salivary gland function

Salivary gland scintigraphy performed in a standardized method as previously described[42, 43] facilitates the quantitative evaluation of salivary gland parenchymal function. It is characterized both by an excellent intraindividual observer variability and reproducibility which enables the detection of changes in parenchymal function in the range of about as less as 5–10 %.[26, 27] This enabled both the early detection of beginning Sjögrens syndrome by salivary gland scintigraphy as compared to other imaging modalities[44] and the detection of parenchymal impairment of salivary glands following low-dose radioiodine therapy.[26, 27] Therefore, quantitative salivary gland scintigraphy proved to be a suitable imaging modality for quantitative evaluation of salivary gland function.

Amifostine

Amifostine was originally developped as a radioprotective agent as a part of the Anti-Radiation Drug Development Program initiated by the United States Army at the Walter Reed Army Institute of Research (Washington) in the early 1950s.[29] Since numerous preclinical studies in cell culture and animal models showed that, following dephosphorilation to its active metabolite WR-1065, amifostine selectively protected normal tissue from damaging effects of irradiation, several clinical studies were initiated confirming its radioprotective potency in various publications.[35–40, 45] This resulted in an approval of amifostine in Germany in 1995 for the supportive therapy of patients with ovarian cancer being treated with cisplatin derivatives in order to minimize myelotoxic side-effects of cisplatin.[30]

The selective radioprotection of normal tissue by amifostine as compared to tumor tissue is mainly caused by two effects. First, amifostine is accumulated much more in normal tissue, and, second, the alkaline phosphatase necessary for dephosphorilation of amifostine and thereby activating amifostine is more active in the alkaline environment of normal tissue than in the acid tumoral tissue.[29, 46–48]

Since amifostin is known to be accumulated extensively in salivary glands[29, 34, 48–51] it seemed reasonable to use amifostine as protecting agent in patients with head and neck tumors receiving external radiation therapy. Takahashi and coworkers[37] studied Ga-67 uptake as an indicator for irradiation-induced damage in salivary glands of patients with head and neck cancer and showed that pretreatment with amifostine resulted in a significantly increased number of Ga-67 negative salivary glands following irradiation. Some studies have been undertaken so far in

head and neck tumors yielding very promising results concerning the reduction of radiation induced salivary gland damage.[35-40]

Since radiation effects of external radiation and radioiodine therapy are in general caused by the same mechanisms,[10] i.e. the production of free radicals, it seemed promising to transfer the radioprotective effect on salivary glands by amifostine to high-dose radioiodine therapy.

Animal studies

In this study 1 GBq I-131 was applied for complete ablation of the thyroid and for concomittant parenchymal impairment of salivary glands. In fact, the activity applied caused a complete thyroid ablation in the animals of the control group as well as in animals treated with amifostine as early as four weeks after application of I-131. Thus, amifostine did not protect thyroid tissue from I-131. This is in accordance with the observation that amifostine is accumulated to an only marginal amount in the thyroid of several specimen.[47, 52-56] This observation yields the prerequisite for the application in differentiated thyroid cancer since protection of thyroid tissue or metastases of differentiated thyroid cancer has to be excluted.

In our animal studies we could show a clear radioprotective effect of amifostine in salivary glands of rabbits treated with-dose radioiodine. This was demonstrated scintigraphically by a significantly reduced parenchymal impairment of salivary glands after pretreatment with 200 mg/kg amifostine. Moreover, in rabbits treated with amifostine we histologically observed a markedly reduced lipomatosis without evidence of an inflammation. This is in accordance with several papers in which lipomatosis is described as a typical late effect of radioiodine treatment.[57-59] Thus, animal studies showed an encouraging radioprotection of salivary glands by amifostine in high-dose radioiodine therapy.

Conclusion

Parenchymal damage in salivary glands induced by high-dose radioiodine therapy can be evaluated quantitatively by salivary gland scintigraphy in a new rabbit animal model introduced. Amifostine significantly reduced salivary gland damage induced by high-dose-radioiodine therapy. These encouraging results need further evaluation in patients since it may help to increase the quality of life in patients with diffentiated thyroid cancer by avoiding xerostomia.

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