Bone metastases result in significant morbidity and poor prognosis. Radium-223 dichloride (Xyproliet®) is an alpha-emitting calcium mimetic that binds to hydroxypatite in bone and via efficient cellularity provides targeted radiation therapy against bone metastases. Xyproliet received recent FDA approval for the treatment of patients with castration-resistant prostate cancer with symptomatic bone metastases. We have previously reported that radium-223 decreases osteolysis and tumor burden in bone in a mouse model of breast cancer bone metastasis in preventive and micro-metastasis settings (Suominen et al. CIBS meeting 2012), as well as in mice with established bone metastases (Suominen et al. AACR Annual Meeting 2011).

Here, we investigated the effects of radium-223 dichloride monotherapy compared to and in combination with either doxorubicin (dox) or zoledronic acid (za) on survival in a mouse model of established breast cancer bone metastases.

Introduction

Materials and Methods

Radiography

Biochemical markers of bone turnover

Figure 3. Rad-223 dichloride improved survival in mice and in combination with zoledronate or doxorubicin. Survival of MDA-MB-231 tumor-bearing Balb/c mice treated with monotherapy or combination therapy with Rad-223 dichloride (223Ra; 300 kBq/mouse), doxorubicin (223Ra; 300 mg/kg, ip) and/or zoledronate (223Ra; 300 mg/kg, ip) for 3 weeks after tumor cell injection. Survival was measured from day 0 to day 28. 

A) Survival of MDA-MB-231 tumor-bearing Balb/c mice treated with (A) Rad-223 dichloride (223Ra; 300 kBq/mouse), (B) doxorubicin (223Ra; 300 mg/kg, ip), (C) zoledronate (223Ra; 300 mg/kg, ip) or (D) combination therapy with Rad-223 dichloride (223Ra; 300 kBq/mouse) and/or doxorubicin (223Ra; 300 mg/kg, ip) for 3 weeks after tumor cell injection. Survival was measured from day 0 to day 28. 

B) Survival of MDA-MB-231 tumor-bearing Balb/c mice treated with monotherapy or combination therapy with Rad-223 dichloride (223Ra; 300 kBq/mouse) and/or doxorubicin (223Ra; 300 mg/kg, ip) for 3 weeks after tumor cell injection. Survival was measured from day 0 to day 28.

Figure 4. Relative changes in serum bone marker values in mice after treating different days (1-21). A) Rad-223 treatment in serum TRACP 5b activity and B) Relative changes in serum PINP concentration.

A) Relative changes in serum TRACP 5b activity in different groups (Mean ± SE) and B) Relative changes in serum PINP concentration in different groups (Mean ± SE).

Figure 5. Relative changes from day 14 to day 21 in bone marker values in mice. At 28 days, change in serum TRACP 5b activity and PINP concentration was determined in tumor-bearing Balb/c mice treated with Rad-223, doxorubicin or both. Mice were sacrificed after 14 days and again after 21 days of treatment. The data were analyzed using ANOVA (Mean ± SE). (A) The percentage change was calculated using day 14 to day 21 relative to day 14 where 14 is set as 100% in each group, and (B) compared to Rad-223 group and (C) compared to doxorubicin group. *P<0.05, **P<0.01 and *** P<0.001.

Figure 6. Rad-223 dichloride induced double-strand breaks in cancer cells in vivo by (A) DAPI staining of the tumor metastatic lesions from the metastasized breast bone metastatic nude. Left panel shows the vehicle control upper panel shows the Rad-223 staining and lower panel shows an overlay of both. (B) The Rad-223 treated group showed increased numbers of cells labelled as DAPI positive compared to the vehicle control group. 

Figure 7. Double strand breaks in osteoclasts from tumor cells treated with Rad-223. A) Vehicle control (left panel), B) Rad-223 treated (right panel). 

A) Vehicle control (left panel) and B) Rad-223 treated (right panel). 

Acknowledgements

Conclusions

Summary

- Animals treated with radium-223 dichloride (alone or in combination with zoledronic acid) had increased body weight on day 24 compared to vehicle control, doxorubicin or zoledronate alone groups.

- Radium-223 dichloride monotherapy extended time to sacrifice (P=0.039) unlike doxorubicin or zoledronic acid monotherapy which did not improve survival compared to the vehicle group.

- Radium-223 dichloride in combination with zoledronic acid (P=0.004) or doxorubin (P=0.001) extended time to sacrifice in comparison to the vehicle but did not provide additional survival benefit compared to the radium-223 dichloride monotherapy. (Fig. 3, Table 1)

- Treatment with radium-223 dichloride in combination with either zoledronic acid or doxorubicin did not have a negative effect on survival demonstrating that binding of both radium-223 dichloride and zoledronic acid to bone does not undermine treatment efficacy and safety in primarily osteolytic metastases.

- Bone formation as measured by serum PINP was higher in the radium-223 dichloride monotherapy group (P<0.01), lower in the zoledronic acid monotherapy group (P=0.003) and did not differ from control in the radium-223 dichloride and zoledronic acid combination group. (Fig. 6a and 7a)

- Bone resorption as measured by serum TRACP 5b activity was lower in both groups receiving radium-223 monotherapy on day 25. (Fig. 6b and 7b)

- A 3-fold increase in the number of osteoclasts with double-strand breaks in the radium-223 dichloride-treated versus the vehicle control group was observed (P<0.001). This finding supports our previous observations that radium-223 dichloride has an inhibitory effect on both tumor cells and osteoclasts. (Fig. 8 and 9).

Figure 8. Mode of action of Ra-223 dichloride is dual inhibition of vicious cycle in bone metastasis.

References

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A 4-fold increase in the number of osteoclasts with double-strand breaks in the radium-223 dichloride-treated versus the vehicle control group was observed (P<0.001).

Rad-223 dichloride in combination with zoledronic acid or doxorubicin increases survival in established breast cancer bone metastasis mouse model.

Rad-223 dichloride has a dual action targeting both tumor growth and osteolysis, both important players in the destructive vicious cycle of bone metastasis (Figure 10).

Our findings strongly support the development of radium-223 dichloride for the treatment of patients with metastatic breast cancer.