Abstract. Aim: To investigate the in vivo efficacy of a novel polybisphosphonate (ODX) in the treatment of bone metastasis from prostate cancer. Material and methods: A rat prostatic carcinoma model was used. Forty-two rats (21 control, 21 treatment) had induction of bone lesions through injection of AT6.1 cells into the distal medullar cavity of long bones (right femur). At day 21 post injection, radiographs were taken and tumor score (severity of lesions, 0-4) and tumor incidence (score >0) were determined. Treatment started at day 23 and lasted until day 49 (four i.v. administrations of ODX during four weeks). Results: ODX reduced the severity of the lesions compared to the control group. Forty-seven percent of the treated rats had regression of their lesions at the study end, including four rats showing disappearance of the lesions i.e. score 0. Osteocondensation at the growth plate was only observed in the treatment group, indicating osteoclast inhibition. Conclusion: In spite of a relatively short treatment period with only four administrations, ODX showed significant efficacy (p=0.0023), with inhibition of tumor progression and osteolysis. The results are encouraging, confirming previous in vitro studies. Clinical research is pending on patients with bone metastasis from castration-resistant prostate cancer.

The vast majority of patients with advanced stage prostate cancer (castration-resistant prostate cancer, CRPC) develop metastasis to the skeleton. Several circumstances make the bone microenvironment favorable for metastatic growth e.g. abundant vasculature with high permeability surrounding the bone marrow and constant remodelling of bone, releasing growth factors that promote tumor growth. Stephen Paget’s seed and soil hypothesis continues to be appropriate (1, 2). The skeletal lesions from prostate cancer are predominantly osteoblastic but also contain osteolytic elements. The consequences of the lesions are well known, with extensive morbidity, such as pain, fractures, nerve root compression due to collapsing vertebra, hypercalcemia, and eventually death.

The comprehension of the interaction between bone cells and tumor cells is critical for the success of the development of new therapies aiming to stop the vicious cycle of progressive bone destruction and tumor growth (3).

Bisphosphonates are standard drugs in the management of patients with CRPC, delaying skeletal-related events (i.e. fractures), improving bone status (4) and alleviating pain associated with the bone lesions (5). In addition, bisphosphonates apparently have direct antitumor effects and interact indirectly through the inhibition of osteoclasts, reducing expression of bone cell-derived growth factors (6-8). The polybisphosphonate ODX described in this report has demonstrated superior antitumor efficacy in comparison to the class leader, zoledronic acid (9).

Most drug development starts in vitro, continues in vivo (animals: toxicology, pharmacokinetics, efficacy), and possibly persists to clinical research. Data obtained from animal models, together with other pre-clinical observations, select drug candidates qualified to progress to clinical research. Although there are no perfect animal models, there seems to be a certain correlation between results in animals and subsequent findings in clinical research (10).

Regarding models for bone metastasis, spontaneous bone metastasis in animals is rare and consequently the majority of experimental models in rodents needs direct injection/implantation of tumor cells into orthotropic locations (11).

In the present investigation, a new model of osteoblastic lesions was used. The lesions were induced by injection of AT6-1 rat prostate adenocarcinoma cells in the femoral medullar cavity of immunocompetent rats. This model should mimic the development of osteoblastic lesions in CRPC (12, 13).
Materials and Methods

Reagents. Dextran 40 PhEur (Pharmacosmos AS, Denmark) was used as conjugate backbone. Sodium meta-periodate (Merck AG, Darmstadt Germany) was used for dextran oxidation (activation). Aminoguanidine and alendronate (Sigma-Aldrich, Stockholm Sweden) were used for the conjugation. Sodium borohydride (Sigma-Aldrich) was used for reductive amination. PD-10 disposable Sephadex G-25 columns were used for separation and purification (GE Bioscience AB, Uppsala, Sweden). Super Acrodisc 25 syringe filters 0.2 μm from Gelman, Sciences (Ann Arbor, MI, USA) were used for sterile filtration.

ODX synthesis. Dextran activation and coupling was carried out as described previously (9). Briefly, 30 mg of activated dextran in 1 ml of 50 mM sodium phosphate buffer at pH 7.5 was mixed with 20 mg alendronate and incubated for 60 minutes. Finally, 80 mg aminoguanidine was added and the incubation continued for an additional 3 hours. After a total of 4 hours incubation, 2 mg of sodium borohydride was added and the mixture incubated for 30 min. The ODX solution was purified on a PD-10 Sephadex G-25 column and eluted with 0.5 M NaCl. Finally, the ODX solution was sterilized by filtration through a 0.2 μm filter.

Determination of conjugation yield. The conjugation yield, i.e. the number of alendronate and guanidine groups coupled to the dextran backbone, was determined by elemental analysis of nitrogen and phosphorus (4 nitrogen=1 aminoguanidine, 2 phosphorus=1 alendronate; Mikrochem AB, Uppsala, Sweden).

Animal model. Four-week-old male Sprague Dawley rats (Elevage Janvier, Le Genest St Isle, France) were housed under pathogen-free conditions at the Experimental Therapy Unit (Medicine Faculty of Nantes, France) in accordance with the institutional guidelines of the French Ethical Committee and under the supervision of authorized investigators. The study was approved by the local Ethics Committee (Comité d’éthique en expérimentation animale des Pays de la Loire).

Animals were anesthetized with isoflurane (2.5% in air atmosphere, at a flow rate of 0.4 l/min) associated with subcutaneous injection of buprenorphine (0.05 mg/kg), and the distal femoral medullary cavity was injected with 50 μl of alginate solution (1.2%, right femur) containing 6x10⁶ rat osteosarcoma cells (AT6.1) after arthrotomy.

To determine the effect of ODX, groups of 21 rats were assigned respectively as controls (untreated) or ODX (2.5 mg/kg intravenous once a week for 4 weeks until the end of the protocol). Before the beginning of the treatment (when first signs of bone metastasis were observed by radiographical analysis), animals were distributed between the two groups (group 1: control group; group 2: treatment group) to ensure similar levels of pathology in each group. Twice a week, the animals were weighed and clinical observations were made. Consequently, when animal showed signs of morbidity, which included cachexia or respiratory distress, during protocol, they were sacrificed by CO₂ inhalation.

Radiographic analysis. To follow-up the development of bone metastases, animals were anesthetized (50 mg/kg Nesdonal; Merial, Lyon, France) and a flat plate radiograph was taken at day -5 (as baseline), at day 21 (before the beginning of the treatment) and at the terminal procedure (day 49, end of the study) with a radiographic device MX20DC12 (Edimex, France).

The degree of lesion severity was evaluated by radiograph scoring between 0 (no bone metastasis) and 4 (severe bone metastasis). The mean score per group is used to demonstrate the severity of the pathologic lesion at the start and at the end of the study.

Statistical analysis. The one-tailed normal distribution approximation of the binomial distribution test was used. Statistics based on the data are treated as ordered classes where each of the five classes is well defined because they are discrete not continuous data. For all experimental animals, the effect over the treatment period is classified as improvement or no improvement with respect to the severity of the lesions. The comparison between the control group and the treatment group is based on the proportion of animals that had an improvement at the end of the study.

Results

Experimental animals that had no sign of bone lesions either at the start or at the end of the treatment period were excluded. Of the remaining 18 animals in the control group, 3 showed an improvement, whereas 9 out of the 19 animals in the treatment group showed improvement. The statistical evaluation showed that the treatment effect was statistically significant (p=0.0023).

Radiographs were obtained before the beginning of the treatment (at day 21 post surgery) and at the end of the study.

The radiographs showed the development of the skeletal lesions. Osteolysis, ectopic ossification and bone deformation, characteristic of bone metastasis from prostate carcinoma, were assessed. In both groups, osteolysis, ectopic ossification and bone deformation were observed. Four animals in the control group showed complete femur deformation, whereas two animals had the same symptoms in the treatment group. In contrast to the control group, the majority of the animals in the treatment group had only small areas with ectopic ossification and osteolysis. Osteocondensation (over bone formation at the growth plate) indicating inhibition of osteolysis was only observed in the treatment group (Figures 1 and 2).

Discussion

An anecdote tells of the pharmaceutical consultant who enthusiastically describes to the seasoned old clinical professor the remarkable effects of a new cancer drug, demonstrated in mice. When the consultant had finished, the professor says "- its all very interesting, however, you see, at this clinic we don’t treat mice". This might illustrate the dilemma, in which animal studies are necessary in the process of development of new drugs, but that the data obtained should be interpreted and extrapolated to humans with great caution.

There are many studies, both in vitro and in vivo demonstrating positive antitumor effects of bisphosphonates (14-17). Very high doses and frequent administrations were
often used and consequently, the clinical relevance has been debated (18).

In spite of these reservations, during the last decade, clinical evidence for antitumor efficacy of bisphosphonates has been accumulating from clinical studies conducted with a large numbers of patients (19-22). The mechanism of bisphosphonate inhibition of osteoclasts is well understood, while the apparent direct anticancer effect remains to be explained. Most probably, the gross effect is a mix of indirect reduction of growth stimulation and direct toxic effects (apoptosis, cytotoxicity). However, at present, all available therapies are palliative and even though current bisphosphonates can curb the proliferation of bone metastasis, eventual progression is inevitable. Forthcoming results from on-going studies will increase knowledge of the adjuvant use of bisphosphonates and further positive results will make changes in clinical practice that will improve outcomes for these patients. Furthermore, inspiration to develop new agents that target constituents of the bone tumor cell microenvironment is stimulated.

ODX is a novel polybisphosphonate, a polymer construct. The fact that it is a polymer facilitates the possibility of incorporating several desirable qualities. The bisphosphonate moiety contributes to the affinity to remodelling bone and induction of apoptosis, while the guanidine group supplies cytotoxicity and efficient cell internalization (9, 22). The antitumor effects of ODX are triggered at sub-micromolar concentrations in contrast to zoledronic acid (used in comparison). This might be of importance in the clinical setting, considering that the achievable concentration is limited by physiological factors and by dose-dependent nephrotoxicity.

Figure 1. Radiographs showing representative lesions from the control group and the ODX-treated group (A), and metastasis incidence at day 21 (B, before treatment) and at the end of the study (C). White arrows, osteolysis; dotted arrows, ectopic ossification; asterisks, osteocondensation.
With hindsight, it might have been desirable to have extended the treatment time in the present study. It seems probable that the difference between control and treatment groups would have been more pronounced with longer treatment duration. Nevertheless, with four i.v. injections during four weeks of a moderately high concentration of ODX, almost 50% of the animals exhibited regression of the skeletal lesions and indications of osteolysis inhibition were observed.

The investigation of ODX will now proceed to a clinical phase 1/2 study on patients with bone metastasis from CRPC.

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References


Figure 2. Radiological score from individual animals of the control group (A) and ODX-treated group (B) at day 21 (before treatment start) and at the end of the study and the mean radiological score at day 21 (C) and at the end of the study (D).


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