In vivo study of serum cancer biomarkers and pain-induced bone metastasis of human prostate tumor in Nude rats

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C. MIGNARD[a], O. DUCHAMP[a], F. BICHAT[a], S. GREGOIRE[b, c], Y. DARBAKY[b], D. ARDID[c]

[a] Oncodesign, 21076 Dijon, France [b] ANS Biotech, 63000 Clermont-Ferrand, France [c] INSERM U766, 63000 Clermont-Ferrand, France

Introduction

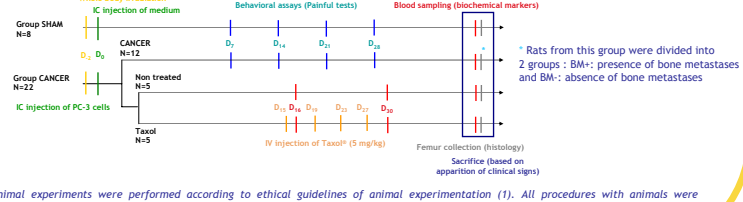
Bone is one of the most frequent sites of spread for many cancers such as breast, prostate, lung or kidney.
Management of metastatic bone disease and associated pain can control the symptoms and prevent further complications such as the decrease of quality of life, pathological fracture or compression of the spinal cord.
Biomarkers of bone turnover, which reflect both formation and resorption of bone, can be used as indicators of bone metastasis in patient and provide valuable insight into the effects of therapy on the dynamic process.
Until recently, investigation of novel drugs in the treatment of cancer-induced bone pain was hampered by a lack of suitable models.
The aim of this study was to characterize biochemical markers and behavioral pain responses in an experimental animal model of bone metastases induced by intracardiac injection of PC-3 human prostate cancer cells in Nude rats.

Material and Methods

- Nociceptive responses in healthy Sprague-Dawley and *Nude* rats
  - Animals : Male Sprague-Dawley (SD) and RH-rnu/rnu rats (125-150 g; n = 8)
  - Weekly session of painful tests as described below:

Painful test	Type of pain evoked	Schedule
Electronic von Frey Test	Mechanical sensitivity	One session *per* week for 4 consecutive weeks (D7, D14, D21 and D28)
Paw Pressure Test	Mechanical sensitivity	
Plantar Test	Thermal (hot) sensitivity	
Acetone Test	Thermal (cold) sensitivity	

- Pharmacological response to an analgesic drug
  - Single SC injection of 3 mg/kg morphine
  - Paw Pressure Test : 0, 15, 30, 60, 90 and 120 min after morphine injection
- PC-3 bone metastases monitoring with nociceptive tests and serum biomarkers
  - Animals : Male RH-rnu/rnu rats (125-150 g)
  - Intracardiac injection of human prostate PC-3 tumor cells in whole body irradiated *Nude* rats
  - Repeated IV injections of Taxol® at 5 mg/kg (Q4Dx4)
  - Weekly body weight measurements and paw pressure test
  - Collection of blood samples for serum biomarker detection : CTX-1 and TRACP-5b for bone resorption and PINP for bone formation
  - Termination of tumor bearing rats when clinical signs such as hind limb paralysis or severe body weight loss appeared
  - Femur collection for histological analysis
  - Study design:



* Rats from this group were divided into 2 groups : BM+: presence of bone metastases and BM-: absence of bone metastases

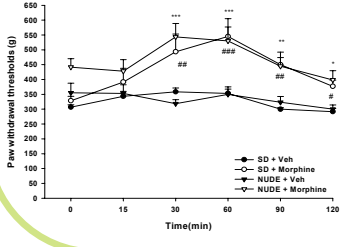
Animal experiments were performed according to ethical guidelines of animal experimentation (1). All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon, France).
(1). Principe d'éthique de l'expérimentation animale. Directive n°86/609 CEE du 24 Nov. 1986, Décrêt n°87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988.

Nociceptive responses in healthy SD and *Nude* rats

Mechanical and thermal sensitivity in *Nude* and Sprague-Dawley rats for four sessions (one session *per* week). Data were analyzed using a two way repeated measures followed by student Newman Keuls test for comparisons. *p<0.05, **p<0.001, ***p<0.001.

Tests	Type of pain evoked	Sessions	Results (seconds and grams)		Significativity
			SD	Nude	
Electronic von Frey Test	Mechanical sensitivity	D7	54 ± 1	51.9 ± 1.6	ns
		D14	60 ± 2.4	56.1 ± 1.9	ns
		D21	59.3 ± 1.1	55.1 ± 1.5	ns
		D28	69.1 ± 2.7	55.9 ± 1.3	p<0.001 ***
Paw Pressure Test	Mechanical sensitivity	D7	345.0 ± 11.6	332.5 ± 10.8	ns
		D14	365.0 ± 13.1	355.8 ± 19.6	ns
		D21	335.8 ± 12.8	375.0 ± 14.7	ns
		D28	454.2 ± 33.2	325.8 ± 15.7	p<0.001 ***
Plantar Test	Thermal (hot) sensitivity	D7	7.4 ± 0.4	8.3 ± 0.8	ns
		D14	7.2 ± 0.6	10 ± 0.5	p=0.002 **
		D21	7.9 ± 0.4	9.0 ± 0.7	ns
		D28	8.1 ± 0.4	9.2 ± 0.3	ns
Acetone Test	Thermal (cold) sensitivity	D7	17.6 ± 0.8	17.4 ± 0.9	ns
		D14	17.3 ± 0.8	17.1 ± 0.6	
		D21	18.2 ± 0.5	16.0 ± 1.0	
		D28	17.9 ± 1.0	16.5 ± 0.6	

Morphine treatment resulted in an increase of mechanical thresholds in both strains. Data were analyzed using a two way repeated measures followed by a Bonferroni test for comparisons *versus* the vehicule SD group: ##p<0.01, ###p<0.001 and Nude group: *p<0.05, **p<0.01, ***p<0.001.



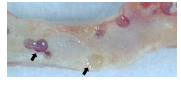
Nociceptive tests in healthy SD and *Nude* rats did not demonstrate any major difference of hypersensitivity between both strains. Morphine (3 mg/kg, s.c.) induced a similar and classically observed analgesic effect in both strains.

Conclusions

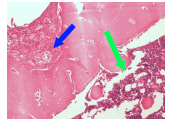
- Intracardiac injection of PC-3 human prostate cancer cells in *Nude* rats associated with bone formation/resorption serum biomarkers monitoring is a useful experimental *in vivo* model for bone metastases study and anti-cancer drug evaluation.
- Surprisingly, pain induced by experimental bone metastases was not demonstrated in this model with classical painful assays.

PC-3 bone metastases monitoring with nociceptive tests and serum biomarkers

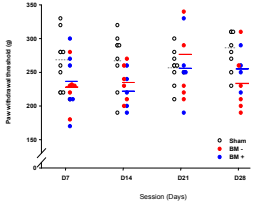
Osteolytic bone metastasis in femur of *Nude* rats after intracardiac injection of PC-3 tumor cells (Take rate = 69%)



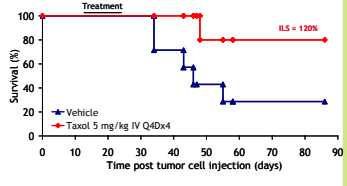
Partial neoplastic envolvement (blue arrow) with persistant hematopoeitic tissue (green arrow) in femur of PC-3 tumor-bearing rats



Presence of bone metastases did not induced any modification of the response in the paw pressure test



Taxol® significantly enhanced survival of PC-3 tumor-bearing rats



Taxol® induced an increase of bone formation marker, PINP (A), and a decrease of bone resorption markers, CTX-1(B) and TRACP 5b (C) in *Nude* rats inoculated with PC-3 tumor cells