CNCSIS PN II – RU PROJECT:

TE_159/ 2010

EVALUATION OF THE ANTIFUNGAL EFFECT OF NANO CONJUGATES OF A NEW PROPICONAZOLE DERIVATIVE WITH BETA-CYCLODEXTRIN

STAGE REPORT 2013

The *in vivo* testing of the antifungical effect of the MXP-4509 nanoconjugates on biofilms on experimental outbred rat models

The *in vivo* antifungal effect was studied on biofilms using the van Wijngaerden et al. (1999) method and performed by subcutaneous implant of catheter fragments previously incubated in a *C. albicans* suspension. The rats were treated with the testing antifungal substance for 6 days, when the catheter fragments were collected and the fungal burden was assessed in comparison with a control set, from untreated animals.

Material and Method

- Wistar rats, females, 200 grams
- *Candida albicans*, SC5314*wild type* strain
- RPMI-1640 medium with 0,9% glucose
- Saline solution 0.9%
- YPD agar in Petri dishes
- Phosphate Buffer Solution (PBS)
- Dexamethasone
- Oxytetracycline hydrosoluble powder
- Multilumen catheters
- Vortex
- Gavage needles

7 days before the implant of the catheters, the rat's immunosuppression was induced by adding oxytetracycline (1g/L) and Dexamethasone (2mg/L) in water. This was added until the animals were sacrificed at the end of the experiment.

Day 0:

- Culture of the SC5314 strain on YPD agar and overnight incubation at 37°C;
- The cutting of the catheters in 1cm long fragments and overnight incubation in bovine serum at 37°C (fig 1)

Day 1:

- *Adhesion phase* (a yeast suspension is made in 1 mL 0,9% saline solution, then is diluted in 1/10 and is adjusted to a turbidity equivalent to 5 x 10^4 CFU/mL in RPMI; the catheter fragments are transferred from serum into

prepared yeast suspension and incubated for 1h and 30 minutes at 37°C (figure 2); after this time period has expired, dilutions are prepared to assess the CFU/ catheter fragment after the adhesion phase: in the first Eppendorf tube containing 1 mL 0.9% saline solution, a catheter fragment is transferred and is mixed for 30 seconds, after that 100 µl are successively transferred into two tubes containing 900 µl 0.9% saline solution each and 100 µl are inoculated on YPD agar, the dispersion is evenly made on the surface of the plate (figure 3).

- *Implantation* (the rat is anesthetised with isoflurane, the lumbar region is shaved and disinfected, an approximately 1 cm long incision – figure 4 - and 3 subcutaneous tunnels are performed where catheter fragments are implanted – figure 5, the cutaneous wound is sutured and disinfected again);
- *Treatment* (the rats are separated in 3 groups with 3 animal each: control group, treated group with MXP-4509 20 mg/kg bw p.o, treated group with MXP-4509 40 mg/kg bw p.o.; the substance was given by gavage, for 7 days).

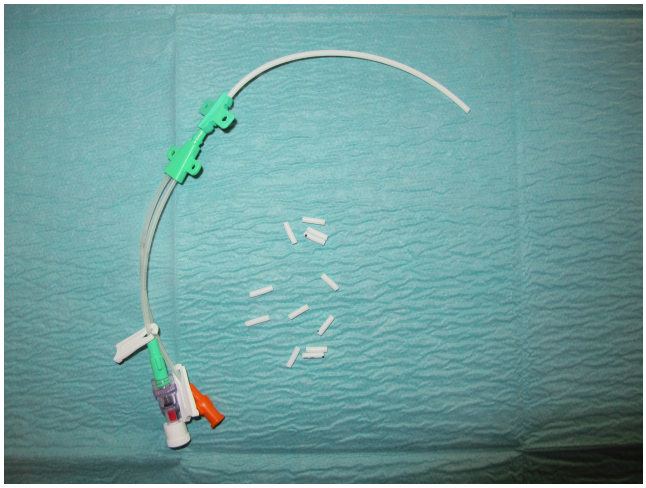

Figure 1. Preparation of the catheter fragments

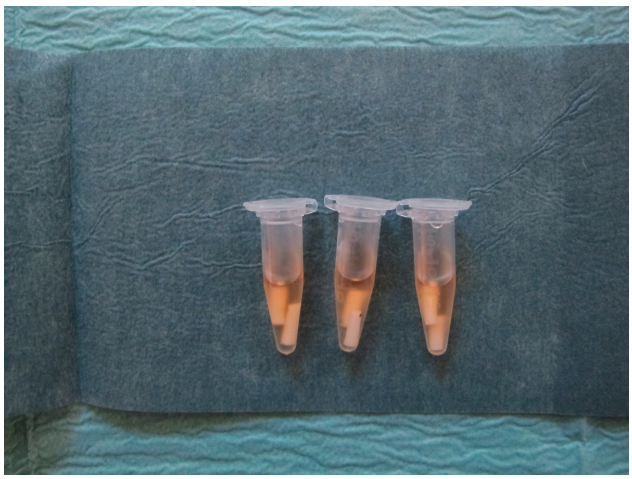

Figure 2. Adhesion phase – incubation of the catheter fragments with yeast suspension in RPMI

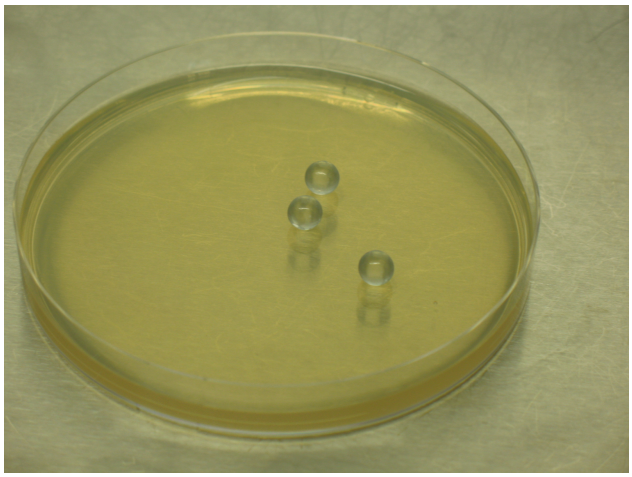

Figure 3. The dispersion of the inoculum on the surface of YPD agar, evenly, to determine the CFU/ catheter fragment

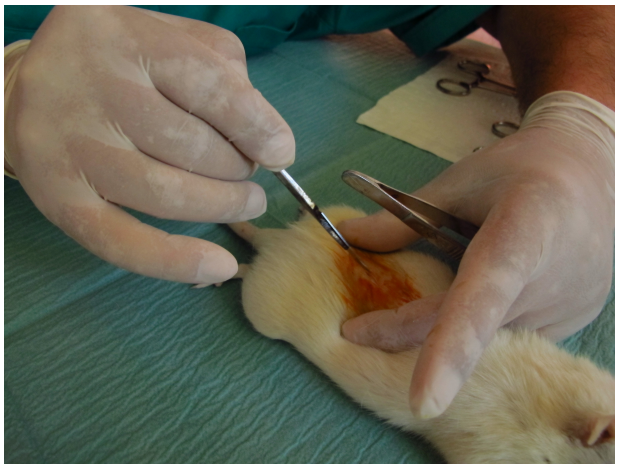

Figure 4. The cutaneous dorso-lumbar incision

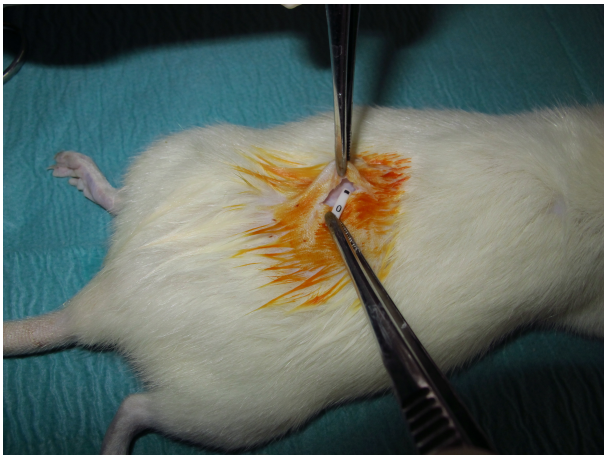

Figure 5. Implantation of the catheter fragments into subcutaneous tunnels made after incision

Day 7:

- The animals are euthanatized, the lumbar area is disinfected, the catheters are removed and placed into 0.9% saline solution;
- The determination of CFU/ catheter fragment is repeated (figure 6 A and B) and the results are compared.

Results

Table 1

Mean fungal burden of the catheter fragments after *in vivo* implantation

Nb. CFU/ control catheter fragment	Nb. CFU/ MXP-4509 20 mg/kg bw catheter fragment	Nb. CFU/ MXP-4509 40 mg/kg bw catheter fragment
1.87×10^4	0.093×10^4	0.054×10^4

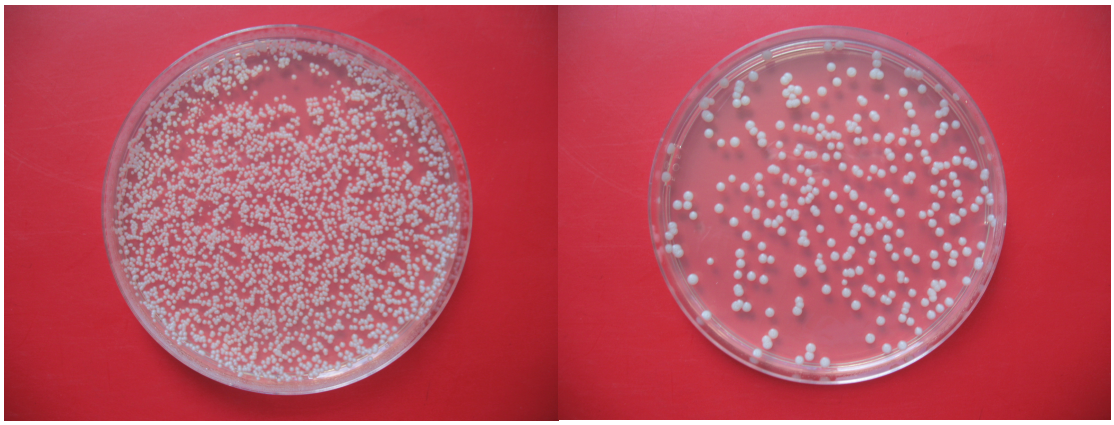

Figure 6: control catheter fragment (A) and catheter fragment from the group treated with antifungal complex 20 mg/kg bw (B)

The mean of yeast cells per catheter fragment (CFU) after 6 days of subcutaneous implantation is presented in table 1. The existent data emphasize a reduction in *Candida albicans* biofilm formation on the fragments implanted in rats treated p.o. with the nanoconjugates of propiconazole derivative substance, in comparison with the one formed on control fragments (to 20.10 times, respectively 34.63 times). This decrease, yet significant, is not sufficient to eradicate the formation of biofilms on catheters, as they were obtained in this experimental model. Furthermore, noteworthy the number of adherent cells to the catheter has increased in comparison to the initial number (assessed at the end of adhesion phase) – from 0.45×10^2 CFU to 0.093×10^4 CFU, respectively to 0.054×10^4 CFU.