434

MICROSPHERE-ENCAPSULATED 4OH-TAMOXIFEN: A NEW SUSTAINED RELEASE DELIVERY SYSTEM WITH ANTITUMOUR ACTIVITY AGAINST DMBA-INDUCED MAMMARY CARCINOMA IN SPRAGUE-DAWLEY RATS

P. Auvray[1], F. Bichat[1], G. Griffon-Etienne[2], A. Bataille[1], N. Guilbaud[1], E. Vuaridel[2], A. Carrio[2], M. Bayssas[2], and P. Genne[1]

[1]ONCODESIGN - 28, Rue de Broglie – B.P. 27627 - 21076 Dijon Cedex, France - [2]DEBIOPHARM - 17, Rue des Terreaux - 1003 Lausanne - Switzerland

Introduction

- ✓ Tamoxifen (TAM), a synthetic non-steroidal anti-estrogenic compound, is considered as the standard treatment of post-menopausal advanced breast cancer
- ✓ 4-hydroxytamoxifen (4OH-TAM), one of its hydroxylated metabolites, may be responsible for a major part of the effects of TAM *in vivo*, with an affinity towards the estrogen receptor (ER) of 10- to 100-fold stronger *(ref.1,2)*
- ✓ Consequently, the use of 4OH-TAM would be of interest in women, but is limited by its inactivation in the liver when administered *per os* (PO) *(ref.3)*

Study aims

- ✓ To investigate the *in vivo* activity of free 4OH-TAM given as repeated daily SC injections for 28 consecutive days in the model 7,12-dimethybenz(a)anthracene (DMBA)-induced rat mammary tumours
- ✓ To investigate the *in vivo* activity of 4OH-TAM encapsulated in biodegradable PLGA microspheres (MS/4OH-TAM, single SC injection) in the model of DMBA-induced rat mammary tumours
- ✓ To compare the antitumour efficacy of free 4OH-TAM and MS/4OH-TAM with TAM in the model of DMBA-induced rat mammary tumours

The DMBA-induced mammary carcinoma model: tumour induction and development

- o Species: female Sprague-Dawley rat, 53-57 days old
  15 rats /group
- o Tumour induction at D0: DMBA (20.0 mg/rat, PO)
  (12.6% of mortality within 3 days after adm.)
- o Randomisation of all rats at D67:
  - % rats bearing tumours : 38-43%
  - Mean No of tumours / rat: 1.0
- o At D103 (control group):
  - % rats bearing tumours: 100%
  - Mean No tumours / rat: 4.2
- o At D151 (control group, end of study):
  - % rats bearing tumours: 100%
  - Mean No tumours / rat: 5.8

Experimental design and treatments

- ✓ Test substance: PLGA MS/4OH-TAM (28.0 mg 4OH-TAM/kg *)
  (Vehicle: water for inj.)
- ✓ Control article: Free 4OH-TAM (0.1, 1.0 and 10.0 mg/kg)
  (Vehicle: absolute ethanol/water (65/35))
- ✓ Reference article: TAM (10.0 mg/kg)
  (Vehicle: water for inj.)
- ✓ Animals: Female Sprague-Dawley (SD) rats, 6-7 weeks-old,
- ✓ Adm. route for MS/4OH-TAM and 4OH-TAM: subcutaneous (SC)
- ✓ Adm. route for TAM: oral administration (PO)

** or 1/10th of 10mg TAM /kg/day X 28 days dose*

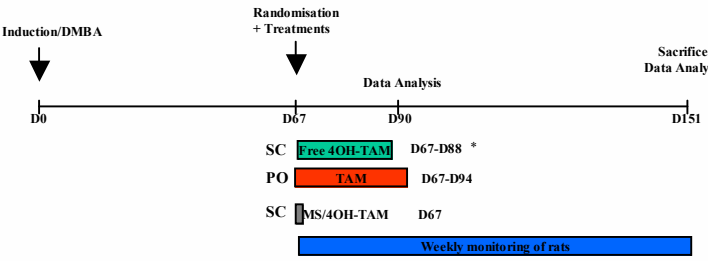
Toxicity results

Summary results of surviving rats and mean body weight change (MBWC)

Groups	Treatments	Dose (mg base 4OH-TAM/kg/adm.)	Total dose adm. (mg base 4OH-TAM/kg)	No. rats alive at D67	MBWC (D67-D90) (g)	MBWC (%)
A	None	-	-	14	+15.7 ± 9.3	+5.3 ± 3.3
B	Vehicle for 4OH-TAM	-	-	13	+7.8 ± 7.6	+2.8 ± 2.6
C	Ovariectomy	-	-	13	+52.9 ± 24.5	+17.1 ± 7.9
D	TAM	10.0	280.0	13	-11.0 ± 13.2	-3.6 ± 4.1
E	4OH-TAM	0.1	2.2	13	-11.8 ± 6.8	-4.0 ± 2.2
F	4OH-TAM	1.0	22.0	13	-15.9 ± 6.9	-5.4 ± 2.3
G	4OH-TAM	10.0	220.0	13	-23.9 ± 11.4	-8.0 ± 3.3
H	MS/4OH-TAM	28.0	28.0	13	-12.7 ± 11.1	-4.0 ± 3.5
I	MS alone	-	-	13	+19.6 ± 11.8	+6.7 ± 4.0

- ✓ No animal died during treatment and post-observation period
- ✓ Free 4OH-TAM induced a significant and dose-dependent body weight loss as compared with control group ($p < 0.001$)
- ✓ MS/4OH-TAM induced a significant body weight loss comparable with that caused by TAM as compared with control and MS alone groups ($p < 0.001$)

Treatment schedule



*treatment had to be stopped at D22, due to local toxicity of the vehicle

Antitumour activity results

Treatment period

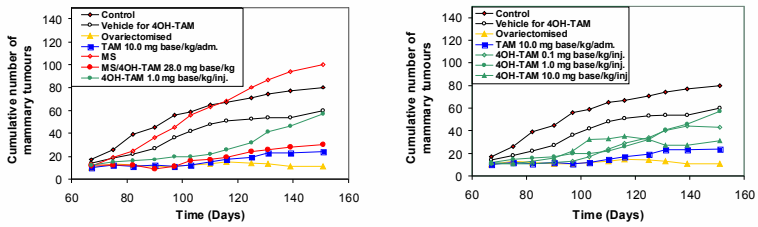
Groups	Treatments	No. regressing tumor* between D67 and D90	Increase of No tumor between D67 and D90 (%)	Increase of tumor bearing rats between D67 and D90 (%)	Increase of tumor volume between D67 and D90 (%)
A	None	4 (9%)	+165	+84	+127
B	Vehicle for 4OH-TAM*	2 (7%)	+80	+35	+152
C	Ovariectomised	8 (73%)	0	-27	+14
D	TAM	4 (33%)	+9	-27	-54
E	4OH-TAM - 0.1 *	5 (42%)	0	0	-31
F	4OH-TAM - 1.0 *	4 (23%)	+42	0	+58
G	4OH-TAM - 10 *	6 (37%)	+45	+42	+181
H	MS/4OH-TAM	6 (67%)	-18	0	+78
I	MS alone	1 (3%)	+260	+142	+67

Treatment and post-treatment period

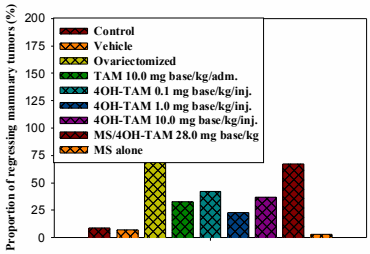
Groups	Treatments	Increase of No tumor between D67 and D151 (%)	Increase of tumor bearing rats between D67 and D151 (%)	Increase of tumor volume between D67 and D151 (%)
A	None	+371	128	+222
B	Vehicle for 4OH-TAM *	+300	+85	+956
C	Ovariectomised	0	+7	+219
D	TAM	+118	+35	+290
E	4OH-TAM – 0.1 *	+258	+100	+399
F	4OH-TAM – 1.0 *	+375	+85	+2197
G	4OH-TAM – 10.0 *	+182	+82	+553
H	MS/4OH-TAM	+173	+82	+656
I	MS alone	+900	+163	+661

* In 4OH-TAM and vehicle treated groups, the treatment was stopped after 22 days (D88 instead of D94 for TAM), due to local toxicity, and therefore we cannot make conclusions on the results.

Cumulative number of DMBA-induced mammary tumour in SD rats



Proportion (%) of regressing DMBA-induced mammary tumours in SD rats between D67 andD90



- ✓ A tumour was considered as regressing when its tumour volume decreased at least 50% between D67 and D90
- ✓ The number of regressing tumors in ovariectomised group was significantly higher than in control group ($p < 0.05$)
- ✓ The number of regressing tumors in MS/4OH-TAM treated-group was significantly higher than in TAM and all free 4OH-TAM treated-groups ($p < 0.05$)

Conclusions

- ✓ A significant antitumor activity was displayed by TAM and MS/4OH-TAM
- ✓ A single SC injection of MS/4OH-TAM displayed an antitumor activity equal/better than that of TAM
- ✓ The number of regressing tumors in MS/4OH-TAM treated-group was significantly higher than in TAM treated-group
- ✓ PLGA/MS may be a promising new class of polymers suitable for 4OH-TAM targeted drug delivery

References

1. Jordan V.C., The Lancet, 2000, 1(1): 43-49.
2. Jordan V.C. and Allen Karen E., Eur. J. Cancer, 1980, 16: 239-251
3. Borgna J.L., Rochefort H., J. Biol. Chem., 1981, 256: 859-868