ANTITUMOR ACTIVITY STUDY OF OXALIPLATIN COMBINED WITH EPINEPHRINE AFTER INTRAPERITONEAL OR INTRATUMORAL ADMINISTRATION ON ADVANCED PERITONEAL CARCINOMATOSIS OR SUBCUTANEOUS TUMOR FROM RAT COLON CANCERS IN BDIX RATS.

P.Genne[1], F. Bichat[1], V.Chiesa[1], G.Griffon Etienne[2], M.Bayssas[2], J.L. Beltramo[3], C. Duvillard[3], [1]Oncodesign, Dijon, France; [2]Debiopharm, Lausanne, Switzerland; [3]Dijon Hospital, Dijon, France.

Abstract

Despite the theoretical advantages of a high local concentration of anticancer drugs, local chemotherapy often fails to produce a complete response. The main reason is the poor diffusion of drugs into tumor mass when injected inside or around tumor tissues. L-OHP has proven to be effective in combination against many tumors in man, without nephrotoxicity. In this work, we determined the antitumor activity, pharmacokinetics (PKs) and biodistribution of single intratumoral (IT) or intraperitoneal (IP) l-OHP injections alone or combined with EP (1 mg/kg), in BDIX rats bearing advanced DHD/K12/PROb peritoneal carcinomatoses or s.c. DHD/K12/PROb tumors.

EP did not modify significantly (p=0.38) the *in vitro* l-OHP cytotoxicity against DHD/K12/PROb cells (IC_{50} l-OHP: 0.16 ± 0.06; IC_{50} l-OHP/EP: 0.19 ± 0.02). L-OHP/EP combination was well tolerated after a single IP injection in healthy Wistar rats. EP greatly reduced the platinum (Pt) peritoneal clearance, inducing a higher local Pt concentration in peritoneal fluid when combined with l-OHP (13 mg/kg) (AUC 5.1 times higher than that obtained with l-OHP alone) with a specific intratumoral accumulation (AUC 4.8 times higher). The Pt PK in plasma was completely modified with a delay [T_{max}: 8.0h (l-OHP/EP) versus 0.083h (l-OHP); C_{max}: 6.79 µg/ml (l-OHP/EP) versus 5.11 (l-OHP)]. A significant increase in the AUC of plasma (2 times higher) correlated with the EP enhancement of the l-OHP antitumor activity (2.65 and 5.30 mg/kg) against advanced peritoneal carcinomatosis (T/C l-OHP:132 and 186%; T/C l-OHP/EP: 164 and 238%). When EP was injected together with l-OHP IT, EP reduced the Pt tumoral clearance (AUC 3.8 times higher), maintaining a high Pt concentration in SC tumor with a significant decrease of the Pt AUC (2 times lower) in plasma and complete tumor regression in the rats.

The EP mechanism of action could be due to its vasoconstrictive effects blocking the clearance of l-OHP from the cavity or tumor into the blood. The results showed a correlation between PKs and tumor efficacy from both IP and IT administrations. The l-OHP/EP combination should be tested as IP chemotherapy of advanced peritoneal metastasis, a common feature of colorectal and ovary cancers for which oxaliplatin showed activity.

Methodology

- **Test substance:** Epinephrine (EP, Fluka, France),
- **Cytotoxic drug:** Oxaliplatin (l-OHP, Debiopharm, Switzerland),
- **Tumor cell line:** DHD/K12/PROb rat colon cancer,
- **Animals:** BDIX rats (Iffa Credo, France),

 # *In vitro* cytotoxic activity of l-OHP associated with 40.0µg/ml EP on DHD/K12/PROb cells:
- Methylene blue assay,
- Three independent experiments each performed in quadruplicate,
- Determination of IC50 (l-OHP concentration which inhibits 50% of cell growth).

 # PK and biodistribution of *Pt* in BDIX rats bearing an advanced peritoneal carcinomatosis after a single IP co-injection of l-OHP/EP:
- IP Injection of 10^6 DHD/K12/PROb cells at D0,
- Treatment start at D20 on advanced carcinomatosis,
- Treatment doses: 1.0 mg base/kg for EP and 6.39 mg base *Pt*/kg for l-OHP,
- Injection volume: 40.0 ml/kg,
- Plasma, IP tumor and organ collection (7 sampling times, 4 rats/time).

 # Antitumor activity of l-OHP/EP combination on BDIX rats bearing an advanced peritoneal carcinomatosis after a single IP co-injection:
- IP Injection of 10^6 DHD/K12/PROb cells at D0 (10 rats/group),
- Treatment start at D10 on advanced carcinomatosis,
- Treatment doses: 1.0 mg base/kg for EP; 2.65 and 5.30 mg base/kg for l-OHP,
- Injection volume: 40.0 ml/kg,
- Monitorin gof body weight and survival rate,
- Determination of treatment toxicity and survival parameters.

 # PK and biodistribution of *Pt* in BDIX rats bearing a SC rat colon tumor after a single IT co-injection of l-OHP/EP:
- SC injection of 10^6 DHD/K12/PROb cells at D0,
- Treatment start at D26 when the mean tumor volumes reach 856 ± 335 mm^3,
- Treatment doses: 1.0 mg base/kg for EP and 6.39 mg base *Pt*/kg for l-OHP,
- Injection volume: 1.0 µl/mm^3 of tumor,
- Plasma and SC tumor collection (7 sampling times, 3 rats/time).

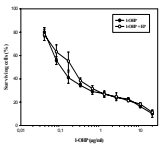
 # Antitumor activity of l-OHP/EP combination against SC DHD/K12/PROb tumors after a single IT co-injection of l-OHP/EP:
- SC injection of 10^6 DHD/K12/PROb cells at D0 (7 rats/group),
- Treatment start at D31 when the mean tumor volumes reach 700 ± 350 mm^3,
- Treatment doses: 1.0 mg base/kg for EP; 2.65 and 5.30 mg base/kg for l-OHP,
- Injection volume: 1.0 µl/mm^3 of tumor,
- Monitoring of body weight, tumor measurement and survival rate,
- Determination of treatment toxicity and tumor growth parameters.

 # Dosage of *Platinum* (*Pt*) in liquids and mineralized samples by Atomic Absorption Spectrometry under GLP validated methods.

 # Determination of PK parameters:
- The area under the Pt concentration-time curve (AUC) was calculated from the sum areas of the individual trapezia using Microgharm software,
- Determination of apparent clearance (ApCl), mean residence time (MRT), experimental *Pt* peak concentration (Cmax) and time to peak (Tmax).

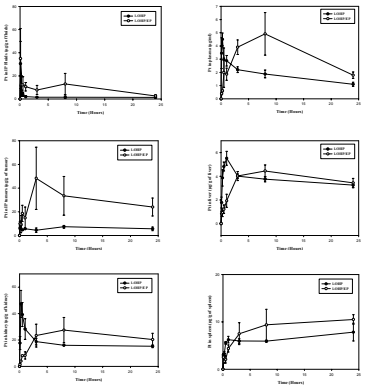
The *in vivo* experiments were performed following the United Kingdom Guidelines for the Welfare of Animals in Experimental Neoplasia (Workman P. *et al.*, Br. J. Cancer, 7, 1-10, 1998.)

Results: *In vitro* cytotoxic activity of l-OHP combined with EP on DHD/K12/PROb rat colon cancer cells



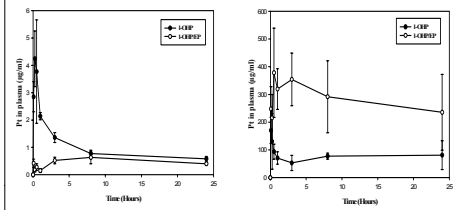
Treatments		l-OHP vs l-OHP + EP	
		l-OHP	l-OHP + EP
IC50 (µg/ml)	Mean	0.16	0.20
	SD	0.06	0.02

Results: PK and biodistribution of Platinum in BDIX rats bearing an advanced peritoneal carcinomatosis after a single IP injection



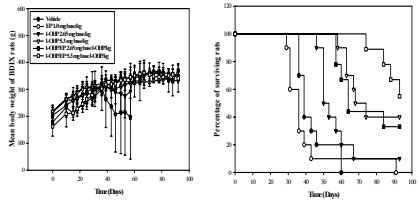
Samples	Treatment	AUC (µg Pt*h/ml (or g))	AUC ratios (l-OHP/EP / l-OHP)	ApCl (ml/h)	Cmax (µg/ml)	Tmax (hour)	MRT (h)
Plasma	l-OHP	42.17	1.96	0.15	4.50	0.25	9.40
	l-OHP/EP	82.89		0.08	4.92	8.0	9.66
Tumor	l-OHP	148.52	4.85	0.04	8.79	0.25	NA
	l-OHP/EP	720.05		0.01	48.22	3.0	NA
Liver	l-OHP	89.18	1.02	0.07	5.52	1.0	NA
	l-OHP/EP	91.45		0.07	4.45	8.0	NA
Kidneys	l-OHP	416.11	1.31	0.01	47.29	0.25	NA
	l-OHP/EP	546.73		0.01	27.43	8.0	NA
Spleen	l-OHP	756.78	1.38	0.04	84	24.0	NA
	l-OHP/EP	215.60		0.03	10.52	24.0	NA
Colon	l-OHP	121.35	0.90	0.05	10.51	1.0	NA
	l-OHP/EP	109.08		0.06	5.72	3.0	NA
IP fluids	l-OHP	40.68	5.13	0.16	30.58	0.083	NA
	l-OHP/EP	140.84		0.04	34.98	0.083	NA

Results: PK and biodistribution of platinum in BDIX rats bearing a SC rat colon tumor after a single IT injection



Samples	Treatment	AUC (µg *Pt* *h/ml (or g))	AUC ratios (l-OHP/EP / l-OHP)	ApCl (ml/h)	Cmax (µg/ml)	Tmax (hour)	MRT (h)
Plasma	l-OHP	22.70	0.53	0.28	4.24	0.25	8.55
	l-OHP/EP	12.00		0.53	0.63	8.0	11.28
Tumor	l-OHP	1810.10	3.75	0.0035	170.2	0.083	NA
	l-OHP/EP	6794.40		0.0009	378.3	0.5	NA

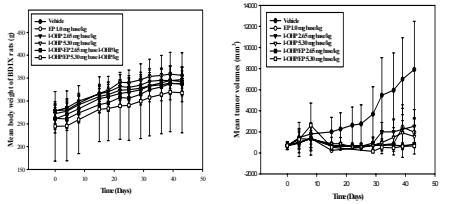
Results: Antitumor activity of 1-OHP/EP combination on BDIX rats bearing an advanced peritoneal carcinomatosis in BDIX rat after a single IP injection.



Groups	Treatments	Dose of l-OHP (mg base/kg)	No. of treated rat	No. of rats dead at DI by toxicity of treatment	No. of survival rat at D93	Survival rate at D93 (%)	MWBC ± SD (g)	MBWC (%)
1	Vehicle	0.00	10	0	0	0	+ 19.50 ± 4.86	+ 8.10 ± 1.36
2	EP	0.00	10	0	0	0	- 1.52 ± 6.66	- 0.58 ± 2.60
3	l-OHP	2.65	10	0	1	10	+ 12.22 ± 5.70	+ 4.68 ± 2.16
4	l-OHP	5.30	10	0	4	40	+ 10.53 ± 5.75	+ 4.11 ± 2.37
5	l-OHP/EP	2.65	10	1	3	33	- 23.27 ± 23.51	- 9.69 ± 9.98
6	l-OHP/EP	5.30	10	1	5	55	- 9.02 ± 10.65	- 3.69 ± 4.28

Groups	Mean survival time ± SD (days)	Median survival time (days)	ILS (%)	T/C (%)
Vehicle	43.40 ± 9.31	39.0	NA	NA
EP	40.50 ± 18.94	36.0	- 7.69.	92.31
l-OHP	57.60 ± 13.88	51.5	+ 32.05	132.05
l-OHP	77.20 ± 14.12	72.5	+ 85.90	185.90
l-OHP/EP	73.89 ± 16.42	64.0	+ 64.10	164.10
l-OHP/EP	89.33 ± 6.58	93.0	+ 138.46	238.46

Results: Antitumor activity of 1-OHP/EP combination against SC rat colon tumor after a single IT injection.



Groups	Treatments	Total dose of l-OHP (mg base/kg)	No. treated rats	No. of rats dead at DI by toxicity of treatments	No. of measurable tumors at D42	Not measurable tumors at D-43 (%)	MBWC ± SD (g)	MBWC (%)
1	Vehicle	0.00	7	0	7	0	+ 10.04 ± 4.33	+ 3.88 ± 1.81
2	EP	0.00	7	0	2	72	+ 1.44 ± 8.28	+ 0.43 ± 3.06
3	l-OHP	2.65	7	0	6	14	+ 7.01 ± 3.50	+ 2.57 ± 1.38
4	l-OHP	5.30	7	0	3	57	+ 5.04 ± 1.95	+ 1.80 ± 0.77
5	l-OHP/EP	2.65	7	1	2	66	- 0.02 ± 3.41	+ 0.01 ± 1.23
6	l-OHP/EP	5.30	7	0	1	86	+ 1.01 ± 2.38	+ 0.41 ± 0.96
7	CDDP/EP	2.00	7	1	0	100	+ 4.80 ± 3.61	+ 2.25 ± 1.29

Groups	Treatments	Mean DT ± SD (days)	Mean SGD ± SD	Mean tumor volume ± SD (500-2700 mm^3)	Mean time to reach V ± SD (days)	Median time to reach V (days)	T/C (%)
1	Vehicle	10.2 ± 2.6	NA	1785.0 ± 326.9	13.7 ± 14.0	8.0	NA
3	l-OHP	17.0 ± 4.0	0.67 ± 0.39	1279.8 ± 365.2	34.00 ± 5.7	32.0	25

Conclusions

- **EP did not modify the *in vitro* l-OHP cytotoxicity against DHD/K12/PROb cells,**
- **EP decreased the *Pt* peritoneal clearance of l-OHP IP injected in BDIX rats bearing an advanced advanced peritoneal carcinomatosis of DHD/K12/PROb rat cancer cells,**
- **EP increased the accumulation of *Pt* in IP tumors,**
- **EP delayed the presence of *Pt* in the systemic bloodstream and increased the AUC of *Pt* in plasma,**
- **EP enhanced the antitumor activity of l-OHP against advanced peritoneal carcinomatosis of DHD/K12/PROb rat cancer cells,**
- **EP decreased the *Pt* clearance of SC tumors to the systemic bloodstream,**
- **EP enhanced the antitumor activity of l-OHP against SC DHD/K12/PROb rat tumors,**
- **The main EP mechanism of action may be due to its vasoconstrictive effect, blocking the clearance of l-OHP from the IP cavity or tumor into the blood**

ONCODESIGN S.A. Parc Technologique de la Toison d 'Or - 28, rue Louis de Broglie - 21000 DIJON - E-mail: contact@oncodesign.fr - Tel. 03 80 78 82 60 - Fax: 03 80 78 82 61