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In vivo screening of anticancer agents for intratumor (IT) treatment of human glioma in combination with Epinephrine (EP).

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Introduction

The IT route of anticancer drug administration has been investigated with a substantial increase in tumor drug exposure without compromising systemic exposure. The main reason for IT chemotherapy failure is because fluids diffuse poorly into the tumor mass when injected inside or around the tumor tissues. Epinephrine (EP) has been shown to facilitate the diffusion, accumulation and antitumor activity of cisplatinum (CDDP) into rat colon tumors after local administration (Duvillard *et al*, 1999). Malignant gliomas are the most common primary brain tumors in adults. The purpose of the present study was to test the possible potentiation of the antitumor activity of conventional anticancer agents IT co-injected with EP against human subcutaneous (SC) glioma tumor xenografted in *Nude* mice and glioma cells intracranially injected in the *Nude* rat brain. 10 human gliomas have been studied for their ability to grow in *Nude* mice and rats after SC implantation. 7/10 gliomas obtained SC tumors in *Nude* mice and 10/10 in *Nude* rats with take rates between 75-100% and 66-100%, respectively. Histology and expression of the main genes related to chemoresistance mechanisms (MDR1, TS, LRP1, MRP1, GSTπ, Bcl2 and TK) were determined. We have selected the TG-8 glioma to test the anticancer activity of drug combinations after single IT co-injections.

Methodology

➤ **Animals** :

-6 week-old female athymic-*Nude* mice were used for the subcutaneous (SC) tumor studies.

-5-6 week-old male RH-*rnu Nude* rats were used for the intracerebral tumor studies.

-Animals were housed and manipulated under SPF conditions.

➤ **Tumor origin and cell line** :

-The TG-8 tumor is a human glioblastoma purchased from Dr. MF. Poupon (Curie Institute, France). The primary tumor was dissociated and cells were SC injected in *Nude* mice. The resulting TG-8 tumor was maintained by serial SC passages.

-The CGL-3 cell line was obtained by mechanical dissociation from a TG-8 SC tumor. The tumor cell line was grown in RPMI-1640 medium supplemented with 10% bovine serum and 2 mM L-glutamine.

➤ **Drugs** :

Cisplatin (CDDP), Bleomycin (BLM), Thiotepa (TTP), Methotrexate (MTX), Cytarabine (ARA), 5-fluorouracil (5-FU) and Epinephrine (EP)

➤ **Tumor induction** :

-SC tumor induction by tumor grafting on the right flank of *Nude* mice.

-Intracerebral (IC) tumor induction by CGL-3 cell injection with a stereotaxic apparatus (Kopf Instrument). 10^5 cells / 5 µl were injected in the right frontal lobe of *Nude* rats.

➤ **Morphology and chemoresistance profile characterization of the TG-8 tumor** :

-Histological sections of SC or IC paraffin-embedded TG-8 tumors.

-Immunohistology of the CD31antigen on cryostat sections.

-Expression level of 6 chemoresistance genes by RT-PCR.

➤ **Growth characteristics of SC TG-8 tumors on *Nude* mice and rats** :

-Tumor take rate and doubling time were determined for 3 consecutive SC passages.

➤ **Treatment schedule** :

-SC tumors were treated by the intratumoral (IT) route with a single injection of 0.2 mg of CDDP, BLM, TTP, or 2.0 mg of ARA or 5-FU alone, or co-injected with 2.0 µg EP. Tumor sizes at the start of treatment were 72-177 mm^3.

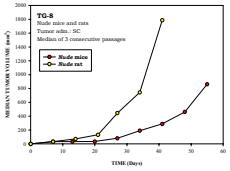
-IC tumors were treated by the IT route with a single injection of 33.0 µg of CDDP or 166.0 µg of BLM or TTP alone or co-injected with 1.7 µg EP at D21 after injection of glioma cells.

➤ **Activity parameters** :

-Tumor volume measurements with caliper, standard growth delay (SGD) and growth inhibition (T/C%) calculation for SC tumors.

-Survival time, increased life span (ILS%) and tumor size measurements on paraffin sections by micrometric histological analysis for IC tumors.

Growth characteristics of SC TG-8 tumors xenografted in Nude mice and rats

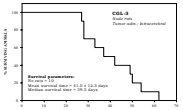


	Nude mice	*Nude* rat
No. of animals	125	20
Tumor take-rate	90 %	95 %
Mean DT (days) ± SD	9.7 ± 1.5	5.3 ± 0.5
Time to reach 250 mm^3	38.1 ± 4.3	36.0 ± 11.0
Time to reach 800 mm^3	53.8 ± 11.6	38.3 ± 10.4
Observation of metastases	25 % lymph nodes and lung	6 % lymph nodes

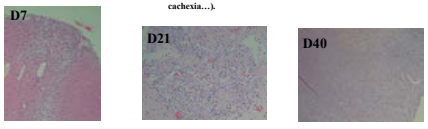
DT : Doubling time
SD : Standard deviation

Growth characteristics of intracerebral CGL-3 tumors in Nude rats

Brain of a *Nude* rat intracranially injected with 10^5 CGL-3 glioma cells



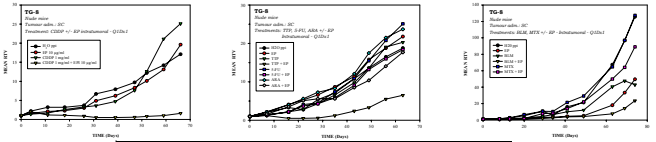
Survival curves of *Nude* rats intracranially injected with 10^5 CGL-3 glioma cells (n=10). Animals were sacrified when clinical signs appeared (behavior, paraplegia, cachexia…).



Kinetic development of CGL-3 glioma cells intracranially injected in *Nude* rats (HESx50)

Antitumor activity of 6 different anticancer agents alone or in association with Epinephrine (EP) in Nude mice bearing SC TG-8 tumors.

(Animals were IT treated when tumor sizes reached 71-177 mm^3 [n=5 for each control and treated group])

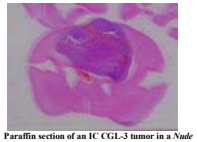


No exp.	Groups	Treatment day	Mean tumoral volume treated (mm³) ± SD	Mean volumes administered (µl) ± SD	Drug concentration (mg/ml)	EP concentration (mg/ml)	Mean DT (days) ± SD	SGD (days)	T-C (days)	T/C (%)
1	H2O	D44	115.83 ± 17.19	233.65 ± 34.38	-	-	15.6 ± 2.8	-	-	-
	EP	D44	162.24 ± 114.84	324.48 ± 229.68	-	0.01	15.9 ± 2.2	0.02	28	75.6
	CDDP	D44	177.42 ± 121.99	354.84 ± 243.98	1.0	-	21.5 ± 7.7	0.38	35	54.4
	CDDP+EP	D44	126.68 ± 61.42	253.35 ± 122.85	1.0	0.01	-13.3 ± 8.3	-4.34	>35	1.1
2	H2O	D13	84.91 ± 85.79	169.83 ± 171.59	-	-	11.3 ± 3.1	-	-	-
	EP	D13	84.66 ± 85.35	169.32 ± 170.71	-	0.01	9.6 ± 2.4	-0.15	16	28.4
	BLM	D13	90.28 ± 91.64	180.55 ± 183.29	1.0	-	10.6 ± 0.3	-0.06	12	54.8
	BLM+EP	D13	92.17 ± 90.77	184.34 ± 181.54	1.0	0.01	16.7 ± 7.7	0.48	>23	14.8
	MTX	D13	82.81 ± 80.80	165.62 ± 161.60	1.0	-	12.9 ± 1.4	0.34	17	96.9
	MTX+EP	D13	79.32 ± 77.46	158.63 ± 154.92	1.0	0.01	13.4 ± 1.0	0.27	0	80.8
3	H2O	D23	71.34 ± 17.02	142.67 ± 34.04	-	-	16.2 ± 3.0	0.11	-	-
	EP	D23	75.97 ± 22.82	151.93 ± 45.64	-	0.01	21.6 ± 3.4	0.21	0.0	118.7
	TTP	D23	75.15 ± 23.00	150.29 ± 46.00	1.0	-	12.9 ± 1.2	-0.52	0.0	138.3
	TTP+EP	D23	73.89 ± 24.38	147.78 ± 48.76	1.0	0.01	7.8 ± 2.5	-0.52	>20.0	21.0
	5-FU	D23	74.62 ± 27.21	149.23 ± 54.43	10.0	-	13.3 ± 1.4	-0.18	0.0	158.3
	5FU+EP	D23	77.27 ± 37.61	154.54 ± 115.22	10.0	0.01	14.2 ± 1.9	-0.13	20.0	51.2
	ARA	D23	78.01 ± 82.26	156.02 ± 165.51	10.0	-	16.2 ± 3.9	0.00	0.0	73.7
	ARA+EP	D23	78.01 ± 89.15	156.01 ± 178.30	10.0	0.01	19.0 ± 4.9	0.17	20.0	54.3

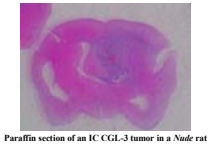
DT : Doubling time
SGD : Standard growth delay
T-C : Growth delay
T/C : Tumor growth inhibition
SD : Standard deviation

Antitumor activity of 3 different anticancer agents alone or in association with Epinephrine (EP) in Nude rats bearing intracerebral CGL-3 gliomas.

(Animals were IT treated at D21 after glioma cell injection [n=3 for each control and treated group])



Paraffin section of an IC CGL-3 tumor in a *Nude* rat from the control group (1A) sacrificed at D35.



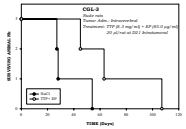
Paraffin section of an IC CGL-3 tumor in a *Nude* rat from the TTP+EP treated group (4A) sacrificed at D35.

Group	Treatment	Drug dose (µg/inj)	Epinephrine dose (µg/inj)	Mean tumor volume (mm³) ● D35 ○ D35	T/C (%)	Surviving animals at D35
1A	Control	-	-	1304.6 ± 172.8	-	3/3
2A	EP	-	1.7	61.2 ± 21.6	24.7	3/3
3A	TTP	166.0	-	74.7 ± 66.4	62.8	3/3
4A	TTP+EP	166.0	1.7	62.1 ± 16.8	14.9	3/3
5A	CDDP	33.0	-	7.4 ± 0.9	2.8	1/3
6A	CDDP+EP	33.0	1.7	0.0 ± 0.0	0.0	3/3
7A	BLM	166.0	-	31.7 ± 51.8	16.1	3/3
8A	BLM+EP	166.0	1.7	22.1 ± 16.2	19.7	2/3

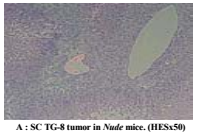
T/C : Tumor growth inhibition
SD : Standard deviation

Group	Treatment	Drug dose (µg/inj)	Epinephrine dose (µg/inj)	Mean survival time (days)	Median survival time (days)	T/C (%)	ILS %
1B	Control	-	-	34.3 ± 3.8	-	-	-
2B	TTP+EP	166.0	1.7	71.7 ± 31.8	63	221.4	321.4

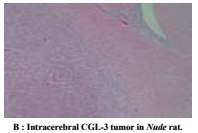
A : Experimentation was stopped at D35. B : Animals were sacrificed when clinical signs appeared.



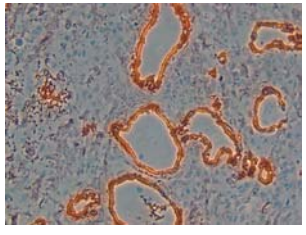
Morphology and chemoresistance profile characterization of TG-8 tumors



A : SC TG-8 tumor in *Nude* mice. (HESx50)



B : Intracerebral CGL-3 tumor in *Nude* rat. (HESx50)



C : Immunostaining of a human TG-8 tumor with anti-rat CD31 antibody (dilution 1/300') (x100).

Cellular morphology

➢Small cells with densified nucleus and typical pseudopalissadic aspect of multiform glioblastoma (Photos A and B).
➢Abondant mitotic figures.
➢Cellular morphology unchanged after several SC passages.

Vascularization

➢Numerous thin capillary vessels (Photos A and B).
➢High expression of CD31 (Photo C).

Gene	Expression Level
TS	++
LRP1	++
MRP1	+
GST π	+
Bcl2	+
TK	+

Conclusions

↖ **We have developed a useful human orthotopic CGL-3 glioma model in *Nude* rats for the evaluation of new anticancer treatments (i.e, local chemotherapy, anti-angiogenic agents…).**

↖ **We have shown an anticancer activity of IT injected CDDP and BLM alone. The combination with EP increased the antitumor activity of these agents in *Nude* mice bearing SC glioma.**

↖ **Single stereotaxic injections of TTP at 166 µg was well tolerated, but not CDDP at 33 µg and BLM at 166 µg in *Nude* rats bearing IC glioma.**

↖ **The combination with EP did not increase the local toxicity of TTP at 166 µg.**

↖ **IC injection of EP combined with TTP, CDDP and BLM showed a significant antitumor activity against orthotopic CGL-3 glioma in *Nude* rats.**

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