Interest of liposomal doxorubicin as a radiosensitizer in malignant glioma xenografts

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Malignant glioma patients have a life expectancy reduced to about 15 months despite aggressive surgery, radiotherapy (RT), and chemotherapy. Doxorubicin has shown a marked cytotoxic effect against malignant glioma cells *in vitro*. The brain exposure to this drug is, however, hindered by the blood–brain barrier. Encapsulation of doxorubicin in liposomal carriers has been shown to reduce toxicities and to improve brain tumors exposure to doxorubicin. In this study, we evaluated the radiosensitizing properties of a nonpegylated liposomal doxorubicin (Myocet, MYO) on two subcutaneous (U87 and TCG4) and one intracranial (U87) malignant glioma models xenografted on nude mice. Doxorubicin biodistribution was assessed by a high-performance liquid chromatography method. Antitumor efficacy was investigated by tumor volume measurements and mice survival determination. We showed that (i) encapsulation of doxorubicin ensured a preferential deposition of doxorubicin in tumoral tissue in comparison with free doxorubicin; (ii) doxorubicin accumulated in both subcutaneous and intracranial tumors during repeated injections of MYO and this accumulation was linked to the potentiation of RT efficacy on two subcutaneous models; (iii) MYO was unable to improve the antitumoral efficacy of RT on an intracranial glioma model. Finally, this study emphasizes the importance of performing preclinical studies on models closer as possible of human tumors and localization to be more predictive of therapeutic effects observed in humans. *Anti-Cancer Drugs* 19:991–998

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Introduction

Malignant brain tumors are still a therapeutic challenge: despite aggressive surgery, radiotherapy (RT), and chemotherapy, prognosis of high-grade glioma patients remains poor and their life expectancy is reduced to few months [1]. For several years, the concomitant administration of RT and temozolomide, followed by adjuvant temozolomide has been the first-line therapeutic standard for newly diagnosed glioblastoma multiforme patients [2]. This new clinical protocol underlines the fact that concomitant chemoradiation could be a relevant strategy for the treatment of high-grade gliomas, the antineoplastic molecule exerting its own cytotoxic activity and acting as a radiosensitizer.

Doxorubicin has shown a marked cytotoxic effect against malignant glioma cells *in vitro* [3]. Doxorubicin, however, does not reach therapeutic concentration in the central nervous system because of its low lipophilicity and the blood–brain barrier that presents active drug efflux mediated by multidrug–resistance-related proteins [4,5]. Moreover, doxorubicin presents significant toxicity to normal tissues, in particular acute mucosal toxicities and long-term cardiac toxicity [6,7]. For about 20 years, liposomal formulations of doxorubicin have been developed to overcome these obstacles. Two types of liposomal doxorubicin have entered clinical trials and are approved for cancer treatment: conventional liposomes (Myocet, Elan Pharmaceuticals, Inc., Cedar Knolls, New Jersey, USA) and 'stealth' pegylated liposomes (Caelyx, Alza Pharmaceuticals, San Bruno, California, USA), which differ from the first preparations by the presence of a polyethylene glycol (PEG) coating on their surface [8]. In both cases, the liposome protects the drug from metabolism and, because of size limitations in the transport of carriers across healthy endothelium, doxorubicin accumulates to a reduced extent in healthy tissues, hence limiting drug toxicities [9,10]. Moreover,

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discontinuities in the endothelium of tumor vasculature have been shown to result in an increased extravasation of liposomes, and, in combination with impaired lymphatics, an increased accumulation of liposomal doxorubicin at the tumor site [8,11–13].

To date, almost all published data dealing with the effects of liposomal doxorubicin in glioma models have used the only commercialized pegylated liposomal form of doxorubicin, Caelyx. In preclinical studies, an increased concentration of doxorubicin has been obtained in intracerebral tumor models [14,15], and was linked to an improved therapeutic efficacy for pegylated liposomal doxorubicin in intracranial gliosarcoma models [15,16]. These promising results have, however, not been translated into clinical benefit: pegylated liposomal doxorubicin only produced a modest increase in survival when administered alone in patients with recurrent high-grade glioma [17,18], despite enhanced tumor exposure to doxorubicin, as shown by Koukourakis *et al.* [19]. It is not surprising that, in highly resistant tumoral types (such as high-grade glioma), a cytotoxic drug delivered alone cannot markedly improve overall therapeutic response and it is expected that a multiple-modalities treatment would give better results. As radiosensitizing properties of doxorubicin have been well documented [20], some researchers have investigated the interest of combining RT with pegylated liposomal doxorubicin and have ascertained the superiority of the association in the treatment of osteosarcoma [21] and head and neck [22] xenografts. To date, no study has reported the use of such an association in malignant glioma treatment.

The major difference between pegylated liposomes and conventional liposomes is the reduced uptake by the reticuloendothelial system (RES) of pegylated liposomes. Indeed, conventional liposomes are cleared from the circulation by macrophages of the RES, in particular those of the liver and spleen. The attachment of PEG to the surface of the liposome reduces the rate of uptake by the RES [8]. This PEG coating considerably prolongs the circulation half-life of doxorubicin but also leads to its accumulation in skin and induces grade 3 palmar–plantar erythrodysesthesia in approximately 20% of the patients, requiring reductions in the doxorubicin dose [23]. In this regard, doxorubicin entrapped in conventional liposomes seems to be much safer. To our knowledge, however, preclinical studies using conventional liposomal doxorubicin alone or in combination with RT have never been reported yet.

As (i) doxorubicin has been shown to be efficient against malignant glioma cells *in vitro*, (ii) liposome encapsulation of doxorubicin has been shown to trigger tumoral tissue, and (iii) because earlier studies have shown the radiosensitizing properties of doxorubicin, we postulated that a conventional liposomal form of doxorubicin (Myocet, MYO) will be of interest in concomitant administration with RT for the treatment of human high-grade glioma xenografts. It is the first study designed to evaluate the radiosensitizing properties of MYO on two subcutaneous and one intracranial malignant glioma models xenografted on nude mice.

Materials and methods

Animals and tumors

Athymic NCr/Sed nude *(nu/nu)* female mice, 7–8 weeks of age, were obtained from Charles River (Saint-Germain-sur-l'Abresle, France). Animal procedures were performed according to institutional and national guidelines (EC directive 86/609/CEE, French decree no. 87–848). All surgical and MRI procedures were carried out under general anesthesia obtained by intraperitoneal (i.p.) injection of xylazine (8 mg/kg) and ketamine (90 mg/kg).

Tumors xenografts were obtained as described earlier [24]. In brief, the first human malignant glioma model (U87) was originally obtained by subcutaneous (s.c.) injection of a suspension of U87 cells (10^6 cells in 0.1 ml of 0.9% NaCl) into the hind leg of mice (HTB-14, American Type Culture Collection, Manassas, Virginia, USA). The second model (TCG4) was derived from an anaplastic oligodendroglioma (according to the WHO grading, 2007) of a 72-year-old man. Pieces of the patient's tumor were directly subcutaneously transplanted into the hind leg of mice, providing the first xenografts. U87 and TCG4 glioma models were then maintained *in vivo* by sequential passages of tumor fragments in nude mice.

Grafts

For the experiments, source tumors were excised, cleaned from necrotic tissue, cut into small fragments and subcutaneously or intracranially implanted into each experimental mouse. For the subcutaneous model, tumor fragments were subcutaneously grafted in the inguinal pit, against the femoral vessels. In the case of the intracranial graft, a craniotomy flap was made and raised, after opening the mouse's skull skin. A 1-mm^3 graft was then slid under the meninges and into the brain cortex in its temporo-occipital area.

MRI imaging and image processing

MRI measurements were conducted on a Bruker Biospec Avance 24/40NMR spectro-imager (Bruker Biospin, Ettlingen, Germany) at 2.35 T using a T1-weighted spin–echo sequence and gadolinium contrast enhancement. The sequence parameters were as follows: TR = 500 ms and TE = 18 ms. For each experiment, 10 images were acquired, corresponding to 10 slices of 1.3 mm in thickness, spaced regularly at 1.8 mm, in the transverse plane, with a field of view of 6.54 cm, and 192 phases. In this way, the total acquisition time was 8 min. Gadobenate dimeglumine (Multihance, Bracco Altana Pharma

Laboratories, Konstanz, Germany) was administered intravenously (0.4 mmol/kg) 10 min before image acquisition. Anesthetized mice were placed on a bed, which were lent totally into the antenna (sensitive volume: 3 cm diameter–4 cm length).

Treatment protocols

Treatments started when tumors reached a volume of about $250 \pm 50\,mm^3$ (V0) for subcutaneous model and when tumor presence was assessed by MRI examination for intracranial model. In a preliminary study, serial MRI were undertaken to assess the presence of the tumor and to enable tumor volume measurements. By MRI examination, U87 tumors became observable as a distinct entity 13–17 days after implantation, hence defining the beginning of the treatments (D0).

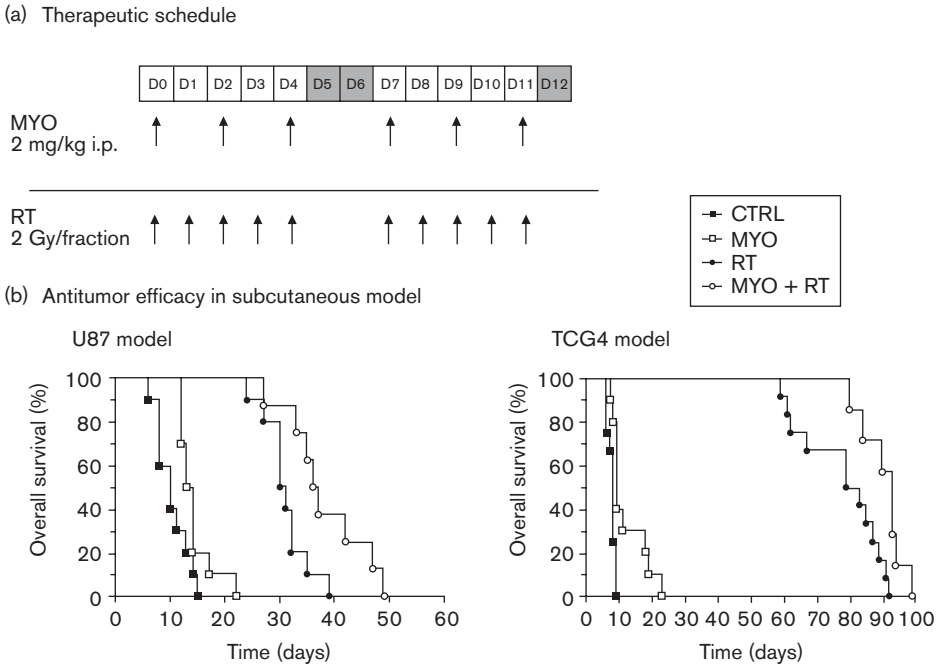
At D0 (corresponding to the first day of treatment), mice were randomly assigned into four groups. In the intracranial model, animals were randomized to have a similar range of small, medium and large tumor volumes in each group. In the control group (CTRL), the mice received i.p. injection with saline (0.9% NaCl). In the MYO group, the mice received i.p. injection with conventional liposomal doxorubicin at a daily dose of 2 mg/kg 3 days/week for two consecutive weeks, leading to a total dose of 12 mg/kg. In the RT group, the tumors were irradiated at the total dose of 20 Gy (10 fractions of 2 Gy, once daily, 5 days/week over 2 weeks). A Pantak Therapax SXT 150 apparatus (Pantak Medical Systems, AGFA NDT, Limonest, France) was used for local irradiation with a 2.0 aluminum filter and a collimator of 1.5 cm in diameter. The dose rate was 2.77 Gy/min. The dose was calculated on the skin (100%). In the MYO + RT mice groups, MYO was injected according to the earlier schedule during the entire ionizing radiation treatment (Fig. 1).

Treatments effects

To follow tumor growth in the subcutaneous model, tumor volume was determined three times per week. Two perpendicular diameters were measured with a caliper. Tumor volume (V in cubic millimeters) was calculated as $V = (\text{length} \times \text{width}^2)/2$. Mice were anesthetized and killed by cervical dislocation when the tumors reached three times their initial volume ($3\,V_0$), thus defining the 'survival times'.

For the intracranial model, the mice were observed for body weight reduction. They were killed by cervical dislocation when they presented signs of neurological disorders or when their body weight reduction was more

Fig. 1



(a) Treatment schedules. Treatments began when tumor volume reached $250 \pm 50\,mm^3$ for subcutaneous tumors and when tumors were objective by MRI examination for the intracranial ones (D0). Myocet (MYO) was injected at the dose of 2 mg/kg intraperitoneally (i.p.) 3 days/week. Radiation therapy (RT) consisted of a daily fraction of 2 Gy, 5 days/week. Treatments were administered for two consecutive weeks. (b) Response of subcutaneous glioma xenografts to antitumoral treatments. (a) U87 model (at least eight mice per group). (b) TCG4 model (at least seven mice per group). Mice were randomly assigned into four groups: control (CTRL) (■); MYO (□); RT (●), and MYO + RT (○). Results are expressed as Kaplan–Meier plots, considering the percentage of tumors not having reached 3 V_0 as the survival end point.

than 20% of their original weight, the time to this moment being referred to as 'survival time'. The response to the different treatments was also assessed by MRI examination once a week until death to evaluate tumor growth. Tumor volume was estimated according to the following method: two perpendicular diameters were measured on the MRI slice, which shows the largest area of tumor and tumor volume was then calculated using the formula described for subcutaneous tumor.

For both the models used, the survival times of mice were recorded. The percentage of increase in the life span was calculated as $\{[(T-C)/C] \times 100\}$, where T and C are the survival times of the treated and control animals. We also calculated the radiopotentiation ratio (RP), defined as $T_{MYO+RT}/(T_{MYO} + T_{RT})$, corresponding to the comparison between the observed effect for the association and the theoretical additive effect of MYO alone and RT alone. If the ratio equals 1, it corresponds to an additive effect between the two treatments. Under 1, the interaction is infraadditive, above 1, RP corresponds to a synergistic interaction between the two treatments.

Tissue distribution

Doxorubicin concentrations were measured in tissues of mice receiving i.p. injection of MYO at the dose of 2 mg/kg. Animals were killed after one or six injections of chemotherapy, in the 6 or 30 h after the last injection. Tissues (subcutaneous tumor, normal brain hemisphere, tumoral brain hemisphere) were excised, washed in saline and immediately frozen at -20°C until processed. At least five organs per treatment groups were analyzed. Similar experiments with nonliposomal doxorubicin-receiving mice were performed as control. On account of toxicity of the repeated free doxorubicin injections, we did not evaluate drug concentration after six injections.

Doxorubicin concentration was determined by a reversed phase high-performance liquid chromatography method after liquid–liquid extraction as described earlier [25]. In brief, tissues were homogenized in four parts of water. Homogenized tissue (0.5 ml) was then mixed with 500 ng daunorubicin as an internal standard, with 20 µl of $AgNO_3$ (33% in water), and with 8 ml of chloroform/isopropanol (1 : 1). After shaking for 20 min, the samples were centrifuged at $1500 \times g$ for 10 min, and the separated organic phase was evaporated under vacuum. Analyses were performed by reversed phase high-performance liquid chromatography on a C18 column (250×4.6 mm I.D., YMC, Interchim, France), under isocratic elution conditions with a mobile phase of acetonitrile/0.01 mol/l KH_2PO_4 pH 2.6 (30 : 70) at a flow rate of 1 ml/min. Doxorubicin and daunorubicin were detected by a fluorescence detector (RF 10A XL Shimadzu, France) with excitation and emission wavelengths of 480 and 590 nm, respectively. The limit of detection of the assay was 2 ng/g of tissue.

Statistical analysis

Kaplan–Meier curve analysis was performed using the log-rank test; P value of less than 0.05 was considered as significant.

Results

Liposomal encapsulation improved doxorubicin deposition in both subcutaneous and intracranial tumors

Our objective was to evaluate whether liposomal doxorubicin could enhance the efficacy of a fractionated ionizing radiation protocol. We first investigated the ability of liposomal doxorubicin to reach tumor tissue and its potential accumulation during the treatment course. Then, we determined doxorubicin concentration in U87 subcutaneous and intracranial tumors, 6 or 30 h after one injection of MYO, corresponding to the theoretical times of the first and second irradiations (Fig. 1). Nonliposome-encapsulated doxorubicin (DOXO) given at the same dose was used as a control. Doxorubicin concentration was also investigated at the end of chemotherapy regimen.

Earlier studies in our laboratory and elsewhere have shown the toxicity of the repeated administration of free doxorubicin [26]; therefore, we did not determine doxorubicin level after six injections of nonliposomal doxorubicin (Table 1). Considering the subcutaneous tumor model 6 h after one injection, administration of DOXO led to a 50% greater concentration of doxorubicin than injection of MYO: doxorubicin concentrations were 139 and 72 ng/g of tissue for DOXO and MYO groups, respectively. We also observed that doxorubicin level increased between 6 and 30 h for the MYO group (72 vs. 164 ng/g of tissue, respectively), whereas it remained stable for DOXO-receiving mice (139 vs. 115 ng/g). Moreover, repeated injections of MYO led to an accumulation of drug in tumoral tissue: doxorubicin concentration 6 h after six injections of MYO was 2.8-fold greater (204 ng/g of tissue) as compared with one injection after 6 h (72 ng/g of tissue).

Using the intracranial model (Table 1), we showed that doxorubicin uptake was always higher in the tumor-bearing hemisphere than in the normal one, for both MYO and DOXO groups. Indeed, 30 h after one injection, doxorubicin concentrations were about two-fold greater in the tumor brain hemisphere: 94 versus 45 ng/g for MYO group and 75 versus 40 ng/g for the DOXO group. Moreover, when considering MYO-receiving mice, we showed that the doxorubicin amount remained stable in normal brain hemisphere between the first and the sixth injection (65 vs. 57 ng/g), whereas doxorubicin concentration increased in the tumor-bearing hemisphere (79 vs. 131 ng/g).

Finally, when comparing subcutaneous versus intracranial xenografts, we noticed that doxorubicin concentrations for both drugs were higher for subcutaneous tumors than

Table 1 **Doxorubicin concentrations in subcutaneous and intracranial U87 xenografts**

	Doxorubicin concentration (ng/g of tissue)							
	One injection						Six injections	
	6 h			30 h			6 h	
	MYO	DOXO	Ratio MYO/DOXO	MYO	DOXO	Ratio MYO/DOXO	MYO	DOXO
Subcutaneous tumor	72 ± 14	139 ± 11	0.52	164 ± 29	115 ± 19	1.43	204 ± 15	ND
Normal brain hemisphere	65 ± 19	42 ± 13	1.55	45 ± 11	40 ± 13	1.13	57 ± 18	ND
Tumor brain hemisphere	79 ± 32	47 ± 8	1.68	94 ± 32	75 ± 16	1.25	131 ± 26	ND

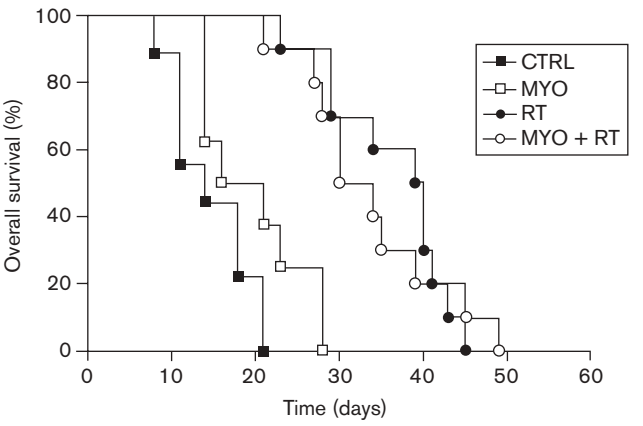
Doxorubicin concentrations were measured after one or six injections of DOXO and MYO at the dose of 2 mg/kg (intraperitoneal). For each time, results are expressed as mean ± SD of at least five organs per treatment.
DOXO, doxorubicin; MYO, Myocet; ND, nondetermined.

for intracranial tumors. For example, 30 h after one injection of MYO, doxorubicin concentration was only 94 ng/g in intracranial tumors, compared with 164 ng/g in subcutaneous tumors. Moreover, we showed that doxorubicin accumulation after the sixth injection of MYO is less important in the case of intracranial versus subcutaneous location (131 vs. 204 ng/g).

Therapeutic efficacy studies in subcutaneous models

Given the exposure of tumor tissue to doxorubicin during the 2 weeks course of our therapeutic schedule, we first evaluated the potential radiosensitizing effect of MYO using two subcutaneous glioma models (U87 and TCG4). The response of the xenografts to therapies was assessed using Kaplan–Meier analysis (Fig. 2, for U87 and TCG4 models, respectively). Preliminary studies have shown that repeated administration of nonliposomal doxorubicin alone or in association with RT led to a significant toxicity (major weight loss during treatment course, data not shown). Hence, antitumor effect of both nonliposomal doxorubicin alone or associated with RT was not examined in this study.

Fig. 2



Response of U87 intracranial xenografts to antitumoral treatments. Intracranial U87 tumor-bearing mice were randomly assigned into four groups: control (CTRL) (■; $n=9$), Myocet (MYO) (□; $n=8$), radiation therapy (RT) (●; $n=10$), and MYO + RT (○; $n=10$). Treatments effects on survival are represented by Kaplan–Meier plots.

In the untreated CTRL groups, median survival was 10.0 and 8.0 days for subcutaneous U87 and TCG4 models, respectively. In both models, MYO presented a weak antitumor effect: median survival was 13.5 days (vs. CTRL, $P < 0.05$) for U87 and 9.0 days (vs. CTRL, $P < 0.05$) for TCG4 xenografts. This translated to an increase in life span of 35% in U87 model, and 10% in TCG4 model, as compared with the respective CTRL groups. Moreover, RT alone produced a significant antitumor effect: the median survival was significantly prolonged to 30.5 days ($P < 0.0001$) and 81.0 days ($P < 0.0001$) for U87 and TCG4 xenografts, respectively. Results obtained in MYO + RT groups clearly showed that concomitant administration of MYO improved the antitumor effect of RT in subcutaneous tumors: median survival increased up to 36.5 days in U87 tumors (vs. RT, $P = 0.002$) and 93.0 days in TCG4 tumors (vs. RT, $P = 0.008$), corresponding to improvement in life span of about 20 and 15%, respectively, as compared with the RT group.

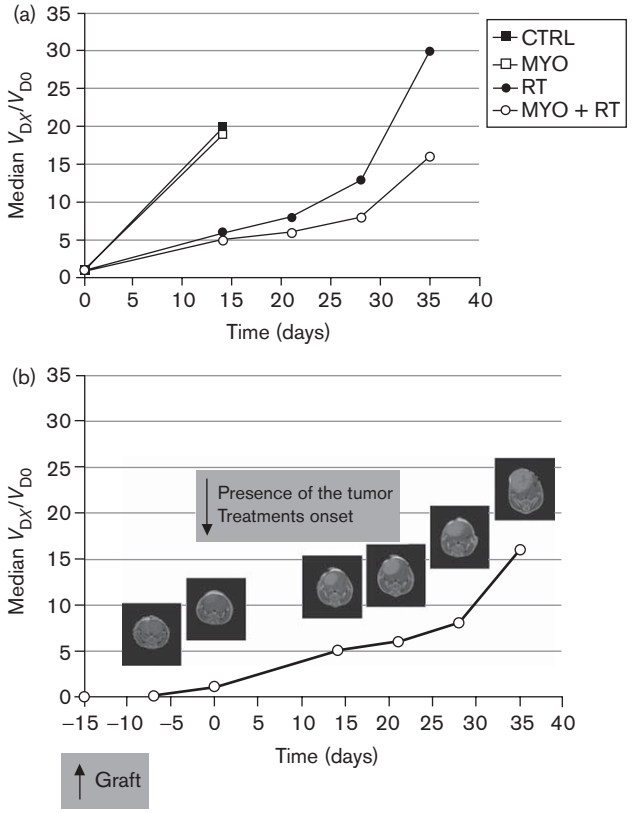
Using median survival, we calculated the RP for each model. RP reached 1.13 for U87 and 1.15 for TCG4 glioma xenografts, illustrating a synergistic effect between MYO and RT in both models.

Therapeutic efficacy studies on U87 intracranial model

The effect of concomitant association MYO + RT on intracranial xenografts was assessed using the U87 glioma model and the same therapeutic schedule as in the subcutaneous model (Fig. 1a).

The first endpoint of this study was the overall survival analyzed by the Kaplan–Meier method (Fig. 3). In the untreated CTRL group, the median survival was 14.0 days. Administered alone, MYO slightly improved mice survival, which increased up to 18.5 days (corresponding to an increase in life span of 32%), although the difference with the CTRL group did not reach the level of statistical significance ($P = 0.068$). Overall survival was significantly enhanced when mice received RT alone or in

Fig. 3



Treatment effects on U87 intracranial xenografts volumes. (a) Intracranial U87 tumor-bearing mice were randomly assigned to four groups: control (CTRL) (■; $n=9$), Myocet (MYO) (□; $n=8$), radiation therapy (RT) (●; $n=10$), and MYO + RT (○; $n=10$). Tumor volume (V_{DX}) was estimate by MRI once a week until death after the end of treatment (D*X*: D14, D21, D28, and D35). Results are expressed as median ratios between tumor volume at D*X* and tumor volume at the onset of treatment (i.e. V_{D0}). (b) MR imaging of the evolution of intracranial tumor in mice receiving MYO + RT regimen. The series of MR images are representative of all mice included in this group.

concomitant association with MYO, as compared with untreated CTRL group ($P < 0.0001$ in both cases). Median survival was 39.5 days for the RT group and 32.0 days for the MYO + RT group. No statistical difference was observed between these two groups ($P = 0.813$).

Mice also underwent weekly MRI examination until death allowing the determination of the intracerebral tumor volume. The effect of treatments on intracranial tumor growth is presented in Fig. 3a and b and shows the volume curve of MYO + RT group with a typical example of a series of MRI obtained for a mouse receiving the association of MYO and RT. Our results showed that MYO alone had no effect on tumor volume as compared with the untreated CTRL group. In the RT group, treatment delayed tumor growth. This effect was even more marked in MYO + RT group, showing a reduced tumor growth as compared with RT group, contrasting with the absence of survival benefit obtained in MYO + RT group.

Discussion

Liposomes are suitable carriers to effectively deliver the drugs to brain tumors. One of the most advanced drug carrier formulation consists of doxorubicin loaded into liposomes. Such a formulation offers the potential for avoiding high-peak concentrations of the bioavailable drug, which are so often associated with pronounced toxicity [8]. Moreover, liposomal encapsulation has been shown to enhance tumor exposure to doxorubicin in several tumor models [8,11]. On the basis of these observations and of the well-demonstrated radiosensitizing properties of doxorubicin [20], we designed this study to determine whether a synergistic effect between liposomal doxorubicin and ionizing radiations could be observed in high-grade glioma xenografted models.

To be close to treatment conditions in humans, we chose to deliver RT as a 2 Gy/day fraction schedule, 5 days a week, as it is usually prescribed for high-grade glioma patients [27]. In our experiments, mice were injected with MYO at a dose of 2 mg/kg/dose, three times a week for two consecutive weeks. This resulted in a weekly dose of 6 mg/kg and a total cumulative dose of 12 mg/kg. Such a doxorubicin dose is in the range of the recommended dosing of 2.5–10 mg/kg/week used in nude mice bearing human xenografts [28]. We first investigated the biodistribution of MYO according to our therapeutic schedule. It is well established that conventional liposomes are trapped by organs of the reticuloendothelial system (i.e. spleen, liver, and bone marrow) [8]. Despite a major sequestration by liver and spleen of liposomes (data not shown), we showed that subcutaneous tumors were similarly exposed to doxorubicin after one injection for both liposomal and free doxorubicin formulations. Hence, our data underscore the fact that the liposomal carrier ensured a better intratumoral deposition of the drug, as shown by others for both conventional [12] and pegylated liposomes [13,29,30].

In our series, we also showed that MYO was not able to slow tumor growth. Nevertheless, when administered in combination with RT, MYO was shown to significantly sensitize TCG4 and U87 xenografts to the antitumor effect of fractionated irradiation, by markedly increasing the life span of mice receiving the association, as compared with RT-receiving mice. Studies combining RT and liposomal doxorubicin are quite rare in the literature: only Harrington *et al.* [31] and Davies *et al.* [21] have described earlier the increase in RT effect by pegylated liposomal doxorubicin on human osteosarcoma and head and neck cancer xenografts, respectively. The

synergistic interaction between MYO and RT obtained in this study may be linked to the accumulation of doxorubicin during RT course. Hence, doxorubicin concentration for the MYO group increased about 2.8-fold between the first and the sixth injection.

The promising results obtained in subcutaneous glioma models prompted us to investigate the antitumoral effect of the concomitant administration of MYO and RT on an intracranial model of brain tumor. Unfortunately, our results showed that no overall survival enhancement was obtained in MYO + RT group, as compared with RT-receiving mice, in spite of an accumulation of doxorubicin concentration in tumor brain hemisphere. Only a trend in a greater delay of tumor growth was observed for the association group. These results are in accordance with data observed in clinical studies showing that objective responses to treatments are not regularly translated into survival rate increase [32,33]. Moreover, an earlier study from Arnold *et al.* [14] has shown that repetitive administration of doxorubicin encapsulated in pegylated liposomes led to an accumulation of drug into an intracranial model of 9L gliosarcoma. Liposome accumulation in tumors proceeds passively and is mediated by enhanced permeability and retention phenomenon. As the blood–brain barrier is disrupted in high-grade gliomas with all the components of the tumor blood vessels showing significant abnormalities (leaky vasculature) compared with normal cerebral vessels, liposomes can extravasate and accumulate in tumor tissue [34].

The discrepancy in our results between subcutaneous and intracranial models could, in part, be explained by the difference in doxorubicin concentration reached in each tumor model. Indeed, doxorubicin in the tumor-bearing hemisphere did not accumulate to the same extent that was obtained in subcutaneous tumors. This effect may be linked to the small tumor volume of intracranial xenografts, as compared with subcutaneous tumors. Moreover, in addition to the blood–brain barrier obstacle, the fact that intracranial tumors grow in a closed compartment with high interstitial pressures [35,36], could play a significant role in the much lower doxorubicin deposition observed for intracranial tumors. To definitely conclude on the radiosensitizing properties of MYO on high-grade glioma and given the great tolerance of our combination schedule, we assume that a higher dosage of MYO would increase doxorubicin deposition in intracranial tumors and then lead to a potentiation of radiation efficacy in the intracranial model. Moreover, special attention should be given to the time interval between chemotherapy doses, as some researchers have shown that higher doses administered less often improved the therapeutic efficacy of pegylated liposomal doxorubicin in tumor-bearing mice, as compared with smaller doses given more often [37]. These points are under investigation in our laboratory.

In conclusion, this study has shown that liposomal encapsulation of doxorubicin improved drug deposition into both subcutaneous and intracranial U87 tumors. We also showed for the first time that concomitant administration of MYO + RT was synergistic on two subcutaneous models but not on the intracranial model. Finally, this study emphasizes the importance of performing preclinical studies on models as close as possible to human tumors and the localization to be more predictive of therapeutic effects observed in humans.

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