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REVIEW

Quaternary ammonium-melphalan conjugate for anticancer therapy of chondrosarcoma: in vitro and in vivo preclinical studies

Caroline Peyrode · Valérie Weber · Emmanuelle David · Aurélien Vidal · Philippe Auzeloux · Yves Communal · Marie Mélanie Dauplat · Sophie Besse · François Gouin · Dominique Heymann · Jean Michel Chezal · François Rédini · Elisabeth Miot-Noirault

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Summary Cartilage tumours present ongoing therapeutic challenges due to their chondrogenic extracellular matrix that potentially hampers drug delivery, their low percentage of dividing cells, and their poor vascularity. In this context, and based on the affinity of the quaternary ammonium moiety for proteoglycans (PG), we developed a strategy that uses the quaternary ammonium function to selectively deliver DNA alkylating agents to the cartilage tumour tissue. We engineered the quaternary ammonium derivative of melphalan (Mel-AQ) and assessed its antitumoural activity in vitro and in vivo. In vitro, micromolar concentrations of Mel-AQ inhibited the proliferation of human HEMC-SS chondrosarcoma and Saos-2 osteosarcoma cell lines. Moreover, 24-h incubation with 20 μM Mel-AQ induced a 2.5-fold increase in S population and a 1.5-fold increase in subG0G1 population compared to controls. In vivo, Mel-AQ demonstrated antitumour activity in the orthotopic model of primary Swarm rat chondrosarcoma. When given to chondrosarcoma-bearing rats (three doses of 16 μmol/kg at days 8, 12 and 16 post-implant), Mel-AQ demonstrated an optimal antitumour effect at day 43, when tumour cell growth inhibition peaked at 69%. Interestingly, the treatment protocol was proved well tolerated, since the animals showed no weight loss over the course of the study. This antitumoural effect was assessed in vivo by scintigraphic imaging using ^{99m}Tc-NTP 15–5 developed in our lab as a PG-targeting radiotracer, and tumour tissue was analyzed at study-end by biochemical PG assay with Alcian blue staining. Mel-AQ treatment led to a significant decrease in the PG content of tumoural tissue. These experimental results highlighted the promising antitumour potential of Mel-AQ as a PG-targeting strategy for therapeutic management of chondrosarcoma.

Keywords Antitumour activity · Swarm Rat chondrosarcoma · Orthotopic model · Proteoglycans · ^{99m}Tc-NTP 15–5 radiotracer · Quaternary ammonium · Melphalan

Introduction

Chondrosarcoma is characterized by the formation of cartilage-like extracellular matrix and accounts for 25% of all bone cancers. Since the tumour is made chemo- and radio-resistant by the poorly vascularized and chondrogenic nature of the tissue, the only therapeutic control of chondrosarcoma currently available is radical surgery [1–3]. Among the numerous strategies aimed at increasing in-target drug accumulation, "vectors" with high specific affinities for one or more targets offer one of the most promising therapeutic opportunities [4]. Our team has built up recognized expertise in vectorization strategies through work identifying chemical carriers able to selectively

C. Peyrode (✉) · V. Weber · A. Vidal · P. Auzeloux · S. Besse · J. M. Chezal · E. Miot-Noirault
UMR 990 INSERM/ UdA, Université d'Auvergne,
Rue Montalembert, BP184, 63005 Clermont Ferrand Cedex, France
e-mail: caroline.peyrode@inserm.fr

E. David · F. Gouin · D. Heymann · F. Rédini
UMR S957 Inserm, Nantes Atlantique Université,
44035 Nantes, France

Y. Communal · M. M. Dauplat
CLCC Jean Perrin,
63001 Clermont-Ferrand, France

deliver radionuclides and drugs to tissues for targeted imaging and therapy [5, 6]. Our chondrosarcoma management-oriented strategy is to exploit the chondrogenic nature of the tumour in order to selectively deliver drugs or radioisotopes to tumoural tissue.

We focused on the proteoglycan matrix of chondrosarcoma, which is mainly composed of aggrecan-type proteoglycans (PG) [1, 7]. Aggrecan is a complex macromolecule that consists of a central core protein to which numerous chondroitin sulphate and keratan sulphate glycosaminoglycan chains are covalently bound. Due to the high sulphate and carboxylate group content of their glycosaminoglycan moieties, the PG of the extracellular matrix (ECM) have strong negative charges that may interact with the positively-charged quaternary ammonium function [8–10].

We have developed cartilage-targeting compounds for therapeutic or imaging purposes by linking a quaternary ammonium function to anti-inflammatory drugs, chondroprotective agents, cytotoxic agents and ^{99m}Tc chelate. Each of these approaches led to high in-joint compound concentrations after in vivo administration [6, 11–13].

Here, we focused on the quaternary ammonium derivative of melphalan (Mel-AQ), which previously been demonstrated to exhibit high accumulation in cartilage in vivo [6].

This study established both in vitro and in vivo that the anticancer activity profile of Mel-AQ enables selective treatment of chondrosarcoma. In this paper, Mel-AQ was assessed on the basis of (a) in vitro growth-inhibitory activity and cell cycle arrest in chondrosarcoma cell lines and (b) in vivo antitumour potency in an orthotopic model of primary grade-II chondrosarcoma. These preclinical results underlined the potential of a melphalan-based PG-targeting strategy as a promising approach for efficacious therapeutic management of chondrosarcoma.

Materials and methods

Chemistry

The quaternary ammonium-melphalan conjugate was prepared as previously described [14], following the four-step scheme illustrated in Scheme 1. First, melphalan was treated by di-*tert*-butyl dicarbonate with Et_3N in methanol to protect the amine function. Subsequently, reaction with 3-(dimethylamino)-propylamine, dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in dichloromethane (CH_2Cl_2) afforded the amide **2** in moderate yield. Treatment with methyl iodide in ethanol gave the quaternary ammonium **3**. Finally, the t-Boc-protecting group was removed by 2 N ethanolic hydrochloride solution, giving the target QA derivative **4** from **1** in an overall yield of 39%.

Drug preparation

For in vitro studies, Mel-AQ was dissolved in dimethylsulphoxide (DMSO) (Sigma Aldrich, France).

For in vivo studies, Mel-AQ was dissolved in normal saline (Versol Sodium Chloride 0.9% 500 mL) with 2% of ethanol.

Cell cultures

HEMC-SS human chondrosarcoma and Saos-2 human osteosarcoma cell lines were ordered from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and the American Type Culture Collection (ATCC, Manassas, USA), respectively. Chondrocytes were ordered from PromoCell (Heidelberg, Germany). The transplantable M4Beu human melanoma cells were obtained from Pr. J. F. Dore (Centre Léon Berard, Lyon, France).

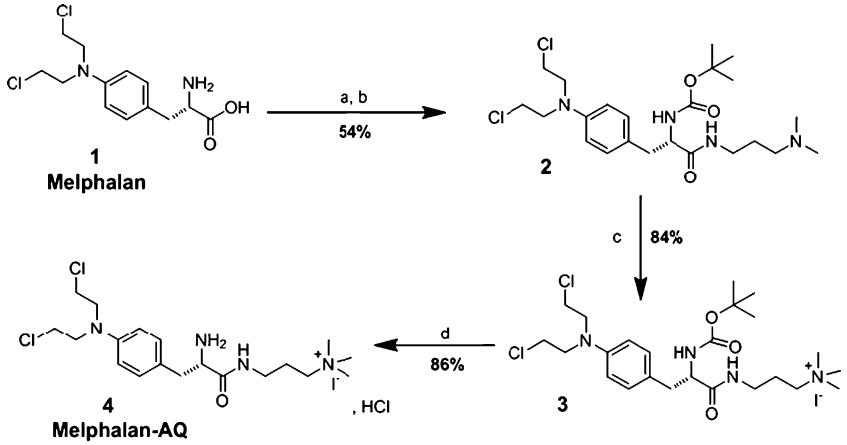
Cell lines were maintained as a monolayer culture in culture media, as follows:

- HEMC-SS cells with DMEM/F12 (v/v) (Dutcher, Brumath, France) supplemented with 10% foetal calf serum (Biowest, Nuaillé, France), 4 mg/L gentamicin (Dutcher) and glutamine 2 mM (Fischer, Illkirch, France).
- Saos-2 cells with McCoy's 5A Medium (Dutcher) supplemented with 10% foetal calf serum (Biowest) and 4 mg/L gentamicin (Dutcher).
- Chondrocytes with Chondrocyte Growth Medium (PromoCell) supplemented with the SupplementMix (PromoCell).
- M4Beu cells with MEM+ glutamax (Fischer) supplemented with 4 mg/L gentamicin (Dutcher), 10% foetal calf serum (Biowest), 1% vitamin solution (Fischer, 100×), 1% non-essential amino acids (Fischer, 100×) and 1% sodium pyruvate (Dutcher, 100 mM).

In vitro evaluation of growth-inhibitory activity and cell cycle distribution

Cells were plated in 96-well microplates (Nunclon™ Surface 167008) at a density of 15×10^3 cells in 150 μL of corresponding medium, and were allowed to adhere for 24 h before treatment. Fifty microliters of increasing concentrations of Mel-AQ in DMSO (Sigma-Aldrich, France) were then added, maintaining final DMSO concentration at 0.5% (*v*/*v*). The cells were incubated for 72 h with or without the drug. The cytotoxic effect of the drug was assessed by the resazurin reduction test as described in [15]. Fluorescence was measured on an automated 96-well Fluoroskan Ascent FL™ plate reader (Labsystems) at an excitation wavelength of 530 nm and an emission wave-

Scheme 1 Synthesis of melphalan-AQ



Reagents and conditions: (a) di-*tert*-butyl dicarbonate, Et_3N, MeOH, room temperature; (b) 3-(dimethylamino)propylamine, DCC, HOBt, CH_2Cl_2, room temperature; (c) methyl iodide, dry EtOH, room temperature; (d) 2N EtOH/HCl anhydrous solution, room temperature.

length of 590 nm. Cytotoxic activity was expressed as the drug concentration that inhibited cell growth by 50% (IC_{50}).

For the HEMC-SS human chondrosarcoma line, effects on cell cycle distribution were evaluated cytometrically. 10×10^4 HEMC-SS cells were plated in 25 cm^2 flasks 72 h before adding DMSO or Mel-AQ at increasing concentrations for 24 h of incubation, or at IC_{50} concentration for different incubation times (from 5 h to 96 h). After incubation, the cells were harvested by scraping. The cell suspension containing both floating and adherent cells was centrifuged twice (400 g, 8 min at +4°C) in phosphate buffer solution (PBS) (DPBS 1× Invitrogen). The dry pellets were then frozen in liquid nitrogen and stored at −80°C until analysis. After thawing, the cell pellets were resuspended in 500 μL of ribonuclease 1 (1 mg/mL) added with 500 μL of propidium iodide (1 mg/mL) (Sigma Aldrich, France). The cell cycle distribution was analyzed using a flow cytometer (Coulter Epics XL, Coulter, Hialeah, Fl, USA) at 488 and 620 nm wavelengths.

Orthotopic model of Swarm Rat Chondrosarcoma (SRC)

The SRC line was a generous gift from Dr. Patrick A. Guerne (Geneva, Switzerland). It was delivered as tissue fragments which were frozen until use. The male Sprague–Dawley rats (Charles River, L'Arbresles, France) used for this study were handled and cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* [16] and Council Directive 86/609/EEC. Protocols were led under the authorization of the French Directorate of Veterinary Services and were conducted under the supervision of authorized investigators in accordance with the guidelines for the use of laboratory animals in cancer research [17].

For tumour implantation, the rats were anaesthetized by inhalation of isoflurane (CSP, Cournon d'Auvergne, France) in air (1.5%, 1 L/min) plus an intramuscular injection of 100 mg ketamine (Imalgène; Rhone Merieux, France) per kilogram of body weight.

Tumour fragments were collected from well-developed tumours on the paratibial area of donor Sprague–Dawley rats. These fragments were immediately stored in ice-cold α-minimum essential medium and manually calibrated to 10 mm^3. Allograft transplantation of a 10-mm^3 SRC fragment was performed on the right paw, the other paw being used as the controlateral control [18]. Using a lateral approach, the cortical surface of the diaphysis was scarified laterally over 10 mm, a 10-mm^3 SRC fragment was placed adjacent to the scarified surface, and the muscular and cutaneous wounds were sutured.

In vivo antitumour activity of Mel-AQ

Chondrosarcoma-bearing rats were randomized into two groups: a control group (n=6) and a Mel-AQ-treated group (n=6). Mel-AQ-treated animals received three Mel-AQ doses (16 μmol/kg per dose) at 4-day intervals (q4d × 3 schedule) injected by i.v. route, beginning on day 8 post-implant. Control animals received the excipient by i.v. route according to the same schedule. Animal weight and tumour size (two perpendicular diameter measurements) were recorded twice a week.

- The Tumour Volume (TV) of each tumour was estimated in two dimensions using the formula:

$$TV(mm^3) = (L \times W^2)/2$$

where L is length in mm, and W is width in mm.

TV values were averaged within each group at each time-point.

The T/C parameter was calculated over the period of study as the ratio of the mean TV of Mel-AQ-treated rats versus controls [19]:

$$T/C = (\text{TV of treated group on Day X}/\text{TV of control group on Day X}) \times 100$$

Tumour growth inhibition percentage (TGI) could therefore be expressed as:

$$TGI = 100 - T/C$$

- Weight variation was calculated according the formula:

$$((\text{Weight at Day x} - \text{Weight at Day 8})/\text{Weight at Day 8}) \times 100$$

Weight variations were averaged within each group at each time-point.

In vivo ^{99m}Tc-NTP 15–5 scintigraphic imaging of chondrosarcoma-bearing rats

Scintigraphic in vivo imaging was performed using a small-animal γ camera (CsI (Na) crystal) equipped with a 1.3/0.2/35 parallel-hole collimator (hole diameter/septum thickness/height in mm) (Gammaimager®, Biospace, France). The energy resolution and intrinsic planar resolution of the system are given as 11% at 140 keV and <2 mm full width at half maximum (FWHM), respectively. All acquisitions were performed with a 15% window centred on the 140-keV photopeak of ^{99m}Tc.

Each animal was submitted to three serial examinations with the ^{99m}Tc-NTP 15–5 radiotracer at Day 7, Day 17 and Day 30 of the protocol. The ^{99m}Tc-*N*-[triethylammonium]-3-propyl-15ane-N5 (^{99m}Tc-NTP 15–5) radiotracer was prepared and radiolabelled as previously published (specific radioactivity of 25 MBq/μmol), and administered by i.v. route to anaesthetized animals (40 MBq/animal) [20]. The animals were anaesthetized intraperitoneally with a mixture of ketamine (50 mg/kg) (Imalgène 500, Merial, France) and xylazine (10 mg/kg) (Rompun 2%, Bayer, France).

Acquisition was performed 15 to 30 min after i.v. tracer administration, with a 10-min planar acquisition for each posterior paw positioned over the camera collimator. Scintigrams were considered positive when tracer uptake areas corresponded to sites of implant. All the scans were evaluated by the same experienced investigator, using fixed-size regions of interest (ROIs) delineated over tumour and muscle patterns. Total activity counts in cpm (counts per minute) per pixel were obtained for each ROI. At each time-point and for each animal, tumour-to-muscle ratio was calculated as follows:

$$T/M = \text{count in tumour}/\text{count in muscle}$$

Data were averaged within each group, and expressed as means ± standard deviation.

Quantification of glycosaminoglycans (GAG)

A biochemical assay for GAG was performed on tumours that had been excised at the end of study then rapidly frozen in liquid nitrogen and stored at −80°C. Each tumour sample was weighed and ground to powder, then solubilized in 300 μL of a digestion buffer containing 0.6 mg/mL papain—0.25 mg/mL dithiothreitol (DTT) in phosphate buffer (20 mM NaH_2PO_4, 20 mM Na_2HPO_4, 1 mM EDTA, pH 6.8) and incubated 24 h at 60°C. Sulphated GAG content was determined using a Blyscan Proteoglycan and Glycosaminoglycan assay kit (Biocolor Ltd, Belfast, Ireland).

Histology

Excised tumours of both control and Mel-AQ-treated groups were assayed for the presence of PG after Alcian blue staining.

Results

Mel-AQ inhibited tumour cell growth in bone cancer cell lines in vitro (Fig. 1)

Mel-AQ was found cytotoxic against HEMC-SS and Saos-2 cell lines at micromolar level, with IC_{50} values of 14.0±8.5 and 37.5±3.5 μM, respectively. Mel-AQ did not show any cytotoxic activity in human chondrocytes (IC_{50} >> 150 μM) or M4Beu human melanoma (IC_{50}>150 μM).

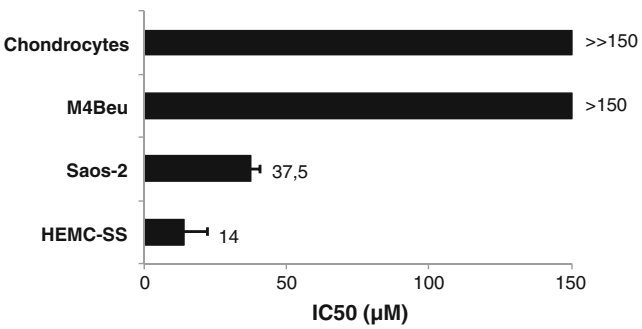


Fig. 1 Cell growth inhibition by Mel-AQ in vitro, in chondrocytes, M4Beu human melanoma, Saos-2 human osteosarcoma and HEMC-SS human chondrosarcoma cell lines. Cells were treated for 72 h, and cytotoxic effect was evaluated by resazurin reduction test. IC_{50}: drug concentration inhibiting 50% of cell proliferation (mean ± SD)

Mel-AQ induced cell cycle arrest in HEMC-SS chondrosarcoma cells in vitro (Figs. 2 and 3)

As illustrated in Fig. 2, Mel-AQ treatment for a 24-h incubation time led to an increase in cells accumulated in the S and subG0G1 phases and a decrease in cells accumulated in the G0/G1 phase. Compared to controls, at 20 μM dose, Mel-AQ induced a 2.5-fold increase in S population, a 1.5-fold increase in subG0G1 population (indicating apoptosis), and a 1.5-fold decrease in G0/G1 population.

The kinetics of cell cycle arrest were studied at a drug concentration of 14 μM (IC_{50} value) (Fig. 3). Significant differences were observed between treated cells and controls for the S, subG0G1 and G2M populations, i.e. at 48 h, a 2.5-fold increase, a 3.8-fold increase and a 2.2-fold decrease in the S, SubG0G1 and G0/G1 populations, respectively, and at 96 h, a 1.9-fold, a 16.3-fold increase and a 3.9-fold decrease in the G2M, SubG0G1 and G0/G1 populations, respectively.

Mel-AQ demonstrated antitumour activity in vivo in the orthotopic model of SRC (Fig. 4)

Mel-AQ was given at 16 μmol/kg according to a q4d × 3 schedule beginning on day 8 post-tumour implant. CTV values were significantly lower in the Mel-AQ-treated group than in controls from day 21 (384±145 vs 1,023±227; p=0.02) (Fig. 4a). Antitumour effect was optimal at day 43, with a T/C value of 31% corresponding to a 69% inhibition of tumoural growth. As shown in Fig. 4b, no weight loss was associated with the treatment.

In vivo ^{99m}Tc-NTP 15–5 imaging of tumours evidenced changes in radiotracer uptake in Mel-AQ-treated animals vs controls (Fig. 5)

At day 7 (before treatment), all animals showed a high and selective in-tumour accumulation of ^{99m}Tc-NTP 15–5, enabling highly contrasted chondrosarcoma imaging in vivo, with T/M values of 1.53±0.23 for the Mel-AQ-treated group and 1.33±0.23 for the control group.

^{99m}Tc-NTP 15–5 imaging was strongly affected by Mel-AQ treatment: when animals were examined at day 17, i.e. 24 h after the last dose of Mel-AQ or excipient, T/M value was significantly lower (p<0.02) than in controls (1.47±0.16 vs 2.13±0.21). This lower radiotracer accumulation in the Mel-AQ-treated group vs controls was still observed at day 30, i.e. 14 days after the last dose, as shown in Fig. 5b and c (T/M values at day 30: 1.73±0.36 for Mel-AQ rats vs 2.34±0.54 for controls).

PG in tumour tissue was lower in Mel-AQ-treated chondrosarcoma vs controls. (Fig. 6)

Biochemical assay highlighted a lower tumour tissue PG content in the Mel-AQ-treated group compared to controls, i.e. 6.1±1.1 vs 7.5±2.4 μg/mg of tumour, respectively (Fig. 6a). This difference in PG content in Mel-AQ-treated tumours vs controls was confirmed by Alcian blue staining, as illustrated in Fig. 6b and c.

Discussion

Improving tumour selectivity is a priority in the development of new anticancer agents designed to deliver an effective high concentration of cytotoxic agent to target tumour tissue while protecting non-target tissue from toxic side effects. In these approaches, established antineoplastic drugs can be chemically modified to improve their selectivity [4, 21]. This was the strategy employed here: by linking a quaternary ammonium function (exhibiting a high affinity for proteoglycans) to a conventional DNA alkylating agent, we expected to enhance both the delivery

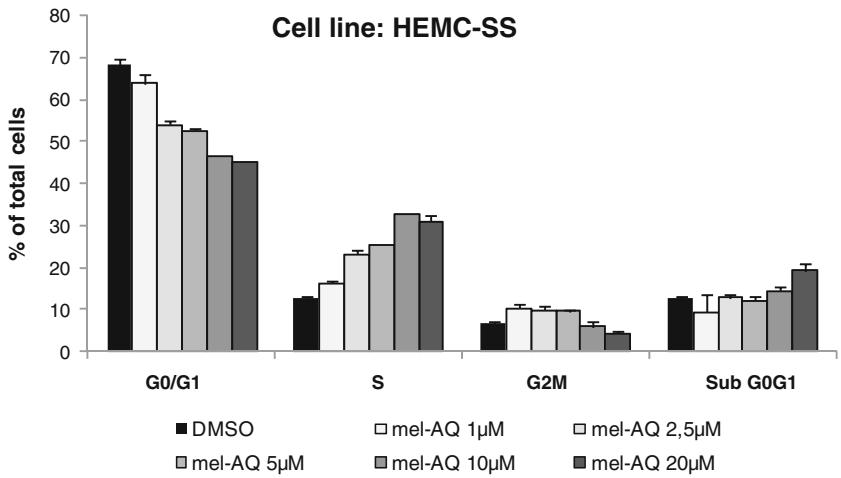


Fig. 2 Cell cycle distribution in HEMC-SS human chondrosarcoma cells treated with Mel-AQ for 24 h in two different experiments (mean±SD). Cells were treated at different concentrations while controls were treated with DMSO

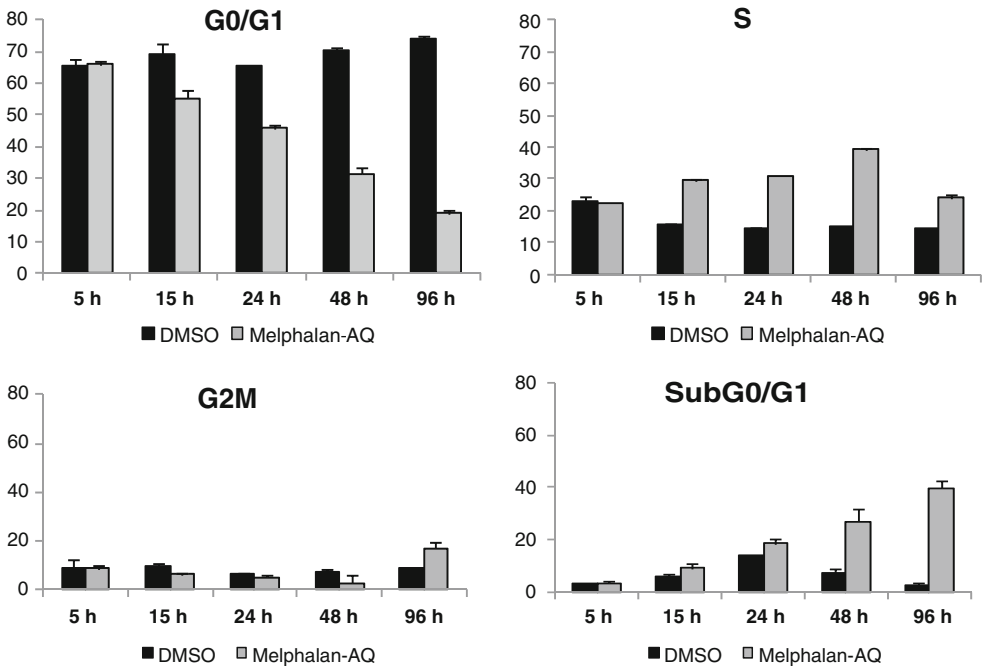


Fig. 3 Cell cycle distribution in HEMC-SS human chondrosarcoma cell line treated with Mel-AQ in two different experiments (mean±SD). Cells were treated with 14 μM Mel-AQ or DMSO for 5 h, 15 h, 24 h, 48 h or 96 h

and the specificity of cytotoxic agents in chondrosarcoma tissue.

The high sulphate and carboxylate group contents of the GAG moieties lends chondrosarcoma ECM PG strong negative charges that may interact with the positively-charged quaternary ammonium moiety of Mel-AQ. Mel-AQ was shown to target cartilaginous tissues in healthy rats as unchanged compound or metabolite while keeping the pharmacologically-active mustard group [4]. Based on these previous promising results, we decided to evaluate the antitumour potential of Mel-AQ.

In a first step, the in vitro cytotoxic profile of Mel-AQ was characterized. Interestingly, Mel-AQ demonstrated a cytotoxic activity in bone tumour Lines like HEMC-SS and Saos-2 cell lines, whereas no effect was observed with M4Beu melanoma cells or chondrocytes. For HEMC-SS and Saos-2 lines, IC_{50} values ranged from 10 to 40 μM. Interestingly, the human HEMC-SS chondrosarcoma line exhibited the highest sensitivity to Mel-AQ, with an IC_{50} of 14.0±8.5 μM. When HEMC-SS chondrosarcoma cells were incubated in vitro with Mel-AQ at various concentrations, we observed drug exposure-time-dependent cycle arrest in both S and G2M phases, leading to a significant increase in cell accumulation in SubG0G1 population. Mel-AQ was then assessed for antitumour challenge in vivo using the syngeneic Swarm rat chondrosarcoma (SRC) model. Syngeneic in vivo tumour models are widely used for in vivo initial drug discovery research, since the animals have the same genetic background as the tumour cell line, thus precluding of risk of graft rejection [22]. We chose the SRC model as it has been extensively characterized with large aggregating proteoglycans that have been described as a major component of the chondrosarcoma ECM of SRC [23, 24]. Since experiments with the SRC model have

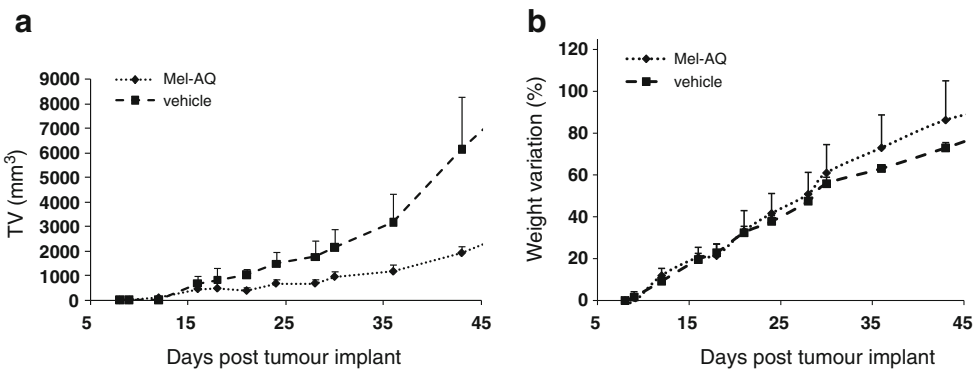


Fig. 4 In vivo assessment of antitumour activity (**a**) and tolerance (**b**) of Mel-AQ in SRC models. Treatments were given by i.v. route according to a "q4d x 3" schedule beginning on day 8 post-implant. Values are presented as means±SD

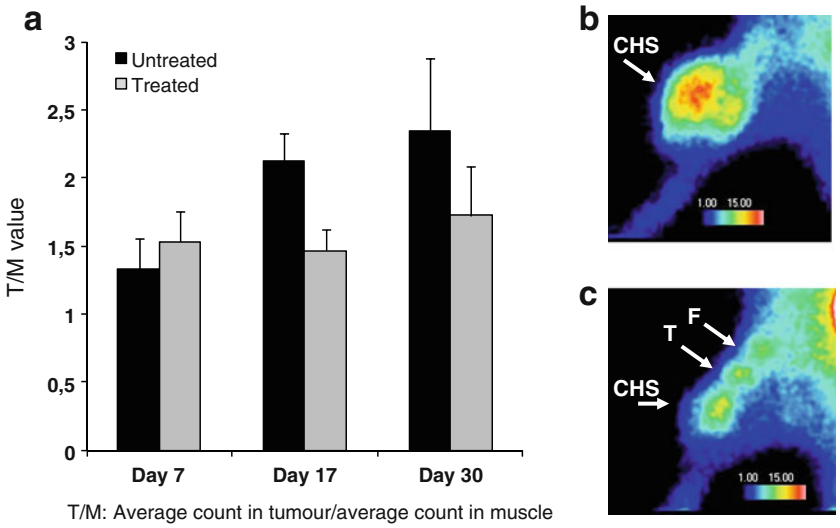


Fig. 5 ^{99m}Tc-NTP 15–5 scintigraphy. **a** Quantitative analysis of ^{99m}Tc-NTP 15–5 accumulation in tumours versus muscle at day 7 post-implant (before treatment) and at day 17 and day 30 post-implant (after treatment). **b** Scintigraphic images obtained at day 30 post-implant on a representative control. **c**: Scintigraphic images obtained at day 30 post-implant on a representative Mel-AQ-treated rat. Abbreviations: *F* femoral condyle, *T* tibial plateau, *CHS* chondrosarcoma

demonstrated that transplantation site can affect tumour grade and malignancy, we used an experimental approach based on primary orthotopic paratibial location [18, 25, 26]. Paratibial tumour implant has been demonstrated to induce well-differentiated malignant chondrosarcoma that features a characteristic lobulated tumour separated by thin vasculo-connective septa. The histological and clinical behaviour of SRC mimics human grade-II disease, with local tumour growth invading and ultimately destroying host bone in late-stage pathology [18]. Based on our experimental results in SRC-bearing rats, Mel-AQ demonstrated a promising antitumour activity compared to controls: the treatment regimen used (three doses of 16 µmol/kg at days 8, 12 and 16 post-implant) showed a TGI ranging from 42% to 69% from day 18.

Interestingly, the treatment protocol proved well tolerated, since the animals showed no weight loss over the course of the study. Antitumour effect was also assessed in vivo by ^{99m}Tc-NTP 15–5 scintigraphic imaging, which was previously demonstrated in the SRC orthotopic model to provide suitable criteria for monitoring cartilage tumour [20].

Since ^{99m}Tc-NTP 15–5 radiotracer and Mel-AQ contain the same quaternary ammonium function in their structure and are therefore both expected to target PG, thery were not administered simultaneously to the animals in order to avoid any competition. ^{99m}Tc-NTP 15–5 scintigraphic imaging was performed at day 7 (before treatment), day 17 (1 day after the last Mel-AQ dose) and day 30 (14 days after the last Mel-AQ injection). As expected, ^{99m}Tc-NTP 15–5 radiotracer accumulation in tumour tissue enabled

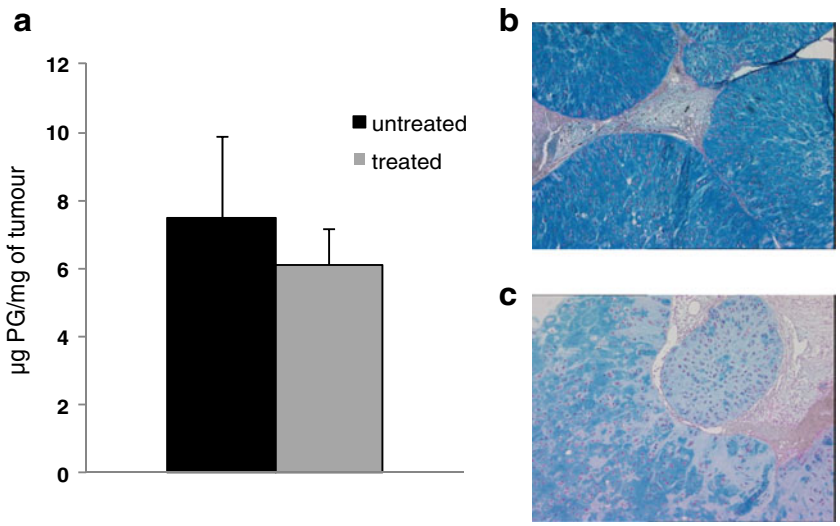


Fig. 6 PG content of chondrosarcoma tissue from Mel-AQ-treated rats and controls at the end of the study. a: PG content in tumoural tissue by biochemical assay. Values are presented as means±SD. **b** Alcian blue staining of chondrosarcoma tissue from an untreated rat. **c** Alcian blue staining of chondrosarcoma tissue from a Mel-AQ-treated rat

highly contrasted scintigraphic imaging of SRC in vivo. Quantitative analysis of scintigraphic scans evidenced a significant decrease in tracer uptake in treated animals compared to controls at day 17 ($p<0.02$). This decrease was still maintained at day 30. We posit that changes in ^{99m}Tc-NTP 15–5 uptake reflect a decrease in the PG content of tumour tissue as a result of a cytotoxic effect of Mel-AQ. The decrease in PG content of Mel-AQ-treated tumours was confirmed by biochemical assay of tumour tissue and Alcian blue staining of histological SRC slices. These results suggest that Mel-AQ treatment could trigger the "downregulation synthesis" of PG in chondrosarcoma tissue in vivo, as already described for other chemotherapy approaches [27].

Conclusion

Cartilage tumours continue to present orthopaedic oncologists with therapeutic challenges. Malignant cartilaginous tumours are generally unresponsive to chemotherapy, with the result that the 5-year survival rate of high-grade chondrosarcoma is only 29% [5]. Based on its high binding capacity to proteoglycans, quaternary ammonium conjugated with melphalan alkylating agent was synthesized, and its potential antitumour activity in chondrosarcoma was tested and characterized in vitro and in vivo. The differential in vitro antitumour activity (tumour chondrocytes versus normal chondrocytes) of Mel-AQ, its ability to inhibit tumour cell growth in vivo, and its good tolerance in animals make Mel-AQ a promising candidate for further work to develop an urgently needed efficacious treatment of chondrosarcoma.

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