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RESEARCH PAPER

Antitumour Efficacy of Two Paclitaxel Formulations for Hyperthermic Intraperitoneal Chemotherapy (HIPEC) in an *In Vivo* Rat Model

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ABSTRACT

Purpose To evaluate the tumour growth delay of a peritoneal carcinomatosis (PC) of colorectal origin after intraperitoneal chemotherapy with paclitaxel/randomly-methylated-β-cyclodextrin (Pac/RAME-β-CD) *versus* Taxol® at normo- and hyperthermic conditions in rats.

Methods Hyperthermic intraperitoneal chemotherapy (HIPEC) was performed 7 days post implantation of the tumour with both formulations at a Pac concentration of 0.24 mg/ml. Tumour evaluation was performed via positron emission tomography (PET) and magnetic resonance imaging (MRI) imaging, measuring tumour activity and tumour volume, respectively. Scans were taken at 2 and 7 days post treatment.

Results PET and MRI data showed a significant reduction in tumour activity and tumour volume for rats treated with Pac/RAME-β-CD (at normo- and hyperthermic conditions), compared to the control group. Treatment with Taxol® did not result in a significant reduction of tumour activity and tumour volume. No significant differences between the normo- and hyperthermic conditions were observed for both formulations, indicating that hyperthermia and paclitaxel were not synergistic despite the direct cytotoxic effect of hyperthermia.

Conclusion Monitoring tumour growth via PET and MRI indicated that Pac/RAME-β-CD inclusion complexes had a significantly higher efficacy compared to Taxol® in a rat model for peritoneal carcinomatosis.

KEY WORDS hyperthermic intraperitoneal chemotherapy · paclitaxel · tumour growth delay · β-cyclodextrin

INTRODUCTION

Peritoneal carcinomatosis (PC) is, in most cases, a secondary cancer. For example, PC is present in up to 80% of patients with terminal colorectal cancer (1), and peritoneal spread is diagnosed in the majority of ovarian cancer patients (2). PC is associated with poor prognosis and low quality of life, with patients having a median survival of six months (3).

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The combination of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) has shown promising results for the treatment of PC (4). Recently, an eight-year follow-up study in patients with PC of colorectal origin indicated a significant increase in survival after HIPEC with mitomycin C: five-year survival of 45% in fully resected patients (5). This treatment, in combination with complete cytoreduction, offers new hope to PC patients.

In HIPEC, higher drug concentrations are administered compared to intravenous (IV), resulting in higher drug concentrations at the cancer nodules. Following intraperitoneal (IP) and IV administration of paclitaxel to humans during a Phase I clinical trial, the IP concentration was 1000-fold higher than the IV concentration (6), making paclitaxel a very promising molecule for IP administration. HIPEC also combines the cytotoxic actions of hyperthermia and chemotherapy, offering in some cases a synergistic antitumoural effect (oxaliplatin, mitomycin C, doxorubicin and cisplatin) (7). Besides the previously mentioned pharmacokinetic advantage, paclitaxel (Pac) is an interesting drug molecule suitable for IP chemotherapy, due to its high molecular weight and its liver metabolism.

However, due to its low aqueous solubility Pac is currently formulated using a Cremophor EL®/ethanol mixture (1/1, v/v), commercialised as Taxol®. This formulation can cause local toxic effects (e.g. abdominal pain) and life-threatening hypersensitivity reactions, prompting the need for a new and safer paclitaxel formulation (6, 8). Therefore, previously a cosolvent- and tensioactive-free paclitaxel formulation was developed consisting of Pac/randomly methylated-β-cyclodextrins (RAME-β-CD) inclusion complexes, which are dissolved in a phosphate-buffered saline solution with 0.1% hydroxypropylmethylcellulose (HPMC) (9). This cyclodextrin-based paclitaxel formulation (Pac/RAME-β-CD) showed an *in-vitro* antitumour efficacy similar to Taxol®, while RAME-β-CD was significantly less cytotoxic compared to Cremophor EL (10). When tested during HIPEC in an *in vivo* rat model, this Pac/RAME-β-CD formulation had a similar maximum tolerated dose (MTD) (i.e. 0.24 mg paclitaxel/ml in the perfusate) as Taxol®, while its bioavailability was significantly higher compared to Taxol® (40-fold increase of C_{max} and AUC_{90min}) (11). This higher bioavailability indicated that *β*-cyclodextrins improved the penetration of paclitaxel through the peritoneum. In order to understand whether the increase in bioavailability affected the antitumour efficacy, this study compares Pac/RAME-β-CD and Taxol® following intraperitoneal administration at normo- and hyperthermic conditions in a rat model with PC of colorectal origin.

MATERIALS AND METHODS

Materials

The following raw materials were used: randomly methylated-β-cyclodextrin (RAME-β-CD) with a total degree of substitution (TDS) of 13 from Cyclolab (Budapest, Hungary), paclitaxel (Pac) from Acros Organics (Geel, Belgium) and Taxol® (containing 6 mg/ml Pac dissolved in Cremophor EL/ethanol (50/50,v/v)) from Bristol-Myers Squibb (Brussels, Belgium).

The inclusion complexes between RAME-β-CD and Pac were prepared, as previously described, via a freeze drying method (9). After freeze drying, a powder for reconstitution was obtained. The reconstitution medium consisted of phosphate-buffered saline (PBS) (Sigma, Bornem, Belgium) with 0.1% hydroxypropylmethylcellulose (HPMC) (Metolose® 60SH-4000, Shin-Etsu, Tokyo, Japan).

HIPEC Procedure

This study was approved by the ethical committee for animal tests of the Faculty of Medicine (Ghent University, ECD 03/23). Adult Wag/Rij rats (Harlan, Horst, The Netherlands) of at least 270 g body weight were kept under the following conditions: access *ad libitum* to food and water, 12-hour day-night cycle and 24°C room temperature. The HIPEC procedure was performed 7 days after implantation of the tumour tissue in the rat. The animals were anaesthetized with isoflurane (Forene®, Abbott, Louvain-La-Neuve, Belgium). An incision was made in the abdomen and in- and outlet tubings (Marprene® and Pumpsil® (Watson-Marlow, Zwijnaarde, Belgium) for Pac/RAME-β-CD and Taxol® administration, respectively) were placed in the peritoneal cavity for perfusion with the cytostatic solution, containing 0.24 mg/ml paclitaxel (= 0.28 mM) for both formulations, during 45 min. The administered dose is the maximum tolerated dose previously determined during HIPEC treatment with Pac/RAME-β-CD and Taxol® (11). A roller pump (Watson-Marlow®, Zwijnaarde, Belgium) circulated the perfusate at a flow rate of 30 ml/min through a heat exchanger, ensuring a temperature of 37°C (normothermic condition) or 41°C (hyperthermic condition, selected based on literature data) (4, 11, 12). During perfusion both body and perfusate temperature were closely monitored by thermosensors (ELLAB®, Roedovre, Denmark), and data were collected using E-Val® 2.10 software (ELLAB®, Roedovre, Denmark). After HIPEC procedure, the solution was removed from the peritoneum, and the incision was sutured.

The paclitaxel concentration in the perfusion solution was monitored (at 0, 15, 30 and 45 min) using a validated HPLC-UV/VIS method. The HPLC-system (Merck-Hitachi,

Tokyo, Japan) consisted of a pump (L-6000), an integrator (D-2000), an autosampler (L-7200) with a 25 μL loop and a UV/VIS detector (L-4200). Detection was performed at a wavelength of 227 nm. Chromatographic separation was achieved with a guard column (Lichrospher® 100-RP-18, 4*4 mm (5 μm), Merck, Darmstadt, Germany) and an analytical column (Lichrospher® 100-RP-18, 125*4 mm (5 μm)). The mobile phase consisted of acetonitrile (Biosolve, Valkenswaard, The Netherlands) and 0.1% (v/v) phosphoric acid in water (Acros Organics, Geel, Belgium) (42:58, v/v). A calibration curve was validated for a concentration ranging from 1 to 100 μg paclitaxel/ml.

Tumour Model

A CC531s rat colon adenocarcinoma cell line, which has previously been used as a model to investigate peritoneal dissemination of a colorectal cancer (13), was obtained from the Laboratory of Experimental Oncology (University Antwerp, Belgium). The cell line was grown in culture flasks (Sarstedt, Newton, NC, USA) at 37°C in a humidified atmosphere with 5% CO_2 using RPMI 1640 medium, buffered with HEPES (20 mM) (Invitrogen Corporation®, Gibco®, Ghent, Belgium) and supplemented with 10% fetal calf serum, 4 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin. Cell suspensions with a cell concentration of 2,000,000 cells in 0.2 mL PBS were injected subcutaneously in the upper hind leg of Wag/Rij rats to induce a tumour. After 4 weeks, tumours of about 1 cm size were excised, and tissue samples (5×5 mm, with 2–3 mm thickness) were transplanted on the parietal peritoneum (covering the abdominal muscle) of an acceptor rat via laparotomy.

Tumour Imaging

Tumour characteristics were evaluated using PET and MRI imaging 6, 9 and 14 days after implantation of the tumour (i.e. 1 day before and 2 and 7 days after HIPEC treatment with paclitaxel as the HIPEC procedure was performed 7 days after implantation). Animals were randomly divided into 6 groups, and each group consisted of 6 animals: (a) Taxol® 37°C, (b) Taxol® 41°C, (c) Pac/RAME-β-CD 37°C, (d) Pac/RAME-β-CD 41°C, (e) blank control group, which received no treatment, (f) HIPEC control group, which received HIPEC treatment at 41.5°C using PBS as perfusion solution.

Determination of Tumour Activity Via FDG-PET

Tumour activity was monitored by positron emission tomography (μPET) imaging after administration (IV) of 1 mCi 2-(fluorine-18)-fluoro-2-deoxy-D-glucose (FDG) 30 min prior to 30 min static μPET acquisition (Supplementary Material Illustration 1). Rats were fasted a minimum of 4 hrs prior to IV administration of FDG and anaesthetized with isoflurane (Forene®, Abbott, Louvain-La-Neuve, Belgium).

Image acquisition was performed with a spatial resolution of 1 mm using Gamma Medica-Ideas labPET 8 (Gamma Medica-Ideas Inc, Northridge, California, USA). Tumour activity was determined based on the ratio of the maximum voxel value of the volume-of-interest (VOI) in the tumour to the maximum voxel value of the VOI in the liver, hereby assuming that the liver has a uniform uptake. To enable the latter, an a posteriori three-dimensional Gaussian filter of 1×1×1 mm kernel width was applied to all reconstructed images. The ratio value of the first measurement of a tumour is considered as a baseline value to which later measurements of the ratio are compared and expressed in percentage. Taking a ratio avoids quantification errors such as extravascular radioactivity in the tail. The maximum voxel value is the truest measure of the actual activity within the region (14). Taking an average value would introduce errors due to partial volume effects, as the counts from the edge of the tumour are reduced because counts from surrounding tissues will be included. Regions-of-interest (ROIs) were accordingly delineated in the tumour and in the liver on several slices generating a VOI. For the liver, each time, the same VOI was replicated throughout the study.

Determination of Tumour Volume Via MRI

Complementary anatomical information about the tumour was acquired with a Trio 3 Tesla MRI (Siemens, Erlangen, Germany). Rats were anaesthetized with xylazine (Xyl-M 2%, VMD, Arendonk, Belgium) and ketamine (Ketamine 100, CEVA Santé Animale, Brussel, Belgium) using a dose of 10 mg/kg and 90 mg/kg, respectively. The rats were placed head first and prone in a wrist coil to measure the tumour volume. A T1-weighted 3D FLASH sequence was applied with a flip angle of 10°, a repetition time (TR) of 13 ms and echo time (TE) of 4.9 ms to obtain a voxel size of 0.19×0.19×0.4 mm^3 (Supplementary Material Illustration 2). The ROI boundaries were drawn based on the tumour contours for each slice where the tumour was present, hereby generating a volume of interest (VOI) of the tumour consisting of a stack of planar ROIs, which were compiled using PMOD software (PMOD Technologies, Adliswil, Switzerland). The first measurement of the tumour volume is considered as a baseline value to which later measurements of the volume are compared and expressed in percentage.

Statistical Analysis

The Statistical Program for the Social Sciences (SPSS 16.0) was used to analyse the results. Data of day 6 were used as reference

(100%) in the PET and MRI study, and a sample size of 6 animals per treatment group was used. A repeated univariate analysis of variances was performed to investigate the time effect and the interaction between time and treatment on body weight, paclitaxel concentration of the perfusate, tumour activity and tumour volume. A Bonferroni-corrected post-hoc analysis was performed to investigate all possible pairwise comparisons between the repeated measures in each treatment group and between the treatment groups at each time point.

RESULTS

Treatment Characteristics

All treatments (at a dose corresponding to the previously determined maximum tolerated dose for HIPEC in this model (11)) were well tolerated: no mortality occurred, and 2 weeks post treatment all rats had recovered to at least 95% of their starting weight. Weights of the rats (after 2 weeks) in the different treatment groups were 96.8±3.1%, 99.1±3.8%, 102.0±3.0%, 102.2±1.8%, 102.8±2.9% and 103.9±3.6% of their initial body weight for Pac/RAME-β-CD 41°C, Pac/RAME-β-CD 37°C, HIPEC, Taxol® 41°C, Taxol® 37°C and the control group, respectively. The curves displaying the weight per day over a 14-day period after treatment indicated an interaction between time and treatment ($p \leq 0.05$); hence, the different treatments were compared at the individual time points of 14 days post treatment. Despite the limited difference (7.1%) between the minimum and maximum average weights after 14 days, this difference between the control group and Pac/RAME-β-CD 41°C group was statistically significant ($p=0.006$). The other treatments were not significantly different from the control at day 14.

During the HIPEC treatment, a sample of the perfusate was taken every 15 min in order to monitor the paclitaxel concentration for the Pac/RAME-β-CD 37°C, Pac/RAME-β-CD 41°C, Taxol® 37°C and Taxol® 41°C treatments. Statistical analysis of the paclitaxel concentrations at the different time points showed no interaction between time and the different treatments ($p=0.222$). Therefore, the different formulations at the different temperatures could be investigated over the full 45 min. For all treatments, the concentration decreased significantly over time ($p \leq 0.05$), but there was no statistical difference between the different treatments ($p=0.284$). After 45 min treatment with Taxol®, 73.8±11.9% and 71.4±9.3% ($n=6$) of the initial paclitaxel concentration (0.24 mg/ml) remained in the perfusate at normo- and hyperthermic conditions, respectively. Paclitaxel concentrations at the end of the treatment with Pac/RAME-β-CD were 60.2±5.3% and 60.1±9.0% ($n=6$) at normo- and hyperthermic conditions, respectively.

Tumour Growth Delay

Tumour data obtained at day 6 (1 day prior to treatment) were considered as reference values, and the measurements at days 9 and 14 post implantation (i.e. 2 and 7 days after HIPEC treatment) were expressed as a percentage of these values.

Tumours were evaluated via MRI and PET imaging on days 9 and 14. Further time points (i.e. days 21 and 28) were not considered, as in a preliminary study on 3 control animals (= no treatment), PET and MRI data indicated that the tumour growth stopped after 14 days. Based on these observations and the large standard deviation, tumour imaging at days 21 and 28 was excluded from the final study protocol. Furthermore, HIPEC is typically a treatment aimed to have an immediate effect post surgery.

Tumour Activity

Statistical analysis of the data demonstrated that both time and treatment were significant factors ($p=0.03$ and 0.003, respectively). There was, however, no significant interaction between both; therefore, the obtained tumour growth data could be investigated as one single data set. This analysis showed that only Pac/RAME-β-CD (at normo- and hyperthermic conditions) was able to significantly reduce tumour activity ($p=0.003$ and 0.010, respectively), compared to the control group (Fig. 1a). All other treatments did not result in a significant reduction (*vs.* the control group) of tumour activity during the postoperative week (all p-values >0.05) (Fig. 1b, c). Comparison of the different formulations (Taxol® and Pac/RAME-β-CD) at normo- and hyperthermic conditions showed no significant differences (all p-values >0.05), indicating that heat did not have a synergistic effect in either of the two formulations (Fig. 1a, b) during the postoperative week. Although the reduction in tumour activity at both temperatures is higher for Pac/RAME-β-CD than Taxol®, this difference between formulations was not significant.

As the primary analysis identified time as a significant factor, it is evident from the data that the effect of the treatment is more short term. Therefore, this time point (2 days after treatment) was investigated in more detail, and compared to the control group, the tumour activity was significantly reduced with Pac/RAME-β-CD (37 and 41°C) and Taxol® (41°C) (Fig. 2). At this time point, a significant difference was also detected between Pac/RAME-β-CD (37°C) and Taxol® (37°C) ($p=0.046$) (Fig. 2). However, 7 days post operation these differences had reduced and were not significantly different anymore.

Tumour Volume

Tumour volume was monitored via MRI imaging. Analysis of data acquired 2 and 7 days post operation showed that (similar to PET data) time and treatment were significant factors

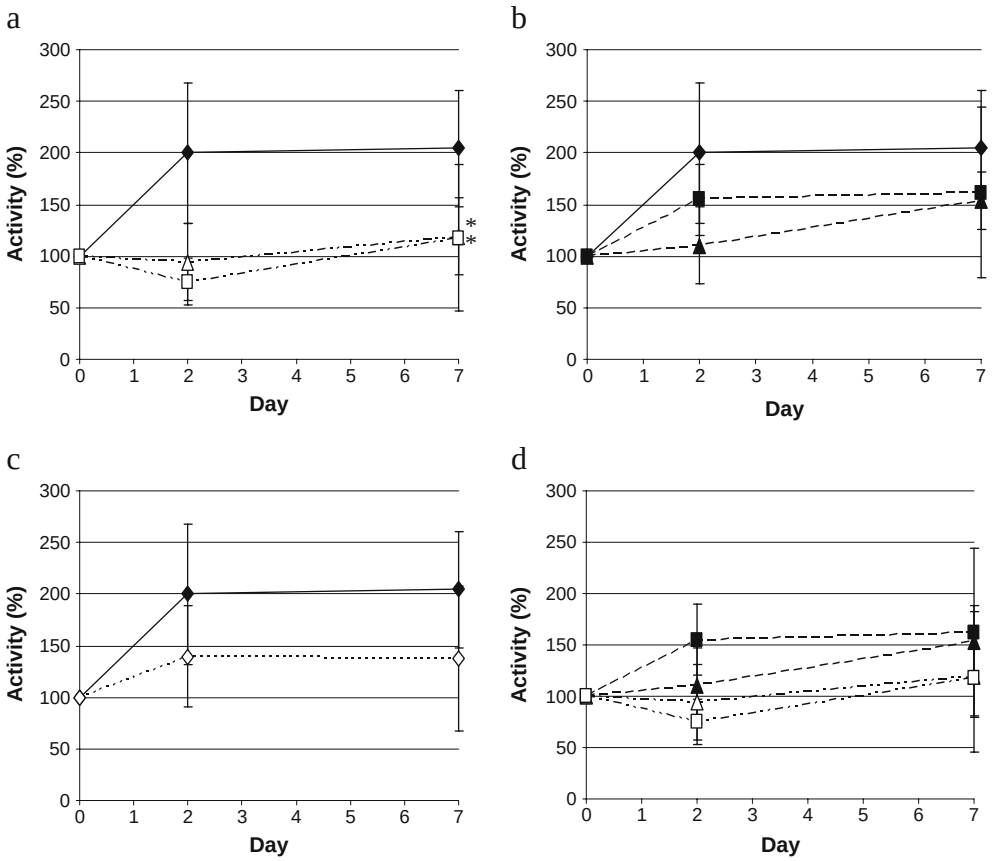

Fig. 1 Tumour activity (%) ± S.D. ($n=6$), measured via PET imaging, during 1 week post treatment in rats with peritoneal cancer. —◆— Control, ·····◇····· HIPEC (saline), — □ ·· Pac/RAME-β-CD 37°C, — △ ·· Pac/RAME-β-CD 41°C, – ■ – Taxol® 37°C, – ▲ – Taxol® 41°C (* treatment *vs* control: $p<0.05$).

($p<0.001$ and $p=0.001$, respectively), but without interaction between both ($p=0.071$), allowing investigation of the data of the postoperative week as a single dataset. MRI data (Fig. 3a, c) showed a significant difference after Pac/RAME-β-CD (normo- and hyperthermic) and HIPEC treatment compared to the control group over a 1-week period ($p<0.0001$, $p=0.003$ and $p=0.025$, respectively). The groups treated with Taxol® (normo- or hyperthermic) had no significant decrease of tumour volume ($p=0.076$ and 0.475, respectively) (Fig. 3b). Furthermore, comparison of the individual treatment groups (excluding the control group) revealed no significant difference between two groups. Similar to the PET data, no significant influence of hyperthermia on the activity of paclitaxel was detected (Fig. 3a, b).

Analysing the data of the individual time points showed again that 2 days after treatment, the effect was the most pronounced, as all treatments reduced significantly the tumour volume (all p-values ≤ 0.05) compared to the control group (Fig. 4), but there were no significant differences between the different treatments (all p-values >0.05). Seven days post treatment only a treatment with the Pac/RAME-β-CD formulation at 41°C significantly reduced the tumour volume compared to the blank group ($p=0.035$).

DISCUSSION

In a previous study, Bouquet et al. (11) demonstrated that paclitaxel formulated with RAME-β-CD had a similar toxicity profile but a higher bioavailability compared to Taxol® after a 45 min HIPEC procedure in healthy rats.

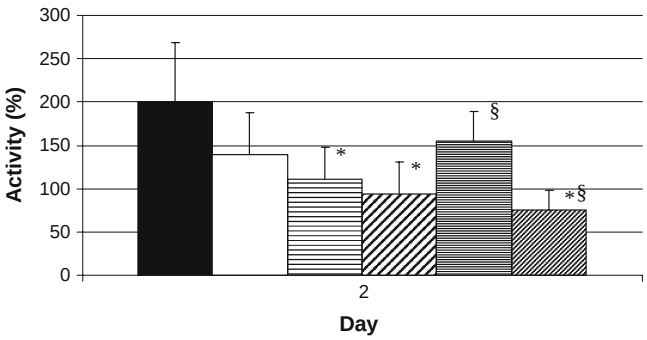

Fig. 2 Tumour activity (%) + S.D. ($n=6$), measured via PET imaging, 2 days post treatment in rats with peritoneal carcinomatosis: ■ Control, □ HIPEC (saline), ▤ Taxol® 41°C, ▨ Pac/RAME-β-CD 41°C, ▤ Taxol® 37°C, ▨ Pac/RAME-β-CD 37°C. (* treatment *vs* control: $p<0.05$) (§ treatment (Taxol® 37°C) *vs* treatment (Pac/RAME-β-CD 37°C): $p<0.05$).

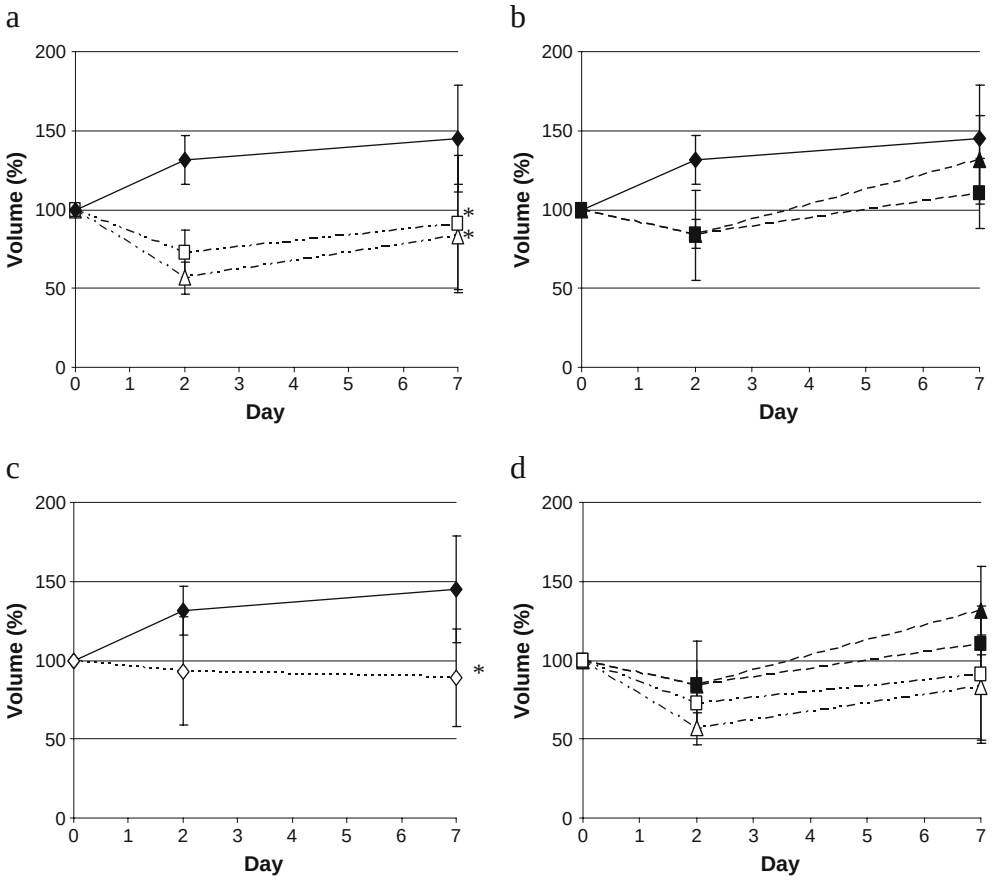

Fig. 3 Tumour volume (%) ± S.D. ($n = 6$), measured via MRI imaging, during 1 week post treatment in rats with peritoneal cancer. —◆— Control, ·····◇····· HIPEC (saline), —·□·· Pac/RAME-β-CD 37°C, —·△·· Pac/RAME-β-CD 41°C, – – ■ – – Taxol® 37°C, – – ▲ – – Taxol® 41°C. (* treatment *vs* control: $p < 0.05$).

The area under the curve (during 90 min) increased 40-fold for the Pac/RAME-β-CD formulation. In the present study, the efficacy of the Pac/RAME-β-CD formulation was evaluated in rats with peritoneal carcinomatosis (PC) of colorectal origin. The weight curves of the rats confirmed the observations of the previous study, as the different treatments were equally well tolerated with all rats reaching ≥95% of their initial body weight 2 weeks after the treatment. Determination of the paclitaxel concentration in the perfusate showed that a similar Pac dose was delivered to the rats, ruling out any differences in efficacy based on differences in administered dose. Bearing this in mind, this study unambiguously shows that after 1 week, only Pac/RAME-β-CD was able to produce a significant reduction in activity and volume of the tumour. In contrast, the treatment with Taxol® produced no significant differences during the post-operative week. The present results also demonstrate that the treatment effect is more pronounced 2 days after treatment than after 1 week.

Although measuring two different parameters (volume and activity), the two biomedical imaging techniques (PET and MRI) provided similar information. When studying the results, it is clear that the MRI results produced lower standard deviations and hereby facilitated statistical analysis of the results. The PET technique is more prone to variation as it consisted of more manipulations in the preparation of the animal. The use of both imaging techniques was here also facilitated by the fact that the

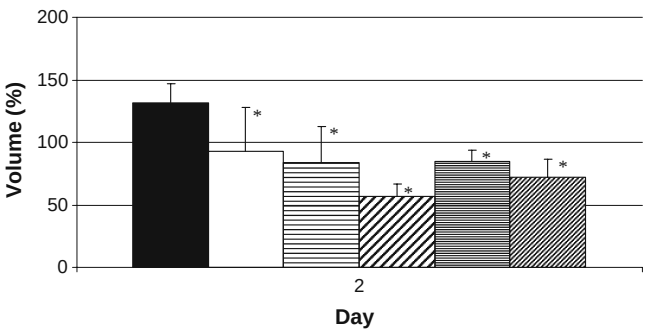

Fig. 4 Tumour volume (%) + S.D. ($n = 6$), measured via MRI imaging, 2 days post treatment in rats with peritoneal carcinomatosis: ■ Control, □ HIPEC (saline), ⊟ Taxol® 41°C, ▨ Pac/RAME-β-CD 41°C, ▤ Taxol® 37°C, ▨ Pac/RAME-β-CD 37°C. (* treatment *vs* control: $p < 0.05$).

PC was limited to one implanted cancer nodule, where in the clinical situation there are numerous nodules which differ in size, making the detection and the evaluation more challenging. This was one of the primary reasons for this simplification of the real situation, as it helped the observation of the tumour. Nevertheless, the observations done on a single tumour nodule can be extended to a series of individual tumour nodules, as it can be expected that similar tumour nodules will react in a similar way.

The difference in effect following treatment with both types of formulations can be linked to the significantly higher paclitaxel bioavailability following HIPEC with the Pac/RAME-β-CD formulation, as it was shown previously that C_{max} was 40 times higher than C_{max} for Taxol® (2.73 mg/ml *vs* 0.05 mg/ml at hyperthermic conditions) (11). As the higher bioavailability demonstrated a better penetration of paclitaxel through the peritoneum when formulated in a RAME-β-CD complex, a similar phenomenon can be expected for tumour tissue, specifically as cyclodextrins have already been shown to enhance the permeability of drugs (15). This higher penetration, in combination with a higher paclitaxel concentration in the blood supply to the tumour (because of the higher bioavailability), can be considered as one of the primary reasons for the higher efficacy of the Pac/RAME-β-CD formulation. The higher bioavailability (11) and higher efficacy were not linked to an increase of toxicity, as red blood cell and white blood cell count, creatinine concentration, alanine amino transferase (ALT) and γ-glutamyl transferase (GGT) concentrations were similar after HIPEC with the Pac inclusion complexes and Taxol (11). The effect of the formulation on the pharmacokinetics and efficacy of Pac have already been reported by Tsai et al. (16), who studied three different paclitaxel formulations, i.e. Cremophor micelles, Cremophor-free paclitaxel-loaded gelatine nanoparticles and polymeric microparticles.

In contrast to the bioavailability-enhancing effect of β-cyclodextrins, a high ratio between the intraperitoneal and intraveous concentrations of paclitaxel was seen when paclitaxel was combined with Cremophor EL® (a major formulation constituent of Taxol®) (17). Due to the high affinity of paclitaxel for Cremophor EL®, release from the Cremophor EL® micelles was reduced (18). Based on these observations, these authors claimed specific benefits from the resulting sustained release of paclitaxel: reduction of systemic toxicity and prolonged IP drug concentrations, which might offer a therapeutic advantage. They also claimed that without Cremophor EL® in the formulation, too high systemic paclitaxel concentrations would be obtained, increasing the risk of severe (haematological) toxicity. Previous results by Bouquet et al. (11) do not support this statement, as Pac/RAME-β-CD increased the systemic drug concentration without increasing the haematological toxicity, as no significant differences in red blood cell counts were detected between the two formulations. In addition, Pac/RAME-β-CD showed the highest antitumour activity, which indicated that a short-term exposure to a high paclitaxel concentration might be more favourable compared to a prolonged exposure to a lower drug concentration. A similar observation was made by Michalakis et al., who showed that a short-term exposure to high doses of paclitaxel induced long-term inhibition of cell proliferation (19).

For several chemotherapeutics (cisplatinum, doxorubicin, mitomycin C and oxaliplatin) the synergism with heat is well established (7). However, reports about the effect of the combination of paclitaxel and heat are not as conclusive, as positive (20, 21) as well as negative effects have been reported (22, 23). In general, synergism was seen at high local concentrations and high temperatures (43°C) for prolonged duration of treatment (≤ 60 min). (24) Here, the effect of heat alone caused a significant decrease in tumour volume, which shows the cytostatic effect of heat. However, the combination of both paclitaxel and heat was unable to produce superior results compared to either of both individually. Therefore, based on the current results and previous *in vitro* data (10), we can not conclude that the addition of heat has an additive effect on the effect of paclitaxel in the current model.

Currently the use of paclitaxel is limited to the early postoperative intraperitoneal chemotherapy (EPIC), mainly due to hypersensitivity reactions following IV administration of Taxol®. To our knowledge, no clinical studies are available of paclitaxel in HIPEC for PC of colorectal origin. The current results show that paclitaxel might have a possible role to play in the intraperitoneal chemotherapy of PC of colorectal origin. However, a limiting factor for this treatment might be that the effect seems to be more short term and that repetitive administration will be necessary to fully eradicate the tumour. Also, in the treatment of PC of ovarian origin, data about HIPEC and paclitaxel are limited (25). In a recent clinical and pharmacokinetic study, de Bree et al. (26) demonstrated the feasibility and safety of HIPEC with paclitaxel after cytoreductive surgery, confirming previous encouraging studies (27, 28). However, more and larger studies are needed to demonstrate the efficacy of paclitaxel in this treatment modality. Nevertheless, these first data demonstrate that paclitaxel could play an important role in the IP treatment of colorectal and ovarian cancer, further increasing the possible applications for the Pac/RAME-β-CD formulation. Further research will be necessary to fully explore the potential of this novel formulation.

CONCLUSION

In conclusion, compared to Taxol®, a newly developed cyclodextrin-based paclitaxel formulation showed superior

antitumour efficacy in a rat model of colorectal carcinomatosis. Thermal enhancement was not observed with either of both formulations. These results suggest that Pac/RAME-β-CD represents an interesting active drug for study in patients with peritoneal carcinomatosis from colorectal origin and possibly ovarian origin.

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