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Paclitaxel tumor biodistribution and efficacy after intratumoral injection of a biodegradable extended release implant

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ABSTRACT

Purpose: The aim of this study was to investigate the effectiveness of paclitaxel controlled release from intratumorally injected polymer.
Methods: The effectiveness of paclitaxel–polymer formulation injected intratumorally was tested in mouse bladder tumor model. To determine paclitaxel biodistribution in tumor at predetermined time periods the tumor was excised, frozen and sectioned, and the paclitaxel concentrations were determined in the tumor tissue and in plasma by HPLC. Histopathological evaluation of the necrosis and inflammation was performed on tumor sections.
Results: In the paclitaxel/polymer group mice were injected intratumorally with 0.2 ml of the 10% (w/w) paclitaxel formulation, the tumor disappeared completely 5 days after injection, and mice survived till the end of the study (50 days post-tumor cells inoculation). In biodistribution studies, the highest paclitaxel concentration in the tumor tissue was 40 μg/mg 1 day after the intratumoral injection and decreased gradually during 10 days to 5 μg/mg that is still high enough to induce cytotoxic effect, and the necrotic effect of paclitaxel on the tumors was confirmed by histopathology.
Conclusions: Treatment with local injection of polymer–paclitaxel formulation inhibited the growth of solid tumors. Distribution studies of paclitaxel after intratumoral injection showed high and effective drug concentrations in tumor.

1. Introduction

To improve the responsiveness of solid tumors to current chemotherapeutic agents, optimization of their delivery that affects their biodistribution and availability has been suggested. Intratumoral chemotherapy increases drug concentration at the tumor target and reduces systemic exposure of toxic chemotherapeutic agents (Walter et al., 1995).

Paclitaxel is one of the best anti-neoplastic drugs found in the past decades. Paclitaxel success is largely due to its unique mechanism of action against tumors and its ability to work in combination with other anticancer therapeutic agents. It has excellent therapeutic efficacy for a wide spectrum of malignancies, especially for ovarian and breast cancers (Ruel-Gariepy et al., 2004). Because of its hydrophobic nature the current clinical dosage form of paclitaxel consists of a 1:1 (v/v) mixture of ethanol and Cremophor EL, a nonionic polyethoxylated castor oil solubilizer, diluted in water for injection prior to infusion. This pharmaceutical formulation, however, is associated with a number of concerns including stability, filtering requirements and use of non-plasticized containers. Moreover, some of the side-effects, such as severe hypersensitivity reactions, observed following paclitaxel administration are considered to be formulation related (Terwogt et al., 1997). An alternative approach to the administration of this drug for the treatment of localized tumors might be via a controlled-release implant that could deliver pharmacologically effective doses of paclitaxel to the tumor site. One of the therapeutic approaches to solid tumors is the surgical removal followed by irradiation and/or systemic chemotherapy to kill malignant cells which may have survived the surgery, and prevent metastasis and re-growth of tumor. Implanting a biodegradable device loaded with anti-neoplastic agent in the cavity created by the tumor removal provides high local concentration of the drug killing the surviving malignant cells. This may also prevent the systemic side-effects of the chemotherapy that is normally associated with the intravenous administration. Injectable device may also provide sustained, controlled delivery to the malignant tumor. In addition, clinicians can pre-treat large solid tumors prior to surgery in order to expose the tumor to large concentrations of the drug (Dordunoo et al., 1997).

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Biodegradable polyanhydrides and polyesters are useful materials for controlled drug delivery (Domb et al., 1997; Langer, 2000; Stephens et al., 2000). They have hydrophobic backbone with hydrolytically labile anhydride and/or ester that may hydrolyze to dicarboxylic acids and hydroxy acid monomers when placed in aqueous medium. Ricinoleic acid based polyanhydrides were synthesized and used as drug carriers (Teomim and Domb, 1999; Teomim et al., 1999). The toxicity, biodegradation and elimination of polyanhydrides and aliphatic polyesters have been recently reviewed (Hakkarainen, 2002; Katti et al., 2002). The fatty acid components of these polymers undergo extensive metabolism in the body and are mainly excreted in the form of carbon dioxide. The *in vitro* and *in vivo* toxicity tests indicate that these polymers are well tolerated by the tissues and can be generally considered as biocompatible (Hakkarainen, 2002).

In our previous publications, we have described the synthesis, characterization and a gelation phenomenon of biodegradable poly(ester-anhydrides) based on sebacic acid and ricinoleic acid (Krasko et al., 2003; Shikanov and Domb, 2006). The aim of this study was to evaluate the anti-tumoral activity of locally delivered paclitaxel. The polymeric formulation with paclitaxel was injected intratumorally to the tumor-bearing mice. The controlled release of paclitaxel from the polymer and its biodistribution in the tumor were monitored and the systemic and local levels of the drug were measured during 10 days after injection. This study proves the hypothesis that locally injected paclitaxel in polymer formulation can be effective in killing tumor without reaching high systemic levels.

2. Materials and methods

2.1. Materials

Poly(sebacic acid-*co*-ricinoleic acid ester anhydride) (P(SA:RA)) 2:8 was synthesized as previously was described (Krasko et al., 2003). The polymer structure is shown in Scheme 1. Ricinoleic acid (RA) (purity >95%) was purified from castor oil (Florish, Israel). Paclitaxel (BioxelPharma, Quebec, Canada), sebacic acid (SA) 99% (Aldrich, Milwaukee, WI), acetic anhydride (Merck, Darmstadt, Germany), were used. All solvents were analytical grade from BioLAB (Jerusalem, Israel) or Frutarom (Haifa, Israel) and were used without further purification.

MBT (mouse bladder tumor) cells were a generous gift from Dr. Ofer Gofrit from Hadassah Ein-Karem Hospital (Jerusalem, Israel). Cell culture medium and fetal calf serum (FCS) were obtained from Beit-Haemek (Israel).

2.2. Preparation of formulations for animal treatment

Formulations of P(SA:RA) 2:8 loaded with 10% (w/w) of paclitaxel were prepared by direct mixing of the polymer with the drug at room temperature. The composition was mixed until a smooth paste was formed and 500 mg of each formulation were filled in 1 ml Luer-lock syringes without heating. The obtained formulations were injectable semi-solid pastes at room temperature.

2.3. In vitro cytotoxicity

The MBT cells were maintained in monolayer cultures in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum. 2×10^3 MBT cells in 100 μl of culture medium were seeded in 96-well plates and were incubated for 24 h at 37 °C. Serial dilutions of paclitaxel in a volume of 10 μl were added, and the cells were incubated for 48 h. All data presented as mean ± STD of triplicate. The data was plotted as a percentage of the data from the control cultures, which were treated identically to the experimental cultures, except that no drug was added.

2.4. In vivo anti-tumor activity

2.4.1. Inoculation of MBT cells

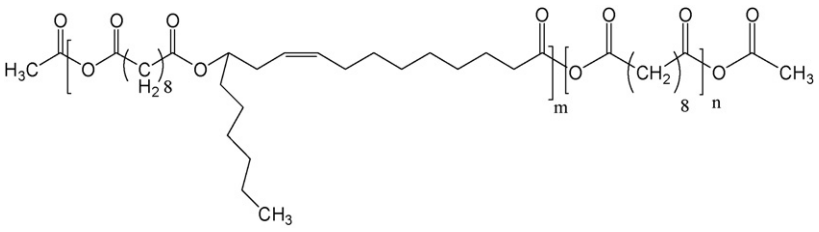
Inbred 8–10 weeks old female C3H mice, weighing about 20 g (Harlan Laboratories, Israel) were kept under specific pathogen free (SPF) conditions and given free access to irradiated food and acidified water throughout the experiment. Mice were injected subcutaneously via a 27-gauge needle in the posteriolateral flank with 5×10^5 MBT cells suspended in 0.1 ml unsupplemented RPMI medium. Tumors were measured using caliper every other day and their volumes were calculated by the formula: length × width × height × 0.523 (Jackson et al., 2000).

2.4.2. Treatment protocols

The treatment was initiated 10 days after inoculation, when the tumor was palpable and reached volume of 0.12 cm^3. The mice were randomly assigned to one of the three treatment groups ($n = 10$ in each group). The two control groups ($n = 10$ in each group) received intratumoral injection of 0.1 ml of the blank polymer or no treatment at all. The first treatment group was injected with 0.2 ml of a formulation containing 10% paclitaxel (equivalent to 1000 mg/kg dose) intratumorally, the second treatment group was treated with intratumoral injection of 0.2 ml paclitaxel solution in diluted Cremophor/ethanol solution at concentration of 1 mg/ml (10 mg/kg) (LD_{50} for paclitaxel in mice is 20 mg/kg). Mice were injected only once during the experiment. The animals were sacrificed when the tumor volume reached 3.5 cm^3.

2.5. Determination of paclitaxel levels in plasma and tumor

For induction of subcutaneous tumors, inbred 8–10 weeks old female C3H mice were injected subcutaneously via a 27-gauge needle in the posteriolateral flank with 5×10^5 MBT cells suspended in 0.1 ml unsupplemented RPMI medium. For determination of paclitaxel distribution in tumor, the injected tumors should partially escape from the treatment; otherwise there would be no



Scheme 1. Structure of poly(sebacic acid-*co*-ricinoleic acid).

tumor tissue to determine the drug concentration in. For this reason the tumors were treated 15 days after tumor cells inoculation with 0.1 ml (half of the dose needed for cure and total tumor elimination) of the injectable polymer/paclitaxel formulation containing 10% paclitaxel only, when the tumors reached volume of 0.8–0.9 cm^3. In this case the tumor could not be totally eliminated and the pattern of the paclitaxel distribution in the tumor could be studied. At each time point 1, 2, 3, 7 and 10 days post-injection six mice were sacrificed. The tumor was excised and frozen at −20 °C, and the blood was collected from the heart, heparinized and centrifuged (2500 rpm, 5 min) to obtain plasma. The obtained plasma was separated from the blood and kept frozen at −20 °C. The analysis of the paclitaxel in the plasma was performed by modification of previously reported methods (Coudore et al., 1999; Gershkovich and Hoffman, 2005). 10 μl of testosterone solution (100 μg/ml) as an internal standard were added to the obtained plasma (600–1000 μl). In order to extract paclitaxel and the internal standard 4 ml of chloroform were added. The tubes were vortexed 3 times for 1 min each time. 3 ml of the lower organic phase were transformed to another glass tube and evaporated to dryness under a steam of nitrogen at ambient temperature. The residue in the tube was reconstituted with 150 μl of mobile phase and 100 μl were injected onto the column. The analysis of the paclitaxel in tissue was performed by modification of previously reported methods (Kim et al., 2005; Song and Au, 1995) Tissue samples were mounted on cryostat chucks with embedding matrix (O.C.T. Compound, Tissue-Tek, Redding, CA) and sectioned into 50–100 μm-thick sections in a cryostat at −20 °C. The sections were weighed and 100 μl of testosterone solution (100 μg/ml) as an internal standard were added to the tissue and left overnight. The tumor tissue was homogenized in 1.5 ml ethyl acetate and then centrifuged at 5000 rpm for 10 min. After that the organic layer (1 ml) was transferred to a clean tube and evaporated to dryness under a steam of nitrogen at ambient temperature. The residue in the tube was reconstituted with 1 ml of mobile phase and 100 μl were injected onto the column. HPLC [Hewlett Packard, Waldbronn, Germany] system composed of an HP 1100 pump, HP 1050 UV detector, and HP ChemStation data analysis program using a C18 reverse-phase column (LichroCart® 250-4, Lichrospher® 100, 5 μm). A mixture of 65% methanol: 35% water at a flow rate 1 ml/min was used as eluent and UV detection at 230 nm. The retention time for testosterone and paclitaxel were 15 and 17.4 min, respectively.

2.6. Macroscopic and histopathological evaluation

Tumors isolated from the animals sacrificed 3 and 7 days post-treatment application were resected, measured and fixed in 4% formaldehyde solution. The tissue slides were processed into paraffin and 3-μm sections were stained with hematoxylin and eosin for histological evaluation. The examination parameters included necrosis total area, inflammatory cell infiltration and intact tumor tissue.

2.7. Statistical analysis

All results are expressed as mean ± the standard deviation (STD) of the mean and statistically analyzed using GraphPad Instat software. p values less than 0.01 were considered significant for all tests.

3. Results

3.1. In vitro cytotoxicity

The IC_{50} value of paclitaxel in MBT cells was found to be 2.6 μg/ml (3 μM). Similar results of IC_{50} for paclitaxel were reported earlier: 2 μM for MBT-2 cells (Nativ et al., 1997). Tumor murine cells are less sensitive to cytotoxic agents then human cell lines. For example, human breast cancer cell lines, MCF-7, MDA-MB-435 and SK-BR-3 show much lower IC_{50} of 0.025, 0.1 and 0.2 μM (Tommasi et al., 2007).

3.2. In vivo anti-tumor activity

The efficacy of paclitaxel delivered intratumorally was investigated in the mouse bladder tumor (MBT) heterotopic model (Fig. 1). The treatment was initiated on the tenth day after tumor cell inoculation. Mice that were not treated and mice injected with the blank polymer were sacrificed 15 days after inoculation because the tumor volume exceeded 3 cm^3. However, the volume of the tumors was smaller in mice injected with the blank polymer and there was a statistically significant ($p < 0.005$, ANOVA) delay in tumor progression. While non-treated mice were sacrificed 15 days post-tumor cell inoculation, mice injected with the blank polymer were sacrificed 18 days post-tumor cell inoculation (8 days post the polymer injection) when the tumor volume reached >3.5 cm^3 (Fig. 1). Intratumoral (IT) injection of paclitaxel solution (0.1%, w/v

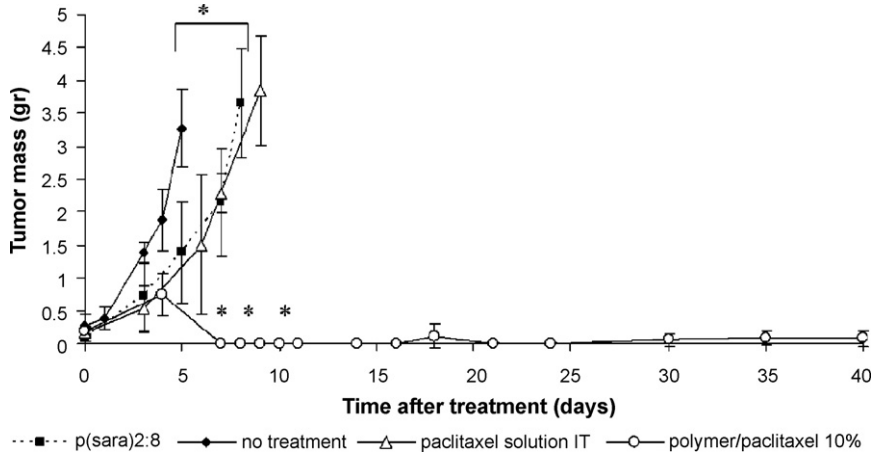


Fig. 1. Effect of paclitaxel on MBT tumor growth in s.c. implanted mice ($n = 10$). Paclitaxel 10% (w/w) in polymer, 200 μl (○, solid line); blank polymer (black square, dashed line); paclitaxel solution (0.1%, w/v in saline, 200 μl injection, 10 mg/kg) (△, solid line) were injected intratumorally. No treatment group is designated as rhomb (♦) with solid line. The tumor volume is expressed as mean ± STD. Statistically significant differences between the groups are signed with a star (*$p < 0.05$).

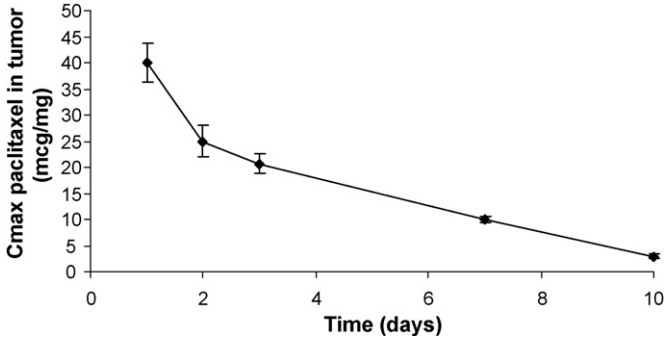


Fig. 2. The time profile of paclitaxel maximal concentration (C_{max}) in the tumor tissue after intratumoral injection of paclitaxel/polymer formulation (10%, w/w, 100 μl). Values are expressed as means ($n = 6$).

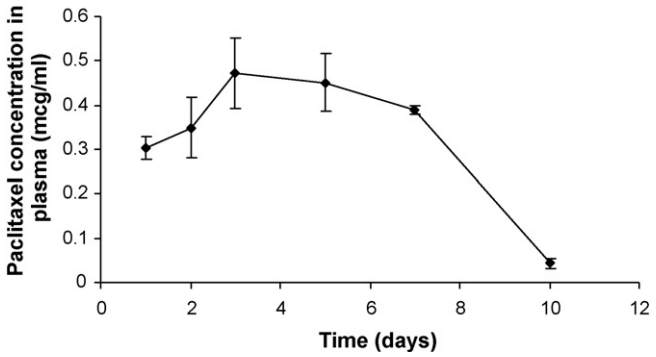


Fig. 4. Paclitaxel levels in plasma. Paclitaxel levels in mice plasma were determined by HPLC. Each data point represents the average of six mice ± STD.

in saline, 200 μl injection, and 10 mg/kg) delayed tumor growth, similar to the blank polymer injection, and mice survived 3 days longer then the non-treated group. In the paclitaxel/polymer group mice were injected intratumorally with 0.2 ml of the 10% paclitaxel formulation 10 days post-tumor cells inoculation. In the first 7 days post-treatment the injected formulation contributed to the measured volume of tumor, but after 1 week the tumor totally disappeared and a small ulcer appeared instead, which also disappeared after 2 weeks. In 8 out of 10 mice the tumor did not appear till the end of the study (50 days post-tumor cell inoculation), in the other two mice a small nodule of volume less than 0.2 cm^3 was measured at the end of the study (50 days post-tumor cell inoculation).

3.3. Paclitaxel tumor distribution

The maximal paclitaxel concentrations (C_{max}) in tumor tissue are shown in Fig. 2. The highest C_{max} was 1 day after the intratumoral injection and reached 40 μg/mg in tumor tissue, while the paclitaxel concentration in plasma was only 0.32 μg/mg. C_{max} decreased gradually during the following 10 days and paclitaxel concentration in tumor was 3 μg/mg that is still high enough to induce cytotoxic effect (Kuh et al., 1999). For comparison, the highest C_{max} found in tumor 2 h after IV injection of 20 mg/kg of Taxol® to mice was 0.21 μg/mg (Kim et al., 2005). Therefore, the highest C_{max} in tumor after IV infusion was lower then C_{max} 10 days after intratumoral injection of paclitaxel/polymer formulation.

Fig. 3 shows paclitaxel distribution in tumor at different time intervals. The paclitaxel/polymer formulation was injected into the tumor therefore the drug distribution is expected to be radial, while the maximal concentrations are found at the injection site. The data is presented in a logarithmic scale from the injection site toward the distant tumor tissue in one direction (Fig. 3). After 24 h post-injection a maximal paclitaxel concentration of 40 μg/mg was found at the injection site, and it decreased gradually up to 0.16 μg/mg at a distance of 6.5 mm from the injection site that is above the therapeutic level (IC_{50} of paclitaxel was determined to be 2.6 μM, which is 0.002 μg/mg tissue). At 2 days post-injection, the highest paclitaxel concentration of 25.5 μg/mg was observed at the injection site and paclitaxel was found at the edge of the tumor (7.5 mm) at concentrations of 0.045 μg/mg. After 7 days, paclitaxel level in the injection site was 21.9 and 0.004 μg/mg at a distance of 8 mm from the injection site. After 10 days in the tumor the paclitaxel level at the injection sites was 5 and 0.005 μg/mg 8.5 mm far from the injection site.

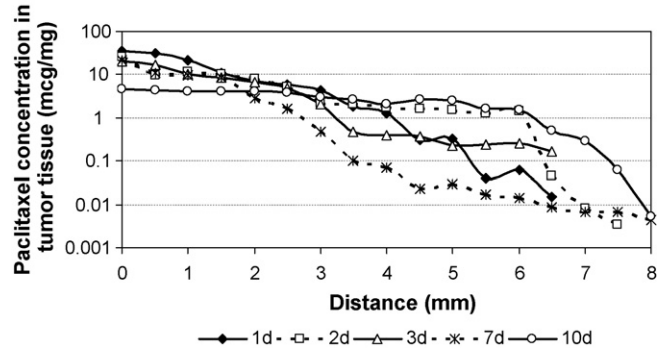


Fig. 3. Paclitaxel tumor tissue distribution after intratumoral injection of paclitaxel/polymer formulation (10%, w/w, 100 μl). Each curve represents time interval when the mice were sacrificed and their tumors processed. Values are expressed as mean ($n = 6$).

3.4. Paclitaxel plasma levels

As was reported earlier by Kim et al. (2005), the paclitaxel plasma levels after IV administration of Taxol® were 80 μg/ml at time zero, and decreased during the following 12 h to undetectable limits (<0.001 μg/ml). The paclitaxel levels after intratumoral injection of paclitaxel/polymer formulation is shown in Fig. 4. The distribution of paclitaxel in plasma is related to events occurring in the tumor, where the polymer releases the incorporated drug. Paclitaxel reached the maximal concentration of 0.47 μg/ml in plasma at days 4 and 5, and decreased to 0.04 μg/ml 10 days after the intratumoral injection of 100 μl of paclitaxel/polymer formulation.

3.5. Macroscopic and histopathological evaluation

Fig. 5 shows the macroscopic view of the tumors resected from mice and sectioned in cryostat. Fig. 5a and b shows the MBT tumor treated with 100 μl of 10% (w/w) paclitaxel/polymer formulation and resected 3 and 7 days post-treatment, respectively. The formulation's color is white because of the paclitaxel color and it is easily recognized (designated as (*)), and contoured with white line). Around the formulation there is a region of a reddish tissue, which is a necrotic area (designated as (◯)), and contoured with green line). Beyond this region, the intact tumor cells region was found (designated as (♦)) (see histopathological evaluation). Fig. 5c shows the tumor with the same treatment, but resected 10 days post-treatment. 7 and 10 days post-injection, 60% and 25% of the formulation were retrieved, but the injection site remained hollow from the polymer and the tumor cells. Around the injection site, the reddish tissue region continued to grow, which implies of continuing cytotoxic activity (designated as (◯), and contoured with green line). Beyond this region the intact tumor cells are found

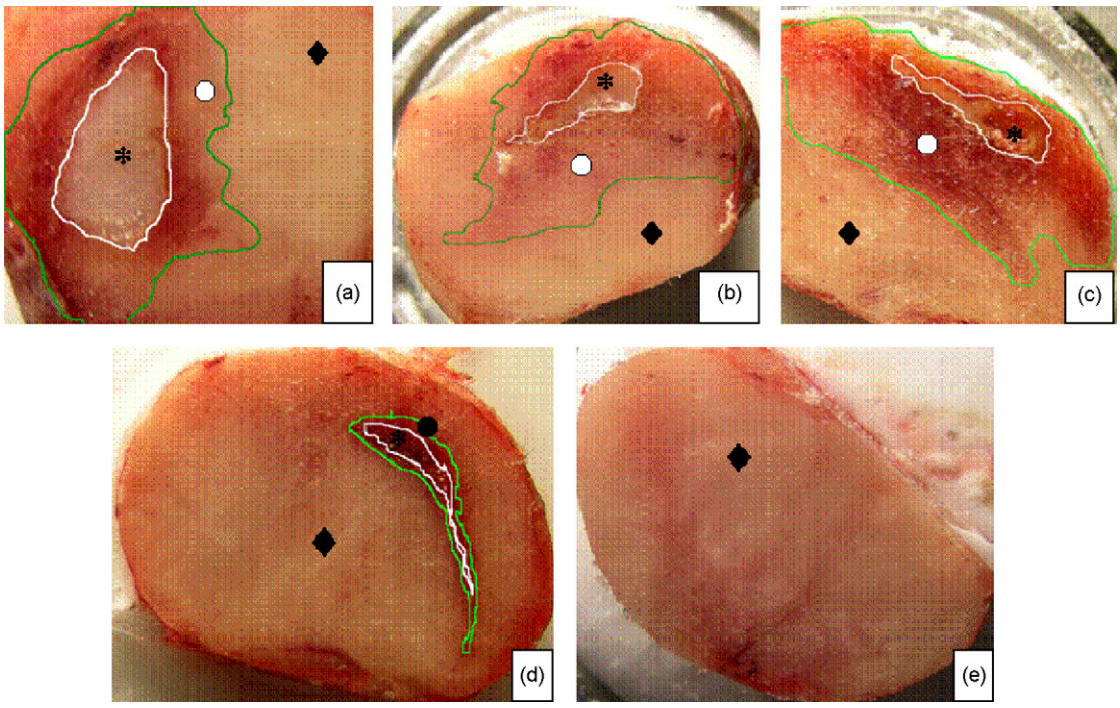


Fig. 5. Macroscopic of the frozen tumors at cryostat sectioning: (a) tumor treated with paclitaxel/polymer formulation and excised 3 days post-injection; (b) tumor treated with paclitaxel/polymer formulation and excised 7 days post-injection; (c) tumor treated with paclitaxel/polymer formulation and excised 10 days post-injection; (d) tumor treated with the blank polymer and excised 3 days post-injection; (e) tumor not treated. The polymeric formulation is assigned with the star (*), the necrotic tissue—with the white circle (○), the infiltration of the inflammation cells with a black circle (●) and the intact tumor cells with a black rhomb (♦).

(designated as (♦)). To exclude the possibility of polymer cytotoxic activity, blank polymer was injected intratumorally and sectioned similarly. Fig. 5d shows the tumor with the blank polymer 3 days post-injection. The polymer region is surrounded by a mild inflammation region, followed by tumor cells. For comparison, Fig. 5e shows the tumor without treatment.

Fig. 6a shows the panoramic view of the histology images of the tumors injected with paclitaxel/polymer formulation at magnification of 10×. The polymeric formulation is assigned with the star, the necrotic tissue and the infiltration of the inflammation cells with a black circle and the intact tumor cells with a black rhomb. The panoramic view of the tumor section is at magnification of 10×. At the formulation-tumor interface only necrotic cells are present (higher magnification, Fig. 6b) and the density of the tumor cells decreases. Along with the necrosis progression inflammation process starts and the region of dead tumor cells appears up to 3.5 mm distance from the paclitaxel formulation. Fig. 6c is a higher magnification of a border region between the necrotic area and intact

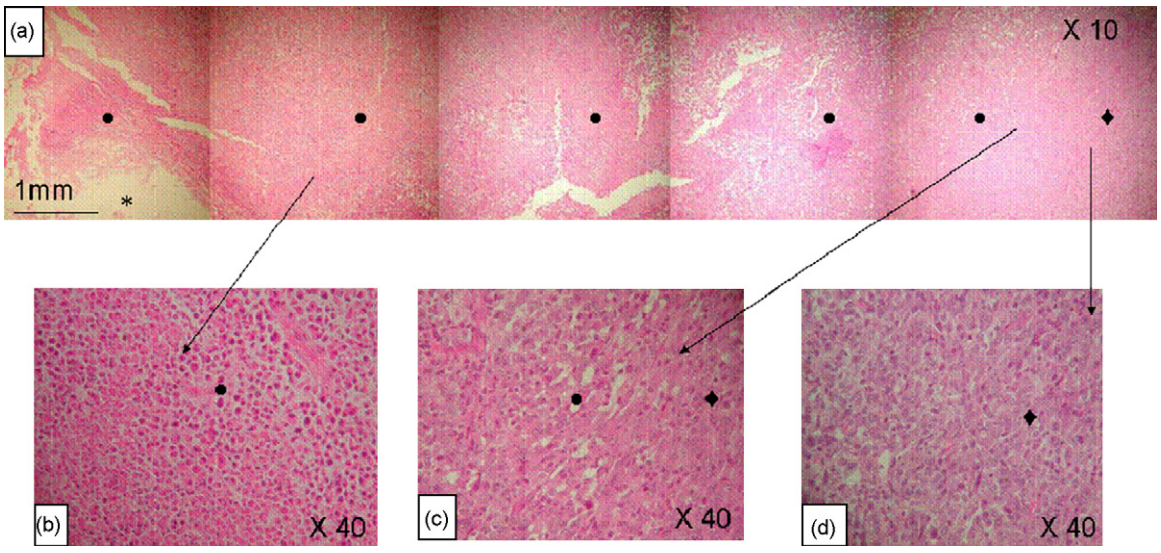


Fig. 6. Histology of the MBT tumor injected intratumorally with paclitaxel/polymer formulation (10%, w/w, 100 μl): (a) magnification 10×, panoramic view of the cut; (b) magnification 40×, enlargement of the paclitaxel/polymer region and the necrotic area around it; (c) magnification 40×, enlargement of the border between the end of the necrotic area and start of the intact tumor area; (d) magnification 40×, enlargement of the intact tumor area beyond the effect of cisplatin. The polymeric formulation is assigned with the star (*), the necrotic tissue and the infiltration of the inflammation cells with a black circle (●) and the intact tumor cells with a black rhomb (♦).

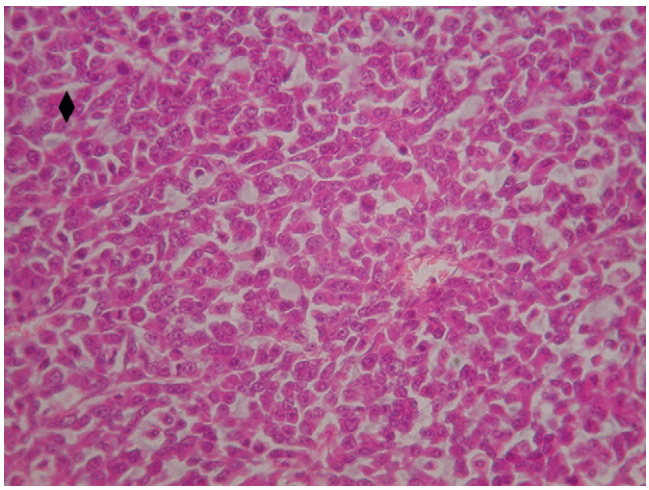


Fig. 7. Histology of the non-treated MBT tumor (magnification 40×), intact tumor cells.

tumor tissue cells. Beyond this border mainly tumor cells can be found (Fig. 6d). Additional observation is that tumor cells density became lower in both necrotic regions and the region beyond the border. The decrease in cells density enhances the drug penetration and the cytotoxic effect (Kuh et al., 1999). Fig. 7 shows the appearance of the MBT tumor without treatment, no necrosis or inflammation process could be detected in the tumor.

Efficacy studies showed that intratumoral injection of a blank polymer caused a delay in tumor growth (Fig. 1). The tumor tissue surrounding the blank polymer is shown in Fig. 8a (magnification 20×) and Fig. 8b (magnification 40×). The polymer is assigned with the star, the infiltration of the inflammation cells with a black circle and the intact tumor cells with a black rhomb. The injection of a blank polymer causes a mild inflammatory reaction and there is an infiltration region around the polymer for 0.3 mm. Beyond the 0.3 mm, intact tumor cells appear again, as shown in Fig. 8b. The injection of a blank polymer into the tumor damaged its structure and delayed its development, but since there was no cytotoxic influence on the tumor cells, the tumor recovered from the physical injury and continued to grow.

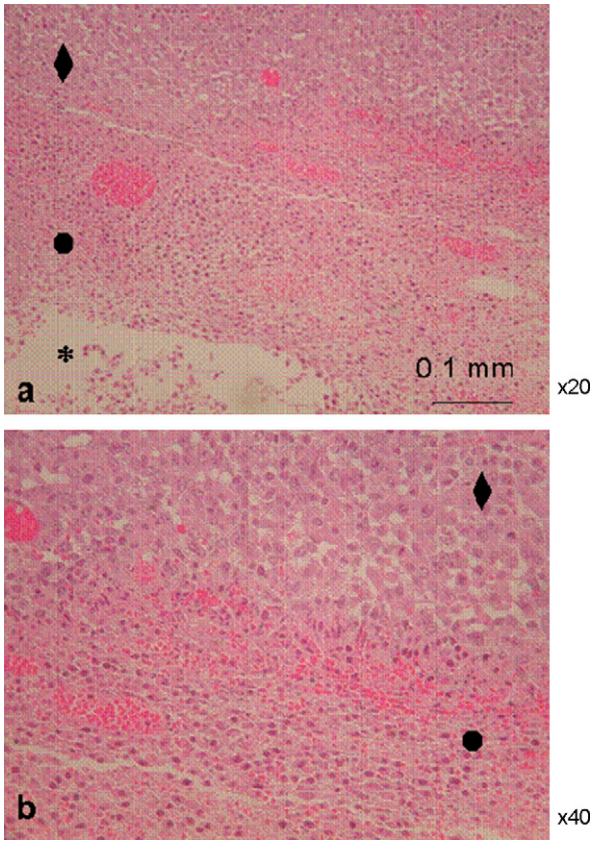


Fig. 8. Histology of the MBT tumor injected intratumorally with the blank polymer: (a) magnification 20×; (b) magnification 40×, enlargement of the blank polymer and the mild inflammation area around it; bar—0.1 mm.

4. Discussion

In regional therapy given for the treatment of localized disease, the drug is applied directly to the tumor bearing organ or cavity. Drug delivery to cells in solid tumors is determined by the ability of the drug to penetrate the multilayer structure of solid tumors (Jang et al., 2001). The barriers and determinants of drug delivery and transport in solid tumors were addressed (Au et al., 2001). One of the most important findings is a high tumor cell density is a barrier to paclitaxel penetration and the apoptotic effect of paclitaxel enhances its penetration in solid tumors (Kuh et al., 1999). There is an evidence for this process in the sections prepared from the paclitaxel treated tumors. There were none live tumor cells found in the interface between the paclitaxel/polymer formulation and the tumor at the injection site. The drug released from the polymer killed the malignant cells and diffused throughout the tumor, up to 7 mm distance from the injection site, as confirmed in the histology evaluation. Additionally, not only apoptotic cells were found in that region, but also a reduction in tumor cells density. This process enhances even deeper paclitaxel penetration. Another finding is the high extend of drug accumulation and retention and a more rapid attainment of a pseudo-steady state between extracellular and intracellular paclitaxel concentrations at higher initial extracellular concentration (i.e. ≥1000 nM) (Jordan et al., 1996). The intracellular-to-extracellular drug concentration ratio as a function of extracellular drug concentration appears as a triphasic process. The first phase, where the intracellular concentration increases linearly with the extracellular concentration, is observed at low extracellular drug concentration or before the saturation of the high affinity intracellular binding site (100 nM). The second phase, where the intracellular drug concentration increases nonlinearly with extracellular concentration, occurs at higher extracellular concentrations and when the high affinity intracellular binding site becomes saturated but remains as the major drug binding site (100–1000 nM). The third phase, where the intracellular drug concentration resumes a linear increase with respect to the extracellular concentration, occurs at even higher extracellular concentrations or when the nonsaturable binding becomes the major mode of intracellular drug binding and the saturable binding becomes negligible (>1000 nM) (Jang et al., 2001). This observation is not useful for systemic treatment resulting in plasma concentrations that saturate the high affinity intracellular binding site because of the systemic toxicity. On the other hand, in tumors treated with paclitaxel/polymer the drug concentration even at the most distant layers was above 1000 nM (0.00086 μg/mg). Additional concern is depletion of paclitaxel from the extracellular medium because of the extensive intracellular accumulation. However, this may appear only at low extracellular concentrations of paclitaxel (<1000 nM) that is not the case in the intratumoral paclitaxel/polymer injection described here.

The toxicity of conventional systemic cancer chemotherapy has severely limited the safety and effectiveness of such therapy and its impact on the quality of life of patients prevents its wider clin-

ical application (Chen et al., 2003; Goldberg et al., 2002). Plasma concentrations are often used a marker of cytotoxic exposure; however, drug delivery to the tumor is determined not only by plasma concentrations but also by the distribution from plasma into the extracellular fluid (ECF) of the tumor and from the ECF into the cells. Solid tumors have several potential barriers to drug delivery that may limit drug penetration, such as alterations in the distribution of blood vessels, blood flow, interstitial pressure, microcirculation of the tumor (Jain, 1996; Zamboni et al., 2000). Therefore, high systemic levels of the cytotoxic drug often cause a related systemic toxicity without reaching the tumor at effective concentrations. Poly(sebacic acid-*co*-ricinoleic acid) 2:8 used in this study is a hydrophobic polymer, built of natural fatty acids, which can be used for the release of hydrophobic or hydrophilic drugs. The polymeric paste formulation with paclitaxel is injectable through a 23-gauge needle and it gels in contact with body fluids without any solvent leach out or temperature change (Krasko et al., 2003; Shikanov and Domb, 2006). The purpose of this study was to evaluate the effect of paclitaxel–polymer formulation injected intratumorally in heterotopic model in mice and to determine paclitaxel distribution in tumor. The levels of paclitaxel in the tumors treated with the paclitaxel/polymer formulation were above the therapeutic threshold during 10 days post-injection, without any signs of systemic toxicity. Additionally, paclitaxel/polymer formulation increased the maximum tolerated dose (MTD) for paclitaxel and was effective in treating MBT tumor in this mice heterotopic model.

5. Conclusion

The results of this work indicate that treatment with the polymer formulation of paclitaxel had a positive outcome and inhibited the growth of the tumors. Histological studies proved the existence of the necrotic process caused by the cytotoxic drug. Distribution studies of paclitaxel after intratumoral injection showed high and effective concentrations in the tumors, while plasma levels were much lower than after systemic administration of paclitaxel.

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