Doctor's Thesis

The United Graduate School of Veterinary Science

Yamaguchi University

A study on high temperature hyperthermia in small animals

小動物における高温温熱療法に関する研究

Hidefumi Takagi, D.V.M.

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Laboratory of Veterinary Surgery,

Department of Veterinary Clinical Medicine,

Yamaguchi University

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Chapter Ⅱ:

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Abstract

High-temperature hyperthermia is an established treatment option for cancer. The aim of the present study was to reveal the exact correlation between high-temperature hyperthermia of 50~70℃ and the resulting antitumor effects, using a glioma rat model. In the 60℃ and 70℃ high-temperature hyperthermia groups, tumor growth rates were significantly suppressed compared with those in the nontreatment group. In the 50℃ high-temperature hyperthermia group, tumor growth rates were not suppressed with those in the nontreatment group. The numbers of terminal dUTP nick-end labeling-positive cells in tumor tissue were significantly higher in the 50℃, 60℃ and 70℃ groups than those in the nontreatment group. The Ki-67-positive areas were significantly decreased in the 70℃ group compared with those in the nontreatment and 60℃ groups. Our data indicate that high temperature hyperthermia at 60 and 70℃ suppresses tumor growth in a glioma rat model. In particular, cell proliferation was significantly suppressed by high temperature hyperthermia at 70℃. We evaluated the effects of high temperature hyperthermia for the treatment of spontaneous tumors in dog. In case 1, an 18-year-old female Papillon presented with a right forelimb rhabdomyosarcoma. Case 2 was a 14-year-old male Golden Retriever with a perianal gland adenocarcinoma surrounding the anus. High temperature hyperthermia wasperformed for10 min at 65℃ with inhaled isoflurane. Case 3 was a13-year-old male English Cocker Spaniel with a right external auditory canal ceruminous adenocarcinoma. In case 1 the tumor disappeared 4 weeks after high temperature hyperthermia therapy. In case 2, the tumor volume decreased by

day 21. In case 3, high temperature hyperthermia was performed three times, and the tumor disappeared after the third procedure. High temperature hyperthermia is a simple procedure with no severe side effects. Consequently, this treatment modality is expected to become a useful alternative therapy for superficial tumors in companion animals.

General Introduction

The companion animal life span has lengthened with the advent of routine vaccination, improved nutrition, improved environment, and advances in veterinary medicine. As a result, the incidence of illnesses associated with aging has increased in pet populations. In particular, cancer is a significant problem. As in human medicine, there are three major treatments for cancer in veterinary medicine, surgery, chemotherapy, and radiation therapy. However, it is difficult to treat all affected patients with these therapies. Therefore, new treatments must be developed. Hyperthermia has long been established as a treatment for cancer, particularly for superficially located cancer [1]. Hyperthermia is used alone or as an adjunct to radiotherapy or chemotherapy [2-5] and has been used to treat spontaneous tumors in veterinary medicine [6-9]. Studies have focused on two common strategies, conventional hyperthermia at mild temperature(42-45℃) [6, 7,8] and ablation therapy at high temperatures(>70℃) [9]. To the best of knowledge, there are no previous studies which aimed to determine the correlation between temperatures of 50-70℃ and antitumor effects.

In the chapter 1, The effects of high temperature hyperthermia at 50~70℃ were evaluated in a glioma rat model by the resulting antitumor effects and histopathological examination. As a result, in the 60℃ and 70℃ high temperature hyperthermia groups, tumor growth rates were significantly suppressed compared with those in the nontreatment group. In the 50℃ high temperature hyperthermia group, tumor growth rates were not suppressed compared with those in the

nontreatment group. The numbers of terminal dUTP nick-end labeling-positive cells in tumor tissue were significantly higher in the 50℃、60℃ and 70℃ groups than those in the nontreatment group. This finding indicates that relatively low temperatures induce apoptosis [10]. Our data also indicate that high temperature hyperthermia at 50~70℃ induces necrosis and apoptosis in a glioma rat model.

Ki-67 is a cell proliferation marker that is detected during all active phases of the cell cycle, but is absent in resting cells [11]. Ki-67 expression increases during S phase until mitosis, when its expression is maximal. Following cell division, cell in G1 phase exhibit decreased Ki-67 expression until they reenter the S phase when the level of Ki-67 increases again [12].Ki-67 expression is useful for assessing the grade of tumors. [13]. The Ki-67-posutive areas were significantly smaller in the 50℃ and 70℃ groups than in the nontreatment groups.

In the chapter 2, we evaluated the effects of high temperature hyperthermia for the treatment of spontaneous tumors in dogs. In case 1, an 18-year-old female Papillon presented with a right forelimd rhabdomyosarcoma. Case 2 was a 14-year-old male Golden Retriever with a perianal gland adenocarcinoma surrounding the anus. High temperature hyperthermia was performed for 10 min at 65℃ with inhaled isoflurane. In case 1, the tumor disappeared 4 weeks after hyperthermia therapy. In case 2, the tumor volume decreased by day 21. In case 3, hyperthermia therapy was performed three times, and the tumor disappeared after the third procedure.

The beneficial effects of high temperature hyperthermia in the treatment of superficial cancer have not yet been reported in veterinary medicine. The high

temperature hyperthermia protocol we used was very simple and was only performed on canine spontaneous tumors. In these three cases, the tumor volumes decreased following high temperature hyperthermia therapy. Furthermore, no severe side effects were observed in any of the cases.

In recent decades, several innovative minimally invasive cancer therapies have been developed as alternatives to surgery. Ablation, which uses high temperature, radio waves, or microwaves, is considered a potent alternative therapy [14].

High temperature (>46℃) can directly damage cells, resulting in severe protein denaturation and DNA damage [15,16] and inducing irreversible changes that ultimately result in cell death. Tumor cells express specific tumor-associated antigens. In high temperature (>46℃) conditions, tumor cells swell and break into pieces, which releases antigens; the large antigen load generates antitumor immunity. The high temperature also cause severe protein denaturation, but this likely destroys the immunogenicity of tumor cells [17-21]. When thermal ablation temperatures (>70℃) are achieved, there is a high risk of shock syndrome induced by the sudden and large production of necrotic tumor material [22]. Therefore, the case for ablation therapy is restricted in human medicine. In general, ablation therapy is performed to the tumor within 3cm in diameter [23].

HTH therapy might indicate the sensitivity to the temperature of HTH vary by tumor types. More extended study which performs HTH to various tumors is necessary. The optimal therapeutic protocol including effective temperature, time, and frequency must be established to expand HTH therapy for routine use in veterinary oncology.

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Chapter I

Antitumor effects of high- temperature hyperthermia on glioma rat model

Abstract. High-temperature hyperthermia (HTH) is an established treatment option for cancer. The aim of the present study was to reveal the exact correlation between HTH at temperatures of 50-70℃ and the resulting antitumor effects, using a glioma rat model. In the 60℃(T-60) and 70℃ (T-70) HTH groups. Tumor growth rates were significantly suppressed compared with those in the nontreatment (NT) group. In the 50℃ (T-50) HTH group, tumor growth rates were not suppressed compared with those in the NT group. The numbers of terminal dUTP nick-end labeling-positive cells in tumor tissue were significantly higher in the T-50, T-60 and T-70 groups than those in the NT group. The Ki-67-positive areas were significantly decreased in the T-70 group compared with those in the NT and T-60 groups. Our data indicate that HTH at 60 and 70℃ suppresses tumor growth in a glioma rat model. In particular, cell proliferation was significantly suppressed by HTH at 70℃. However, differences in the mechanism of HTH at 60 and 70℃ were observed.

1. Introduction

Hyperthermia has long been established as a treatment option for cancer, particularly for superficial types of cancer (1). Hyperthermia is used alone or as an

adjunct to radiotherapy or chemotherapy (2-5). Traditional hyperthermia(41-43℃, or even lower) can synergistically enhance the therapeutic effects of radiotherapy by inducing apoptosis. In hyperthermia with thermal ablation(>60℃), direct Killing of tumor cells occurs by necrosis(6,7).

High temperature (>46℃) can directly induce cell damage, including severe protein denaturation and DNA damage(8,9).These changes are irreversible and ultimately result in cell death. Tumor cells express specific tumor-associated antigens. In high-temperature (e.g.>46℃) conditions, tumor cells swell and break into pieces, allowing antigen release. This creates a large antigen load for the generation of antitumor immunity. Although high temperatures cause severe protein denaturation, this is likely to destroy the immunogenicity of tumor cells (10-14). When thermal ablation temperatures(>60℃) are achieved, there is a high risk of shock syndrome induced by the sudden and large production of necrotic tumor material. However, a relatively low temperature range (46-55℃) can increase the proportion of apoptotic cells among the dead cells, which is likely to reduce the risk of shock syndrome (15). To the best of our knowledge, there are no previous studies which aimed to determine the correlation between temperatures of 50-70℃ and antitumor effects.

Glioma is the most common brain tumor. Surgery, chemotherapy and radiotherapy form the basis of glioma treatment. However, the prognosis of patients with glioma is poor (16). The development of alternative therapies for patients with glioma is essential to improve their prognosis. Previously, it was indicated that hyperthermia can prolong the survival time and rate of patients with glioma (17). Specific reports have revealed the mechanisms of action of hyperthermia on glioma cell lines (18,19).

However, mild-temperature hyperthermia was utilized in these studies.

The aim of the present study was to clarify the exact correlation between high-temperature hyperthermia (HTH) at 50-70℃ and the resulting antitumor effects, using a glioma rat model. The effects of HTH were evaluated in a glioma rat model by histopathological examination.

2. Materials and methods

Treatment device. In this study, a tissue ablation device for veterinary medicine (AMTC 200; AdMeTech Co., Ltd, Ehime, Japan) was used. This device can regulate temperature between 50 and 70℃.

Preparation of the glioma-bearing rat model. F344 rats (female; 31-37 weeks old) were purchased from CLEA Japan (Osaka, Japan). The animals were maintained under conventional conditions. The use of these animals and the procedures they underwent were approved by the Animal Research Committee of Tottori University (Tottori, Japan).

9L cells were maintained in E-MEM (Wako, Inc., Osaka, Japan) containing 10% fetal bovine serum, 0,1 mg/ml neomycin, 0,05 mg/ml penicillin at 5% CO_2 and 37℃ under a humidified atmosphere in an incubator. The rats were anesthetized via inhalation of 3-5% isoflurane (Intervet, Inc., Tokyo, Japan). In total, $2{,}5 \times 10^7$ cells (0,2 ml) were injected subcutaneously into the femoral regions of each rat. Rats with tumor diameters exceeding 10 mm were used for the experiment.

Study design. Rats (n=14) were randomized into four groups: Nontreatment (NT; n=3), 70℃(T-70) HTH(N=3)、60℃(T-60) HTH (n=4) and 50℃ (T-50) HTH (n=4) groups. The tumor growth rates were calculated according to the tumor volumes (mm^3/day). On day 0, HTH was performed for 10 min at 70, 60 or 50℃. On days 0, 3, 6, 9 and 12, the volumes of the tumor tissues were calculated by measuring the mediastinum, transverse length and depth of each tumor. the tumor tissues were removed on day 12 and fixed in 10% buffered formalin.

Histological examination. Fixed samples were embedded in paraffin and sectioned in a routine manner. The sections were subjected to hematoxylin-eosin (HE), Ki-67 and terminal dUTP nick-end labeling (TUNEL) staining.

For Ki-67 staining, tissue sections(3 μ m) on glass slides were deparaffinized, washed with ethanol and water, and soaked in phosphate-buffered saline (PBS). THE sections were autoclaved with 0,01 M citrate buffer (pH 6,0) for 15 min (121℃**).** The sections were then washed with PBS and incubated with rabbit polyclonal anti-Ki-67 antibody (1:50; E0468, Dako, Glostrup, Denmark) for 30 min at room temperature. After washing with PBS, the sections were incubated with rat anti-IgG antibody(1:100; sc-372; Vector Laboratories, Inc., Burlingame, CA, USA) for 30 min at room temperature. The slides were washed with PBS and stained using the ABC method (PK-4000, Vector Laboratories, Inc.) for 30 min.

For TUNEL staining, tissue sections (3 μ m) on glass slides were deparaffinized, washed with ethanol and water, and soaked in diluted water, and soaked in diluted water. TUNEL staining was performed using the *In situ* Apoptosis Detection Kit

(Takara Bio, Inc., Shiga, Japan) according to the manufacturer's instructions. Ten high-power field randomly selected and the positive cells were counted.

Image analysis of Ki-67 staining. Quantitative digital morphometric analysis of the Ki-67-positive area was performed. Ten randomly-selected high-power fields (magnification, x200) were photographed for each cross section using a digital camera attached to an Olympus microscope system (Olympus Corporation, Tokyo, Japan). The color wavelengths of the copied images were transformed into digital readings using Lumina Vision software (Mitani Corporation, Tokyo, Japan), allowing for quantification of the various color wavelengths with pixels as the unit of measurement. The percentage of positive areas in the tumor tissues was calculated by dividing the total pixel area of the positive areas by the total pixel area corresponding to the entire tumor tissue in the field of view. The tumor tissues of three rats were analyzed in each group. The mean scores for 30 fields were used to determine the percentage of positive areas for each group.

Statistical analysis. Data are expressed as the mean±standard error of the mean. Statistical analyses were performed using one-way analysis of variance followed by the Tukey-Kramer test or the Steel-Dwass test. $P<0.05$ was considered to indicate a statistically significant difference.

3. Results

Tumor growth rates. The tumor growth rates of the various groups are shown in Fig. 1.

The tumor growth rates were significantly lower in the T-70 group than those in the NT group on days 3, 6, 9 and 12 ($P<0.05$). The tumor growth rates were significantly lower in the T-60 group than those in the NT group on days 3, 6 and 12. The tumor growth rates were slightly, but insignificantly, lower in the T-50 group than those in the NT group. The tumor growth rates were also lower in the T-70 and T-60 groups than those in the T-50 group on days 3, 6, 9 and 12. The tumor growth rates in the T-60 and T-70 groups were similar on days 3, 6, and 12.

Histological evaluation. The Results of HE staining are shown in Fig. 2. In the NT group, active cell proliferation was frequently observed. Cell proliferation was markedly suppressed in the T-70 and T-60 groups compared with that in the NT group. In addition, in the T-70 and T-60 groups, necrotic cells were widely observed. Cell proliferation was slightly suppressed in the T-50 group compared with that in the NT group, and only a few necrotic cells were observed.

TUNEL staining. The results of TUNEL staining are shown in Fig. 3A. The TUNEL-positive cells are denoted by arrows. The numbers of TUNEL-positive cells were significantly higher in The T-70 (106.1 ± 14.2 cells/field), T-60 (131.4 ± 12.4 cells/field) and T-50 groups (106.7 ± 6.7 cells/field) than those in the NT group (47.1 ± 9.5 cells/field) ($P<0.01$) (Fig. 3B).

Ki-67 staining. The results of Ki-67 stains are shown in Fig. 4A. Ki-67-positive areas are denoted by arrows. The Ki-67-positive area was significantly smaller in the T-70 group (1.7±0.1%/field) than that in the NT (3.9±0.4%/field) and T-60 groups (4.1± 0.2%/field) P<0.01). The Ki-67-positive area was significantly smaller in the T-50 group (2.8±0.2%/field) than in the NT and T-60 groups (P<0.05, vs. NT; P0.01 vs. T-60) (Fig. 4B).

4. Discussion

In the present study, the antitumor effects of HTH were evaluated using a glioma rat model HTH at 60 and 70℃ significantly suppressed tumor growth. Previously, specific reports indicated that HTH has potency as a treatment for melanoma in experimental models (23, 24). Li *et al* (24) previously described that local HTH (≥50℃) inhibited tumor growth and stimulated a favorable antitumor immune response in a malignant melanoma model. However, the authors did not investigate the correlation between higher temperatures (>60℃) and antitumor effects.

Studies of hyperthermia have focused on two commonly applied strategies, conventional hyperthermia at mild temperatures (42-45℃) (1, 20, 21) and ablation therapy at high temperature (>70℃) (22). To the best of our knowledge, no study has examined the difference in antitumor effects between mild (42-45℃) and high temperatures (70℃) under the same conditions as performed in the present study. Necrotic cells were more commonly observed in the T-60 and T-70 groups than in the NT groups. Previous reports indicated that HTH directly induced cell damage and necrosis (6, 7). In the T-50, T-60 and T-70 groups in the present study, the numbers of TUNEL-positive cells in tumor tissues were significantly increased compared with

those in the NT group. This finding indicates that relatively low temperatures induce apoptosis (15). Our data also indicate that HTH at 50-70℃ induces necrosis and apoptosis in a glioma rat model.

Ki-67 is a cell proliferation marker that is detected during all active phases of the cell cycle, but is absent in resting cells (25). Ki-67 expression increases during S phase until mitosis, when its expression is maximal. Following cell division, cells in G1 phase exhibit decreased Ki-67 expression until they reenter the S phase when the level of Ki-67 increases again (26).Ki-67 expression is also useful for diagnosing and assessing the grade of tumors in the central nervous system (27). The Ki-67-positive areas were significantly smaller in the T-50 and T-70 groups than in the NT groups. Our data also indicate that temperature exceeding 70℃ sufficiently suppress cell proliferation. Following suppression of cell proliferation, apoptosis may be induced in circumferential areas. The Ki-67-positive area was significantly smaller in the T-70 group than that in the T-60 group, although the tumor growth rates in these groups were equally decreased. We cannot explain this difference, however one possible explanation may be that there are differences in the percentages of apoptotic cells, as the T-60 group had significantly more TUNEL-positive cells than the other groups. HTH at 60℃ may suppress tumor growth by inducing apoptosis more significantly than HTH at other temperatures. Further studies, including those of other molecules associated with apoptosis, are required to clarify this point.

In conclusion, HTH at temperatures exceeding 60℃ suppressed tumor growth in a glioma-bearing rat model. In addition, HTH at 50-70℃ induced necrosis and apoptosis in a glioma rat model. Further studies is required to clarify the differences in

the mechanisms of action for HTH at 60 and 70℃.

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Figures

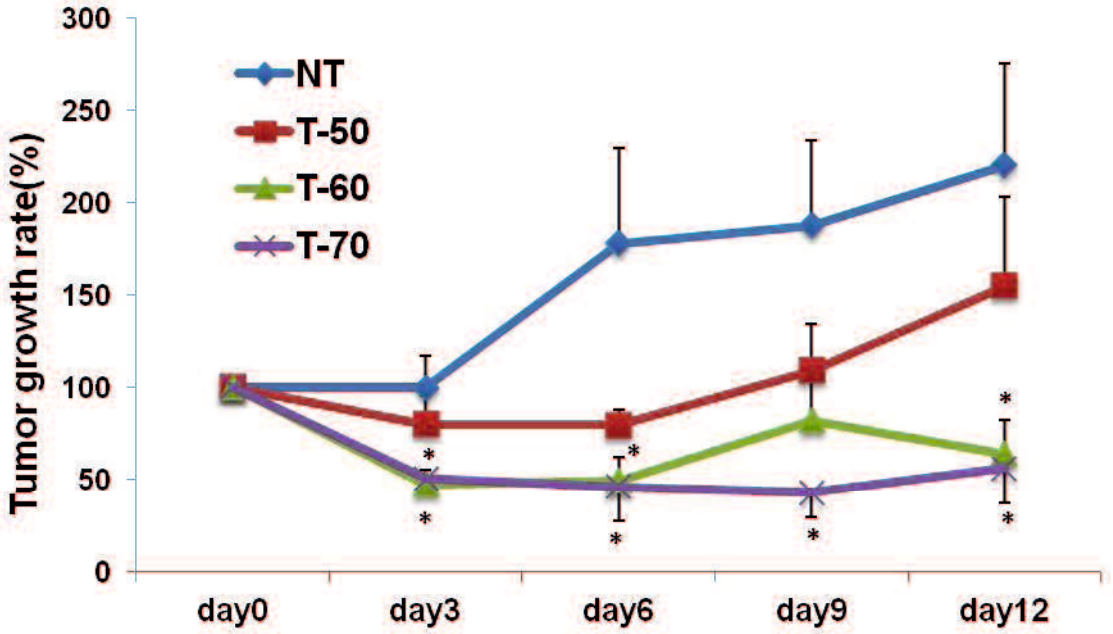


Figure 1. Effects of high-temperature hyperthermia on tumor growth. The tumor volume was measured on days 0, 3, 6, 9, and 12. The tumor growth rates were calculated according to the tumor volumes. The data are presented as the mean ± standard error of the mean for each group. Statistical significance was determined using the Tukey-Kramer test; $P<0.05$ vs. NT group.

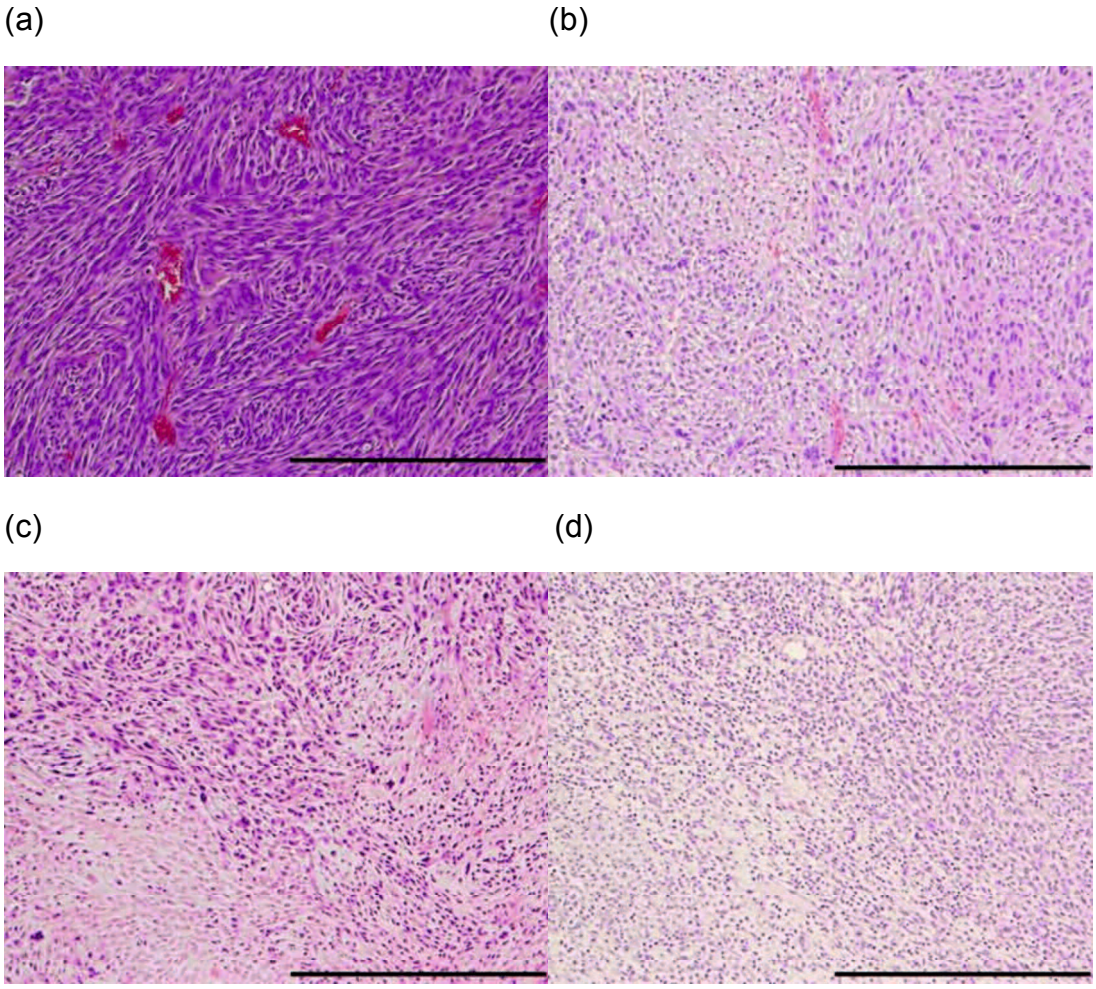
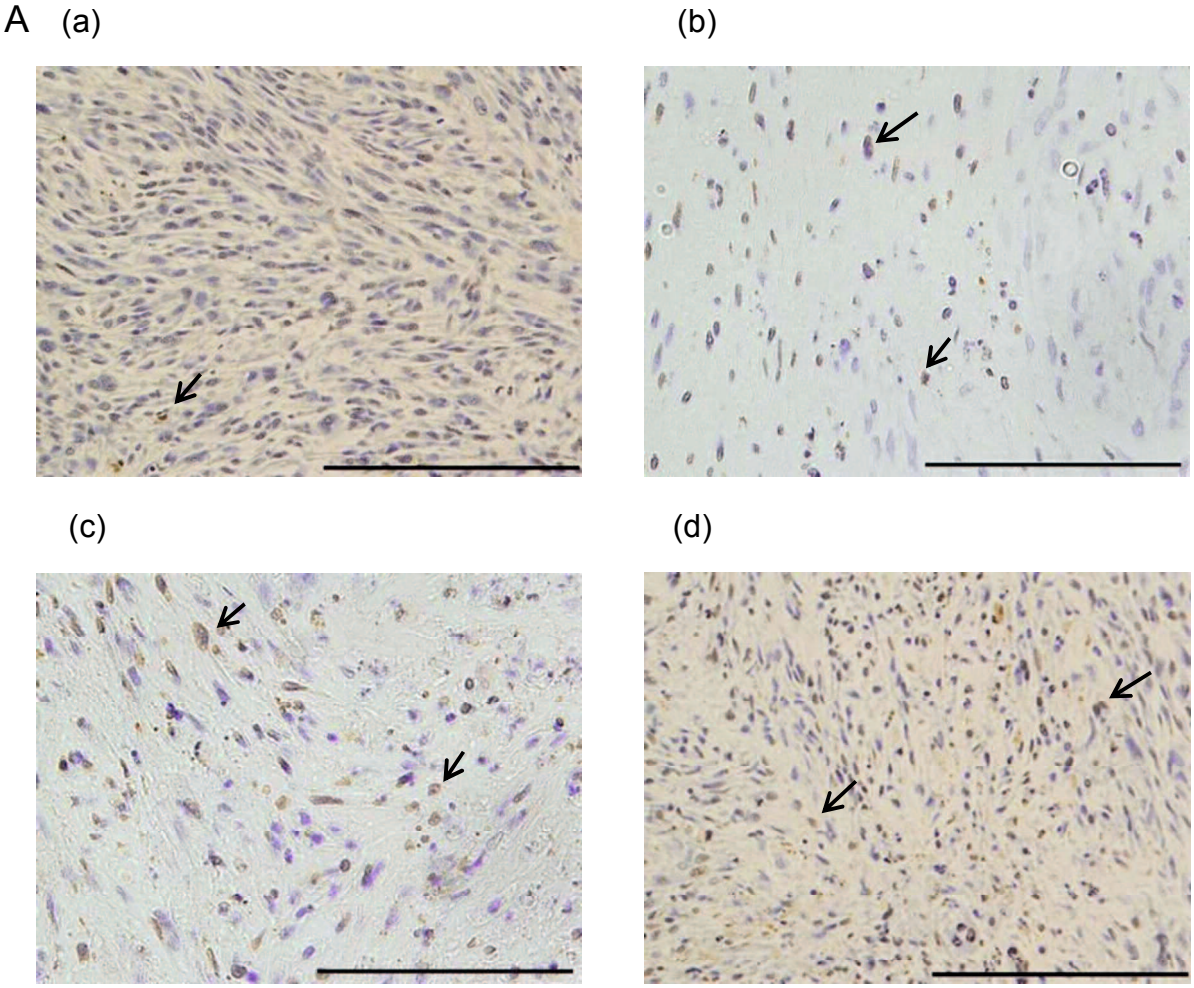


Figure 2. Effects of high-temperature hyperthermia on histological changes.

The tumor tissue sections were stained with hematoxylin and eosin (bar, 200 μ m).

Data are presented for one rat each from the (a) nontreatment ,(b) 50, (c) 60 and (d) 70℃ high-temperature hyperthermia groups.



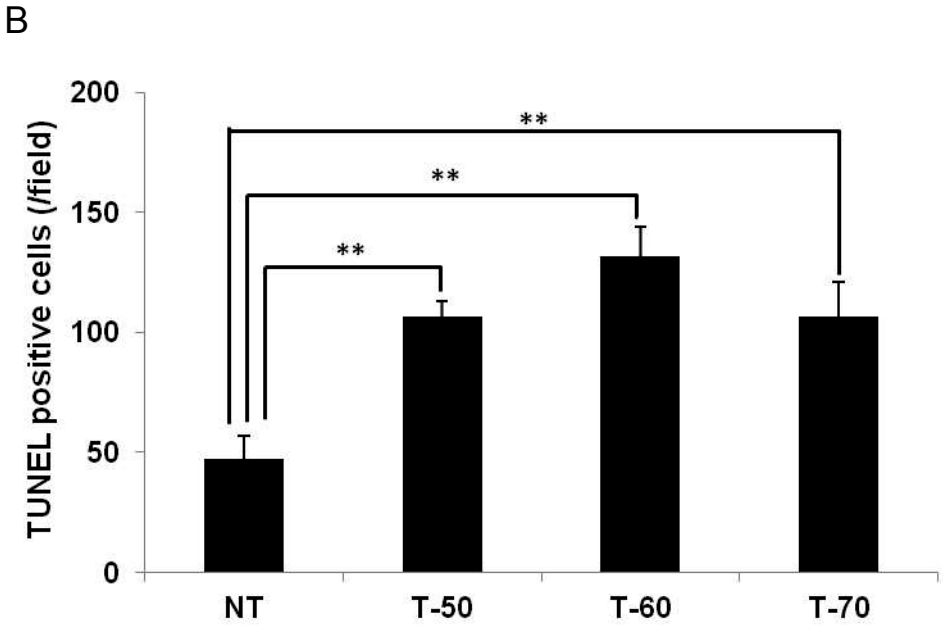
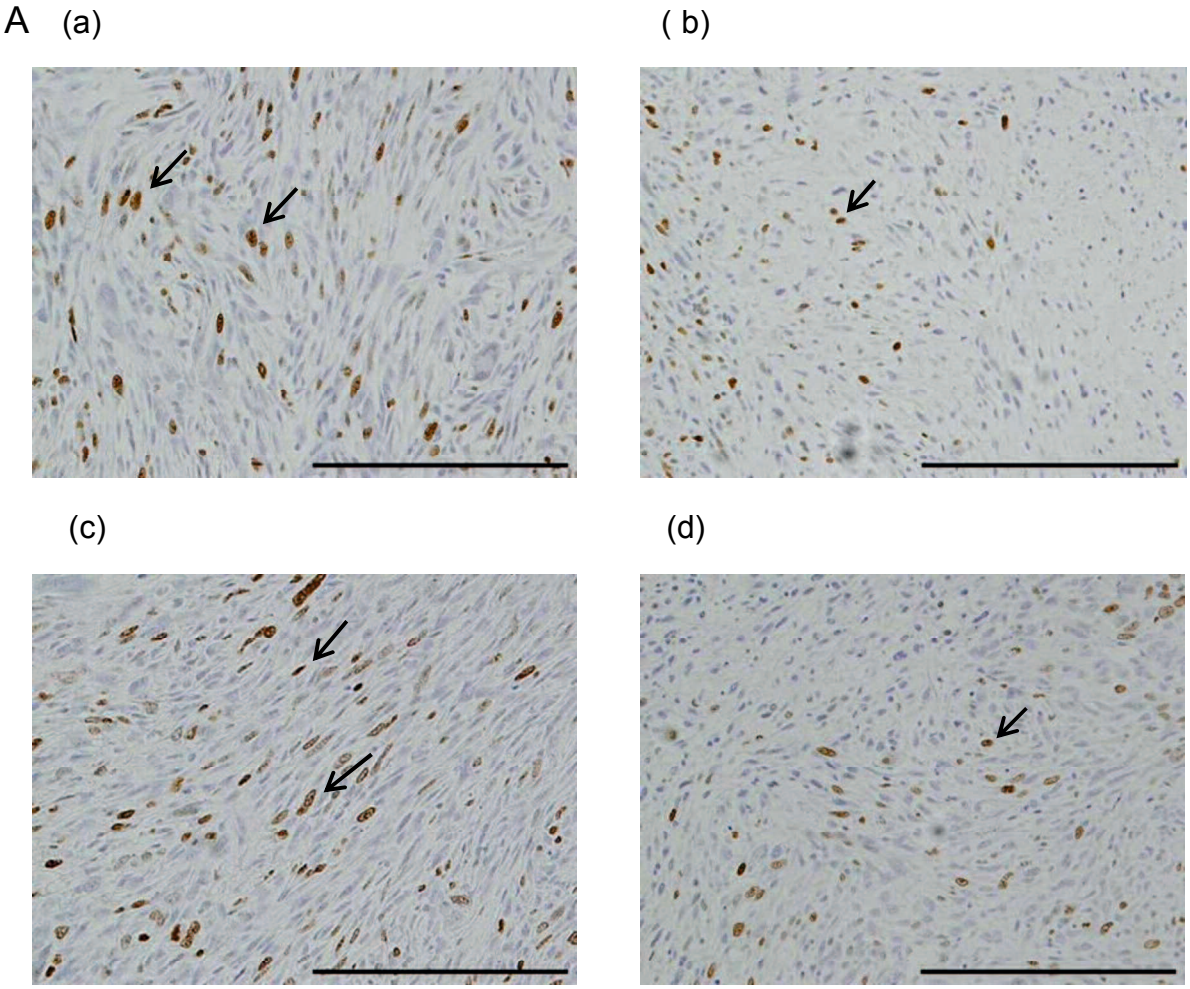


Figure 3. Effects of high-temperature hyperthermia on the number of TUNEL-positive

cells in the tumor tissue. (A) The tumor tissue sections were stained with TUNEL (bar, 100 μ m). Data are presented for one rat each for the (a) NT (b) T-50 (c) T-60 (d) T-70 groups. (B) The numbers of TUNEL-positive cells were calculated. The data are presented as the mean ± standard error of the mean for each group. Statistical significance was determined according to the Steel-Dwass test; **P<0.01. TUNEL, *terminal* dUTP nick-end labeling; NT, nontreatment; T-50, 50℃ high-temperature hyperthermia; T-60, 60 ℃ high-temperature hyperthermia; T-70, 70 ℃ high-temperature hyperthermia.



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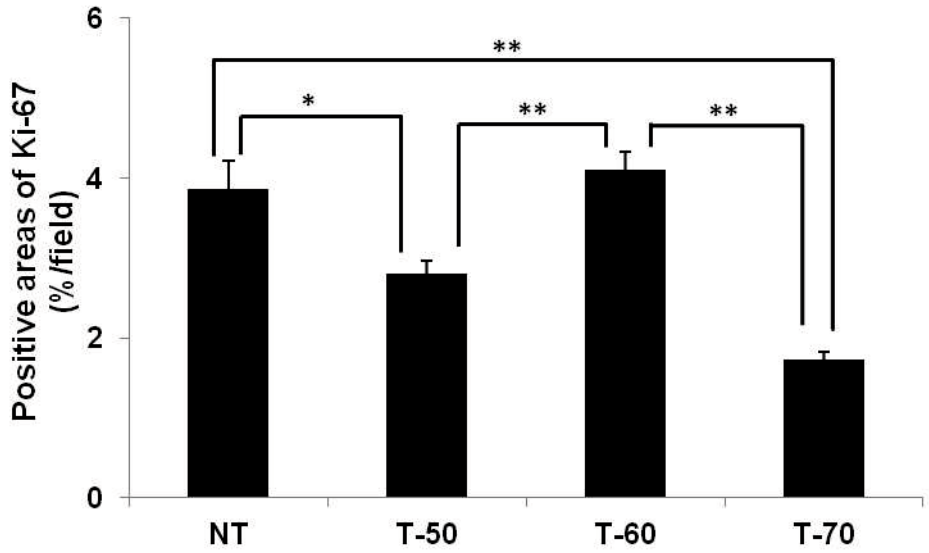

Figure 4. Effects of high-temperature hyperthermia on the size of the Ki-67-positive areas in the tumor tissue (bar, 100 μ m). (A) Tumor tissue sections were immunohistochemically stained with Ki-67. Data are presented for one rat each for the (a) NT (b) T-50, (c) T-60 and (d) T-70 groups. (B) Proportions of Ki-67-positive areas were calculated. The data are presented as the mean $\pm$ standard error of the mean for each group. Statistical significance was determined according to the Steel-Dwass test; *P<0.05; **P<0.01. NT, nontreatment; T-50, 50 ℃ high-temperature hyperthermia; T-60, 60℃ high-temperature hyperthermia; T-70, 70℃ high-temperature hyperthermia.

Chapter II

High temperature hyperthermia treatment for canine superficial tumor: A report of three cases

Abstract

High temperature hyperthermia (HTH) has been demonstrated to suppress tumor growth in a tumor-bearing rat model. In the present study, we evaluated the effects of high temperature hyperthermia for the treatment of spontaneous tumors in dogs. In case 1, an 18-year-old female Papillon presented with a right forelimb rhabdomyosarcoma. Case 2 was a 14-year-old male Golden Retriever with a perianal gland adenocarcinoma surrounding the anus. HTH was performed for 10 min at 65℃ with inhaled isoflurane. In case 1, the tumor disappeared 4 weeks after HTH therapy. In case 2, HTH was performed three times, and the tumor disappeared the third procedure. In case 3 the tumor volume decreased by day 21.HTH is a simple procedure with no severe side effects. Consequently, this treatment modality is expected to become a useful alternative therapy for superficial tumors in companion animals.

1. Introduction

The companion animal life span has lengthened with the advent of routine vaccination, improved environment, and advances in veterinary medicine. As a result, the incidence of illnesses associated with aging has increased in pet populations. In

particular, cancer is a significant problem. As in human medicine, there are three major treatments for cancer in veterinary medicine, surgery, chemotherapy, and radiation therapy. However, it is difficult to treat all affected patients with these therapies. Therefore, new treatments must be developed.

Hyperthermia has long been established as a treatment for cancer, particularly for superficially located cancer [1]. Hyperthermia is used alone or as an adjunct to radiotherapy or chemotherapy [2-5] and has been used to treat spontaneous tumors in veterinary medicine [6-9] Studies have focused on two common strategies, conventional hyperthermia at mild temperatures (42-45℃) [1,10, 11] and ablation therapy at high temperatures (>70℃) [12]. We previously demonstrated that high temperature hyperthermia (HTH) ranging 60-70℃ suppressed glioma tumor growth and induced necrosis and apoptosis in a rat model [13]. In the present study, we evaluated the efficacy of HTH in the treatment of spontaneous tumor in dogs.

2. Case reports

Case 1: An 18-year-old female Papillon (3.2kg) presented for evaluation of a right forelimb tumor (Figure 1A). Surgical removal of the tumor was performed twice previously, but the tumor recurred, A biopsy and histopathological analysis revealed that the tumor was a rhabdomyosarcoma. On initial exam, the caudal right forelimb was covered by the tumor, and the patient was lame in the affected limb. We explained the risk of recurrence and the treatment options to the owners, including surgery, radiation therapy, and chemotherapy. Complete surgical excision was too difficult because the tumor border was unclear. We recommended HTH experimental therapy and the pet was enrolled in a clinical trial, with the owners' signed informed

consent. A tissue ablation device for veterinary medicine (AMTC 200; AdMeTech Co., Ltd, Ehime, Japan) was used to administer the HTH treatment. On day 0, HTH was performed with no anesthesia and sedation. Three needle of the device were inserted into tumor tissue at 6-mm intervals, and HTH was performed for 10 min at 45-65℃. On day 21, the tumor volume decreased from that on day 0, and the lameness improved (Figure 1B). After 4 weeks of HTH, the tumor disappeared.

Case 2: A 14-year-old male Golden Retriever (32.7 kg) presented for evaluation of a tumor surrounding the anus (Figure 2a). Biopsy and histopathological analysis identified the tumor as perianal gland adenocarcinoma. We explained the risk of recurrence and the treatment options to the owners, including surgery, radiation therapy, and chemotherapy. Complete surgical excision was too difficult because the tumor border was unclear. We recommended HTH experimental therapy, and the pet was enrolled in a clinical trial, with the owner's signed informed consent. On day 0, HTH was performed under general anesthesia administered by inhalation of isoflurane. Five needles of the device were inserted into the tumor at 1-cm intervals, and HTH was performed for 10min at 65℃ (Figure 2B) and then repeated one additional time. On day 21,the tumor volume was decreased from that on day 0 (Figure 2C). HTH was repeated using the same protocol, but the dog died 1week later due to senility.

Case 3: A 13-year-old male English Cocker Spaniel (12.3 kg) presented for evaluation of a tumor in the right external auditory canal (Figure 3A). We performed a right total ear canal ablation, and subsequent histopathological analysis revealed a ceruminous adenocarcinoma. Several months after intervention, the tumor recurred

at the surgical site. We explained the risk of recurrence and the treatment options to the owner, including surgery, radiation therapy, and chemotherapy. In particular, surgery presented risks of vestibular disorder and facial paralysis complications. We recommended HTH experimental therapy, and the pet was enrolled in a clinical trial, with the owner's signed informed consent. On day 0, HTH was performed under general anesthesia maintained with inhaled isoflurane. Five needles of the device were inserted into the tumor, and HTH was performed for 10 min at 65℃ (Figure 3B). On day 22, the tumor volume was decreased from that on day 0. On day 28, the HTH was repeated using the same protocol. On day 78, the tumor volume had decreased further, and a third HTH procedure was performed. On day 133, the tumor had disappeared and did not recur.

3. Discussion

The beneficial effects of HTH in the treatment of superficial cancer have not yet been reported in veterinary medicine. The HTH protocol we used was very simple and was only performed on canine spontaneous tumors, In these three cases, the tumor volumes decreased following HTH therapy. Furthermore, no severe side effects were observed in any of the cases.

In recent decades, several innovative minimally invasive cancer therapies have been developed as alternatives to surgery. Ablation, which uses high temperature, radio waves, or microwaves, is considered a potent alternative therapy [14].

High temperature (>46℃) can directly damage cells, resulting in severe protein denaturation and DNA damage [15, 16] and inducing irreversible changes that

ultimately result in cell death. Tumor cells express specific tumor-associated antigens. In high temperature (>46℃) conditions, tumor cells swell and break into pieces, which releases antigens; the large antigen load generates antitumor immunity. The high temperatures also cause severe protein denaturation, but this likely destroys the immunogenicity of tumor cells [17-21]. When thermal ablation temperature (>70℃) are achieved, there is a high risk of shock syndrome induced by the sudden and large production of necrotic tumor material [22]. Therefore, the case for ablation therapy is restricted in human medicine. In general, ablation therapy is performed to the tumor within 3cm in diameter [23]. In the present cases, tumor sizes were over 3 cm in diameter although we did not measure exactly. In a previous study, we reported that HTH administered at 50-70℃ induces necrosis in arat glioma model [13]. However, HTH at 50℃ did not have adequate suppressive effects compared to treatment at 60 and 70℃. In case 1, however, the adequate suppressive effect was showed by HTH at 45-65℃. This result might indicate the sensitivity to the temperature of HTH vary by tumor types. More extended study which performs HTH to various is necessary. The optimal therapeutic protocol including effective temperature, time, and frequency must be established to expand HTH therapy for routine use in veterinary oncology.

In conclusion, HTH is a simple therapeutic option with no severe side effects. This treatment modality is expected to become a useful alternative therapy for superficial tumors in companion animals.

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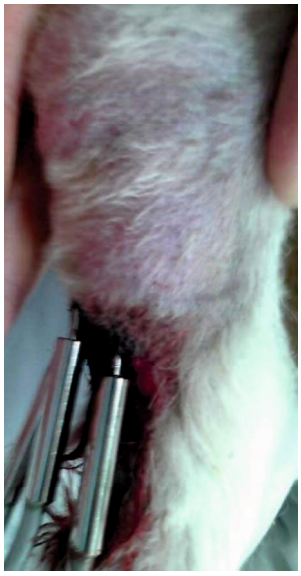
Figure legends

Figure 1. Gross appearance of case 1. (A) A tumor with right forelimb (rhabdomyosarcoma). (B) On day 21, the tumor volume was decreased compared with that on day 0.

Figure 2. Gross tumor appearance in case 2. (A) The tumor surrounding the anus, later diagnosed as perianal gland adenocarcinoma. (B) HTH was performed with inhalation of isoflurane. Five needles of the ablation device were inserted into the tumor, and HTH was performed for 10 min at 65℃. (C) the tumor volume was decreased on day 21.

Figure 3. Gross tumor appearance in case 3. (A) The tumor, diagnosed as a ceruminous adenocarcinoma, recurred in the right external auditory canal after a total ear canal ablation. (B) HTH was performed with inhalation of isoflurane. Fiveneedles of the ablation device were inserted into the tumor, and HTH was performed for 10 min at 65℃. (C) The tumor volume was decreased, and HTH was reported using the same protocol on day 28. (D) The gross appearance of the affected ear on day 133 reveals that the tumor disappeared

(A)



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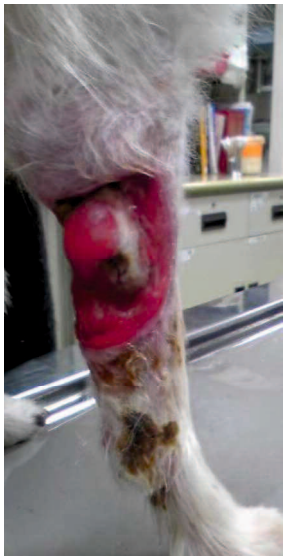

Figure 1.

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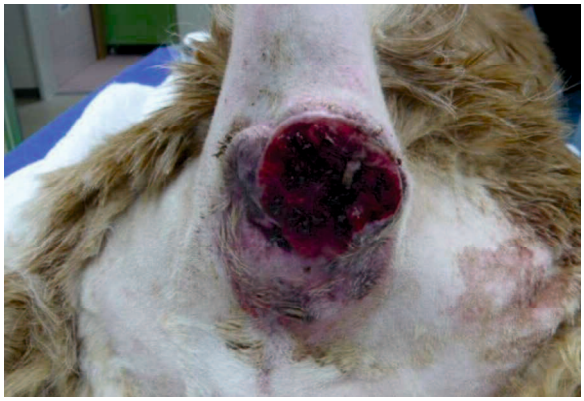

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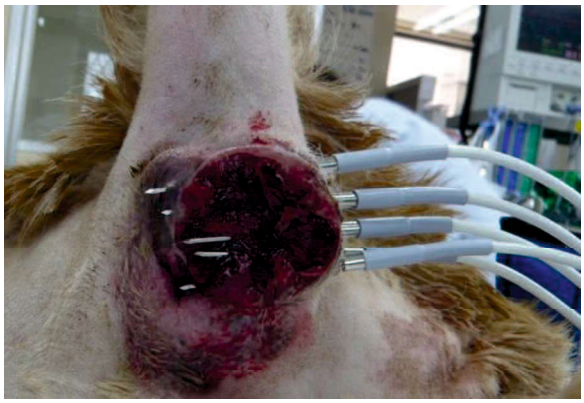

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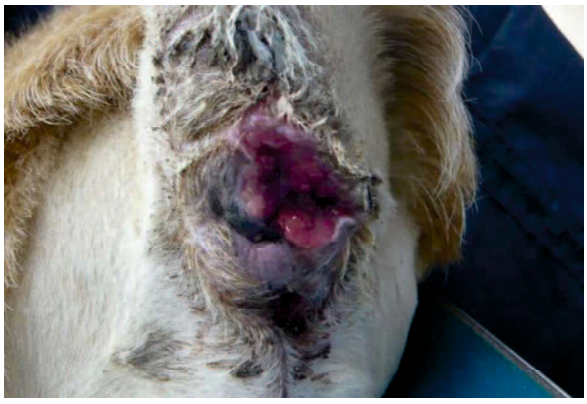
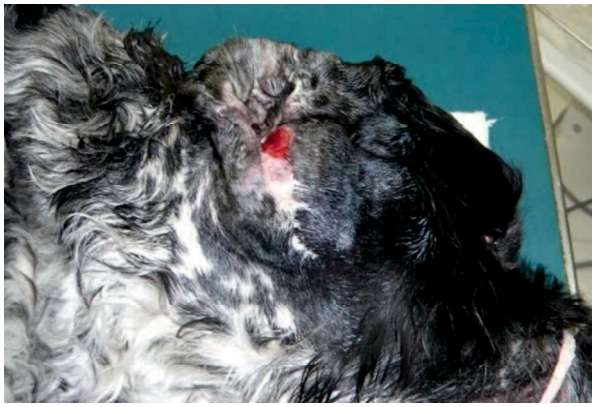


Figure 2

(A)



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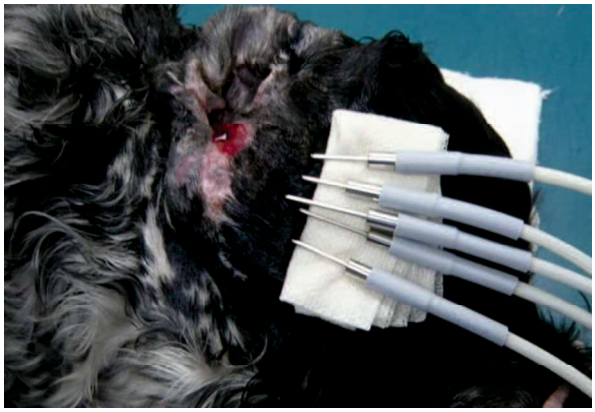

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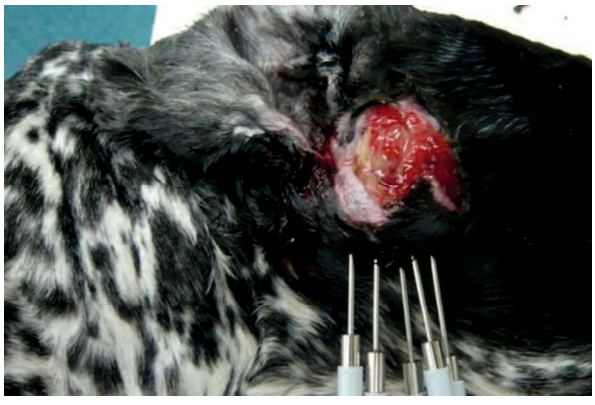

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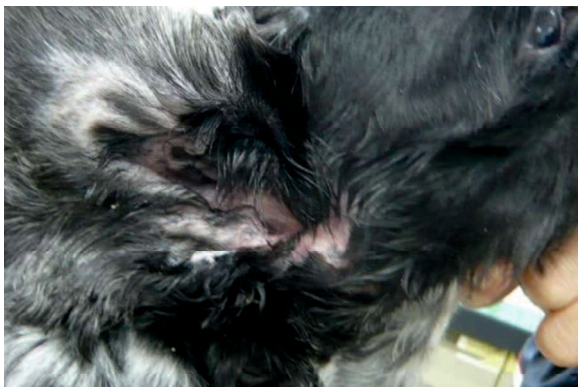

Figure 3

Conclusions

High temperature hyperthermia at temperatures exceeding 60℃ suppressed tumor growth in a glioma-bearing rat model. In addition, high temperature hyperthermia at 50-70℃ induced necrosis and apoptosis in a glioma rat model. Further studies is required to clarify the differences in the mechanisms of action for high temperature hyperthermia at 60 and 70℃.

High temperature hyperthermia is a simple therapeutic option with no severe side effects. This treatment modality is expected to become a useful alternative therapy for superficial tumors in companion animals.

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