Anti-oxidants
Anti-cancer drugs

Introduction

Physiology of cancer cells has been extensively studied, and the understanding of mechanisms for their rapid growth and proliferation has been advanced in the past decade (1-3). Accordingly, various therapeutic strategies in cancer treatment have been developed (4, 5). Although surgical removal of the cancer tissue is still the golden standard for complete cure, it is not always feasible in cases with advanced or metastatic cancer. Surgical stress may be too large for geriatric and/or exhausted patients. In such cases, combination of various therapeutic strategies has been recommended. Among such strategies, hyperthermic therapy may be applied on the top of the conventional cancer chemotherapy or radiation therapy (6, 7). Although it may not achieve complete remission of cancer by itself, clinical studies have demonstrated that the survival and quality of life may be significantly improved (3, 8).

Molecular mechanism of hyperthermic therapy includes the overstimulation metabolism of rapidly proliferating cancer cells, leading to the induction of apoptosis (9). Increased production of reactive oxygen species (ROS) from mitochondria may also be involved (10). Because ROS production may be increased in the presence of anticancer drugs by itself, the combination of chemotherapy and hyperthermic therapy would synergistically increase ROS production, leading to effective cancer cell death (7). However, ROS production is inhibited in the presence of various antioxidants (11). In this regard, various antioxidants, which are also used as dietary supplements, may interfere with the efficacy of such chemotherapy and/or hyperthermic therapy. Unfortunately, however, evaluation of the effect of such antioxidants in the combination of cancer chemotherapy has not been well performed(12, 13). Ascorbic acid, for example, is often used as dietary supplement. Because ascorbic acid may improve immunity or peripheral circulation (14), people, including cancer patients, take this antioxidant. However, the use of ascorbic acid in cancer patients remains controversial; ascorbic acid may enhance (11) or suppress (14) the efficacy of chemotherapy.

In this study, we examined the effect of temperature, anticancer drugs, and antioxidants on ROS production. We used MAT-LU prostate cancer cells since hyperthermia therapy has been often applied to prostatic cancer patients (15, 16), and thus it is necessary to evaluate the effect of hyperthermia on this cancer cell type. We demonstrate their effect on ROS production, and make potential suggestions for future use of antioxidants in cancer patients.

Materials and Methods

Materials

We used the following anticancer drugs; vinblastine (VBL) (Nihon Kayaku, Japan), cisplatin (CIS), (Pfizer, Japan), adriamycin (ADR), (Wako, Japan), docetaxel (DTX), (Sanofi Aventis, Japan). Similarly, as antioxidants, we used N-acetyl-cysteine (NAC), (Sigma, Japan), retinoic acid (Sigma, Japan), quercetin (Sigma, Japan), catechin (Wako, Japan), lutein (Sigma, Japan),β-carotene (Sigma, Japan), and ascorbic acid (Wako, Japan).

Cell culture

Rat prostatic adenocarcinoma cells (R3327-MAT-Lu) were cultured in RPMI-1640 medium supplemented with 10% FBS and 250 nM dexamethasone, which were kindly provided by Dr. J. T. Isaacs (Johns Hopkins University, MD). Cells were incubated at 37°C in 5% CO_2. In some experiments, cells were incubated at 42°C as hyperthermic treatment(see below). Rat cardiac fibroblasts were isolated from adult rats (250–300 g, male) by using a modification

of published methods(17). Fibroblasts were separated from cardiac myocytes by gravity separation and grown to confluence on 10-cm cell culture dishes at 37°C with 90% air with 10% CO_2 in growth media (DMEM with 10% FBS, 1% penicillin, and 1% streptomycin).

Hyperthermic stress and measurement of reactive oxygen species (ROS)

Cells were plated in 24-well culture plates (5.0 × 10^4 cells/well) overnight. Cells were then treated with various agents, including anticancer drugs, at 37℃ for 3 hours. For hyperthermic treatment, cells were further incubated in the presence or absence of various reagents at 42℃ for 1 hour. The intracellular ROS level was then measured using a fluorescent dye 2',7'-dichlorofluorescein diacetate (DCFH-DA)(Invitrogen) as previously described(18). In the presence of oxidant, DCFH is converted into the highly fluorescent 2',7'-dichlorofluorescein. Cells were first washed with PBS, and serum-free DMEM containing 10 μM DCFH-DA was added to each well. Cells were then incubated at 37°C for 45min. ROS production was measured using a microplate reader equipped with a spectrofluorometer (Perkin-elmer ARVO MX) at an emission wavelength of 538 nm and extinction wavelength of 485 nm.

Statistical analysis

Data are expressed as means ± SEM. Data was analyzed by one-way ANOVA followed by Tukey post hoc using Graph-pad Prism software. Statistical significance was set at $P<0.05$.

Results

Effect of temperature on ROS generation

It is known that cancer cells exhibit higher metabolism than normal cells. High metabolic rate may be reflected by increased ROS generation, in particular, upon hyperthermia. Accordingly, we compared the effect of temperature on ROS production between MAT-Lu prostate cancer cells and normal fibroblasts obtained from the cardiac tissue. It is known that fibroblasts grow rapidly and thus possesses high metabolic rate in comparison to other normal cell types.

As shown in Fig 1A, ROS production was lower at 32°C than at 37°C while it was higher at 42°C. Thus, ROS production was increased in a temperature-dependent manner, at least, in prostate cancer cells. In contrast, ROS production in cardiac fibroblasts was not increased at 42°C in comparison to that at 37°C (Fig 1B). Thus, ROS production by hyperthermia was increased only in cancer cells.

Effect of ascorbic acid on ROS production

We then examined the effect of ascorbic acid, which has been used in cancer treatment as part of chemotherapy, but is also known as major antioxidant. In the presence of an increasing concentration of ascorbic acid (10 μM to 100 mM), ROS production was decreased in a concentration-dependent manner at 37°C (Fig 1C). Similar inhibition was observed at 42°C. Thus, ascorbic acid potently inhibited the production of ROS.

Effect of anticancer drugs on ROS production

Anticancer drugs may induce cytotoxicity through various mechanisms. We examined the effect of these

anticancer drugs, which have been widely used in many cancer cell types, including prostate cancer, on ROS production. We first determined the EC_{50} values of these drugs in prostate cancer cells, which were 200 nM for vinblastine (VBL), 15 μM for cisplatin (CIS), 7.5 μM for adriamycin (ADR), and 1 mM for docetaxel (DTX). When prostate cancer cells were incubated with these drugs at the EC_{50} value concentration, ROS production was slightly, but significantly increased with VBL and CIS, but not with DTX and ADR at 37°C (Fig 2A). When hyperthermic treatment at 42°C was added, ROS production by VBL and CIS became even greater (Fig 2A). Thus, hyperthermia by itself can increase ROS production, which is further enhanced in the presence of certain anticancer drugs.

We then examined the effect of ascorbic acid in the presence of anticancer drugs. ROS production was potently inhibited by 1mM ascorbic acid in the presence of any anticancer drugs (Fig 2B). ROS production at 37°C was similar among these anticancer drugs. However, when hyperthermic treatment at 42°C was added, ROS production was significantly greater with VBL (Fig 2B). Thus, ascorbic acid may negate ROS production induced by certain anticancer drugs at 37°C, however, it cannot negate ROS production of VBL at 42°C. Accordingly, anticancer drug-induced ROS enhancement may be retained in hyperthermia for VBL, but not others.

Effect of ascorbic acid on ROS production by Resovist

Resovist is super-paramagnetic iron oxide nanoparticle that has been used as MRI contrast agent. Because of its magnetic property, similar compounds have been used as source of heat production in hyperthermic therapy. We found that the ROS production was increased in the presence of 10 μM resovist at 37°C, suggesting that Resovist can produce ROS with cancer cells. When ascorbic acid was added, ROS production was negated or rather decreased. Thus, ascorbic acid could potently inhibit ROS production induced by Resovist.

Effect of various antioxidants on ROS production

Patients may take various dietary supplements during cancer chemotherapy. In some cases, patients may take supplementary antioxidants on the top of anticancer drugs. We thus examined the effect of these antioxidants and related drugs, namely, N-acetyl cysteine (NAC), retinoic acid, quercetin, catechin, lutein, and β-carotene, on ROS production. We used these antioxidants at concentrations as previously demonstrated to be effective in various assays (12, 19, 20). We examined their effect on VBL and CIS, which increased ROS production in the above assays.

As shown in Figs 4A-F, these antioxidative compounds exhibited various degrees of antioxidative effects. NAC showed the most potent inhibition on ROS production; ROS production was decreased by a quarter in prostate cancer cells. VBL or CIS did not further increase ROS production in the presence of NAC at both 37°C and 42°C, suggesting the ROS production by these anticancer drugs was completely suppressed by NAC. Thus, NAC showed similar to, but perhaps greater antioxidative effect than ascorbic acid. Retinoic acid, quercetin, and lutein showed comparable results with each other.They inhibited ROS production at both 37°C and 42°C. However, both VBL and CIS could increase ROS production in the presence of these antioxidants, suggesting that these antioxidants could not inhibit anticancer drug-mediated ROS production. Catechin and β-carotene are best known as antioxidants in general. However, they did not inhibit ROS production, either 37°C or 42°C, in the absence or presence of anticancer drugs. Thus, the effects of many antioxydants are not always the same.

Discussion

The current study has demonstrated that ROS production was higher in cancer cells than in normal cells, and was further increased with temperature. Ascorbic acid exhibited the potent inhibition of ROS production regardless of temperature. ROS production was also increased in the presence of anticancer drugs, such as VBL and CIS, but not by DTX or ADR. Importantly, ROS production of these anticancer drugs was inhibited in the presence of ascorbic acid regardless of temperature. In contrast, antioxidants, some of which have been used as dietary supplement among general population, showed variable effects. NAC inhibited ROS production regardless of the presence of anticancer drugs while catechin or β-carotene did not inhibit ROS production. Lutein, quercetin, and retinoic acid inhibited ROS production in the absence of anticancer drugs while they did not inhibit the ROS production as induced by anticancer drug. Thus, these antioxidants should be taken carefully by patients since they may variably affect the effect of anticancer drugs, at least, in their ROS production.

ROS as a cause of cytotoxicity of anticancer drugs has been extensively studied in the past(21, 22). CIS may interfere with mitochondrial membrane function and thus increases ROS production. Paclitaxel, which is comparable to DTX, may regulate membrane NOX release, and increases ROS production(23-26). We found that both CIS and VBL increased ROS production in prostate cancer cells. Hyperthermic therapy potentiates ROS production, leading to enhanced cytotoxicity (27). We also found that increased temperature enhanced ROS production by CIS and VBL. Thus, both cancer chemotherapy and hyperthermic treatment enhanced ROS production, at least, in prostate cancer cells.

With increasing public interest in antioxidant therapy, many nutritional supplements have been taken by general public including cancer patients. There have been multiple studies that examined the interaction between anticancer drugs and antioxidants. However, the results of these studies are not in agreement with each other. Anticancer drugs may produce ROS, which may damage cancer cells(28, 29). Thereby, some studies demonstrated that antioxidants reduced the effect of these anticancer drugs (30). In contrast, others demonstrated that ROS production was enhanced by antioxidants(31). More specifically, ascorbic acid can quench ROS within the cell, and thus stabilizes mitochondrial membrane, leading to protection of the cell (14, 27). Although previous studies demonstrated that ascorbic acid increased the effect of anticancer drugs, attenuation of anticancer drug effect was also reported more recently (27).

We found that antioxidants indeed exhibited various effects on ROS production. NAC, which by itself scavenges ROS (19), potently decreased ROS production, and ROS production by anticancer drugs was also negated. Thus, the use of NAC may hamper the effect of anticancer drugs. In contrast, lutein, quercetin, and retinoic acid, which are also known as ROS scavenger, decreased ROS production. However, they were not potent enough to inhibit the ROS producing effect of anticancer compounds. Thus, these antioxidants may be taken safely by cancer patients during chemotherapy and hyperthermic therapy. Catechin and β-carotene are known as antioxidant and are contained in various kinds of foods, such as green tea or carrot (12, 13). However, they did not exhibit inhibitory effect on ROS production regardless of the presence of anticancer drugs, suggesting that they do not interfere with such drug effects. Thus, cancer patients may take these antioxidants as well as foods containing these antioxidants.

Putting together, administration of NAC and ascorbic acid may need caution while other antioxidants may not require major attention, at least, in terms of ROS production in cancer patients. In particular, ascorbic acid is widely used for multiple purposes, including for viral infection. Accordingly, the current study has suggested that the use of ascorbic acid may be considered carefully by both cancer patients and oncologists. Further, with our findings, the effect of ascorbic acid and its related antioxydants need to be clinically examined in future in cancer patients, who will be treated with chemotherapy and/or hyperthermic therapy.

Figure legends

Figure 1 ROS production in cancer cells and normal cells at different temperatures

A) ROS production in cancer cells at 32°C, 37°C, and 42°C. Prostate cancer cells were incubated at different temperatures, followed by determination of ROS production. Means±SEM are shown (n=4, *p<0.05).

B) ROS production in cardiac fibroblasts at 37°C and 42°C. Cardiac fibroblasts were incubated at different temperatures similarly, followed by determination of ROS production. Means±SEM are shown (n=4, *p<0.05).

C) ROS production was determined with cancer cells in the presence of an increasing concentration of ascorbic acid (10μM to 100 mM). Prostate cancer cells were incubated at 37°C, followed by determination of ROS production. Means ± SEM are shown (n=4, *p<0.05).

Figure 2. Effect of anticancer drugs and ascorbic acid on ROS production

A) ROS production was determined at 37°C or 42°C in the presence of 200 nM VBL, 15 μM CIS, 7.5 μM DTX or 1 μM ADR. Means±SEM are shown (n=4, *p<0.05).

B) ROS production was similarly determined in the presence of 1 mM ascorbic acid at 37°C or 42°C. Means ± SEM are shown (n=4, *p<0.05).

Figure 3. Effect of Resovist on ROS production

ROS production was determined in the presence of 10μM Resovist and/or 1 mM ascorbic acid at 37°C. Prostate cancer cells were incubated for 45 minutes, followed by ROS production assays. Means ± SEM are shown (n=4 *p<0.05).

Figure 4. Effect of various antioxidants on ROS production

ROS production was determined in the presence of 200 nM VBL or 15 μM CIS at 37°C or 42°C. Various antioxidants, i.e., 10mM NAC (N-acetyl-cysteine) , 50nM retinoic acid, 100 nM quercetin, 50 μM catechin, 100 nM lutein, and 20 μM, β-carotene, were added. Cells were incubated for 45 minutes, followed by determination of ROS production. Means ± SEM are shown (n=4, *p<0.05).

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Fig 1

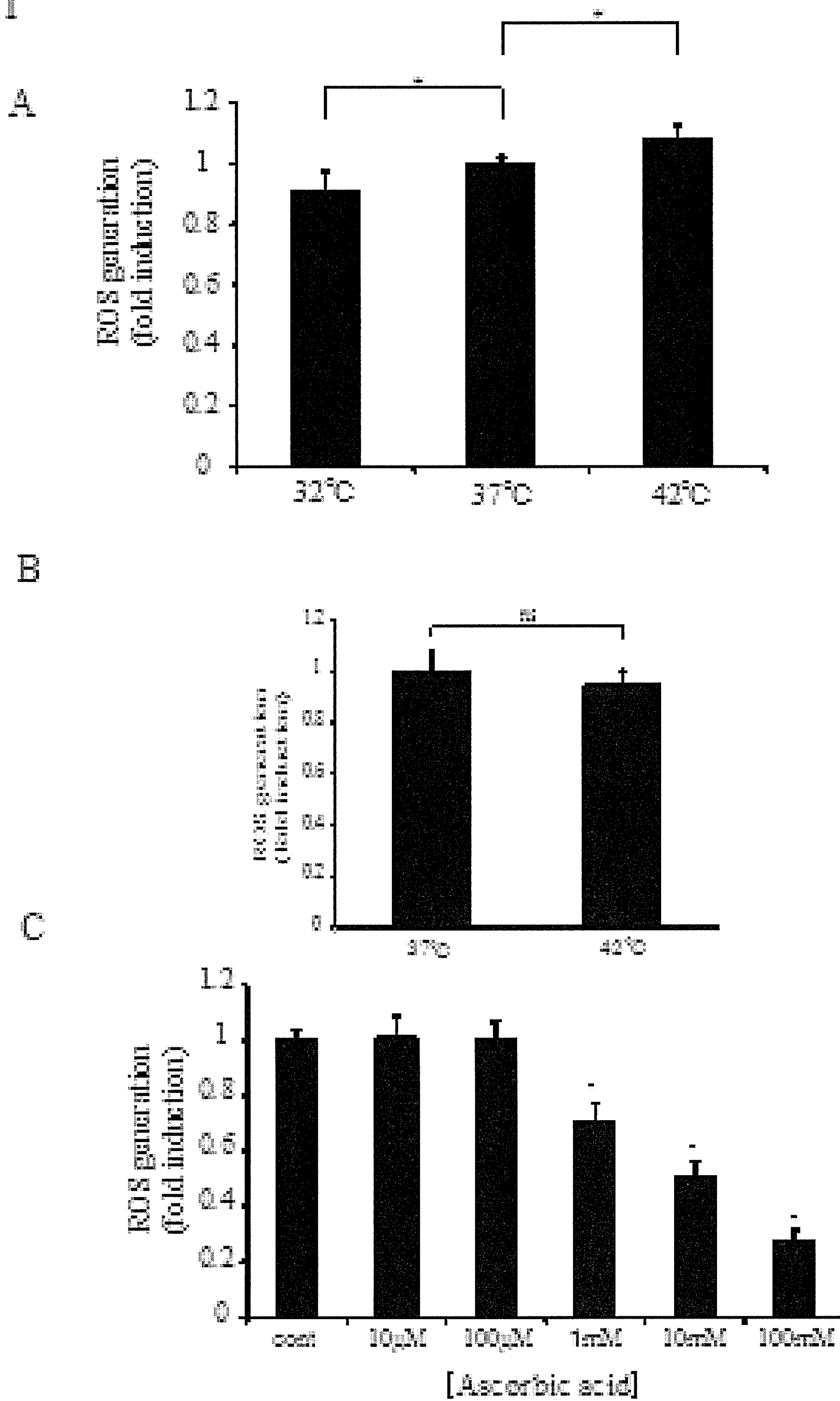

Fig 2

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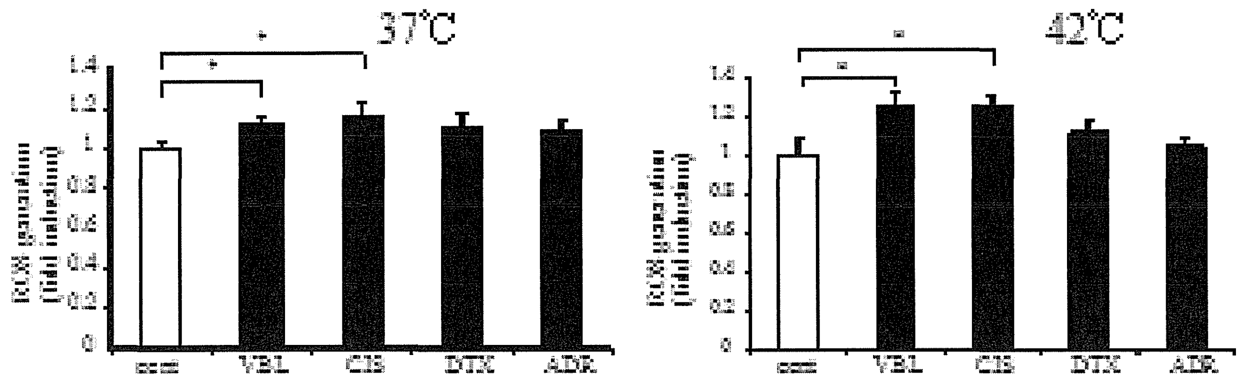


B (+)ascorbic acid

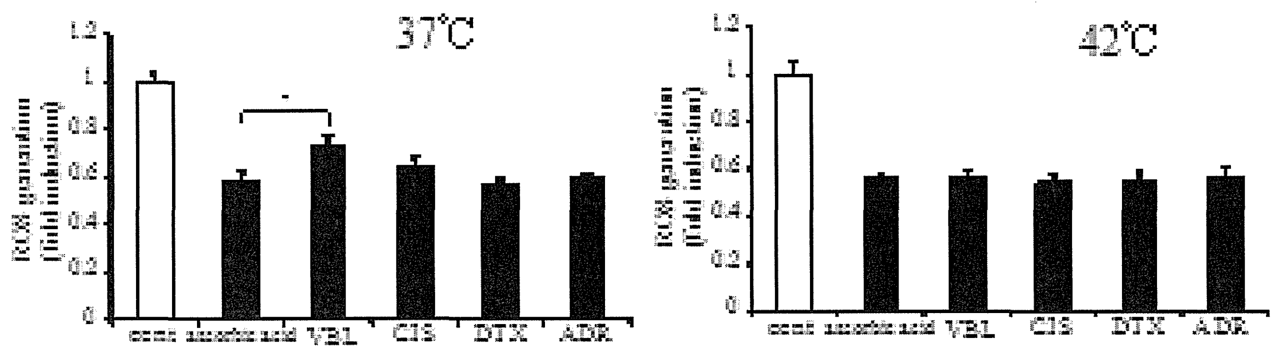


Fig 3

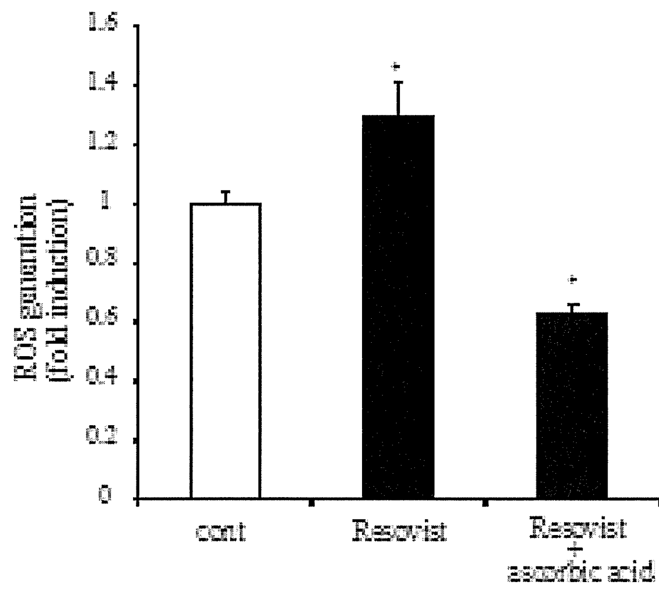
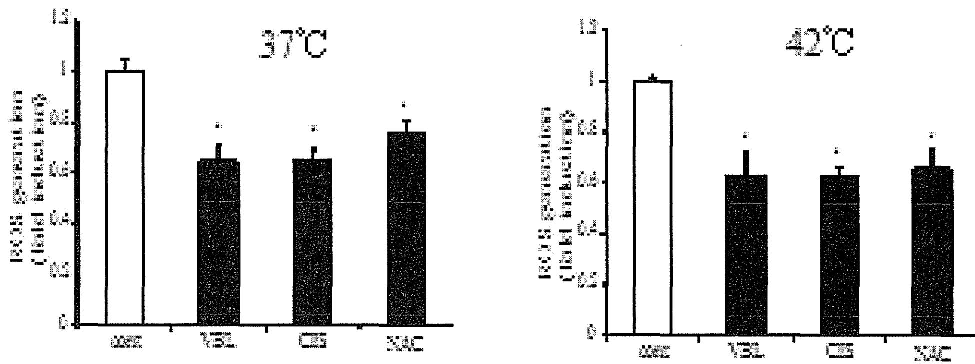


Fig 4

A



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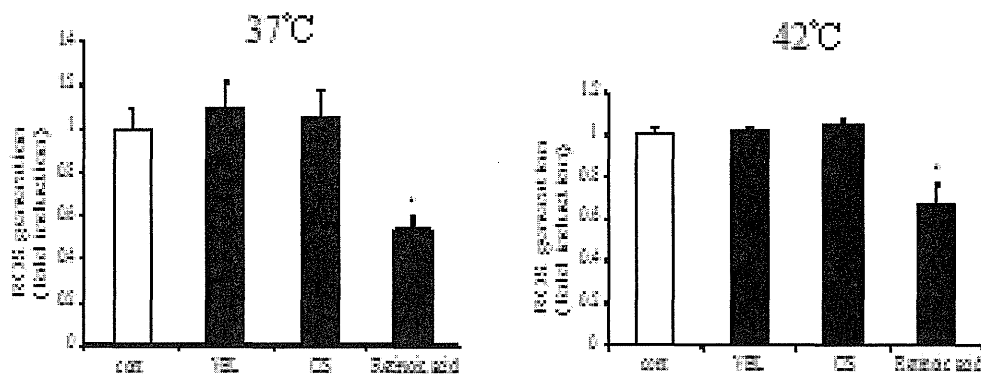


C

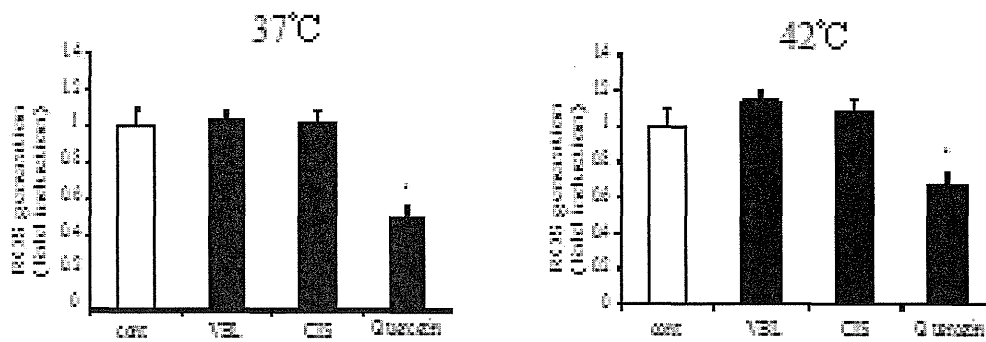
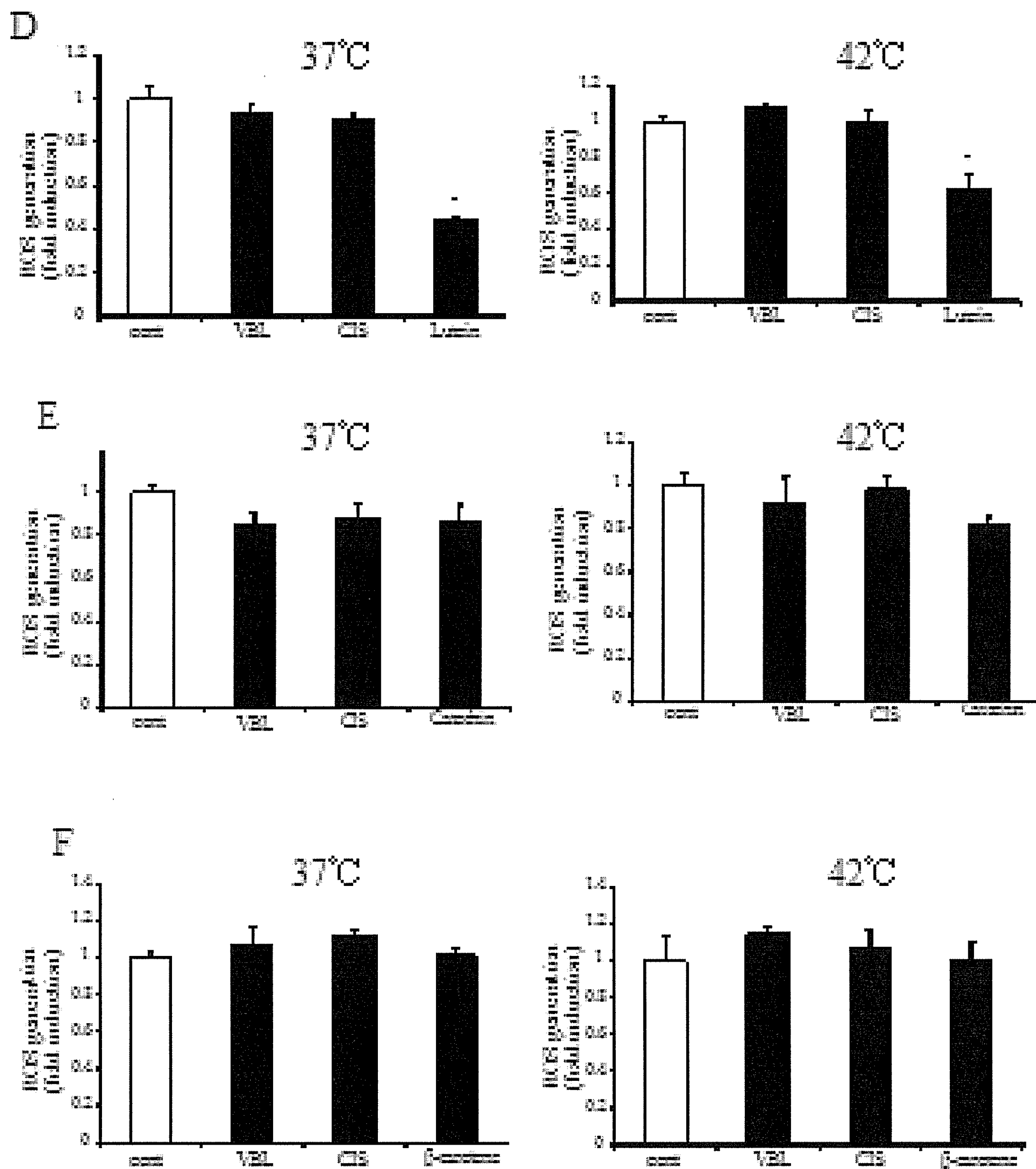


Fig 4



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分担研究報告

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A．研究目的

局所加温が可能なハイパーサーミアの発熱源としては、針状磁性体や感温磁性体といった磁性材料を使用したものや、共振回路を用いたものが研究されている。本研究では磁性ナノ粒子を発熱源として用いたハイパーサーミアの実現に向け、磁性ナノ粒子の発熱機構の解明を行うことを目的とする。本研究を実施することにおり、磁気ハイパーサーミアの励磁装置を設計試作するための知見や、磁性抗癌剤の特徴を顕在化させ、それを実用するための知見を得るものと期待される。

B．研究方法

平成24年度は、マグネタイト（Fe_3O_4）ナノ粒子に、ポリエチレンイミン（PEI）をコーティングさせて分散させたサンプルを用いた。平成25年度は、バイオ医療応用として血管内凝集がないなどの優位性をもつ超常磁性マグネタイトをサンプルとして、発熱特性を評価した。前年度と同様に、発熱のメカニズム、最適な励磁条件を考察した。

（倫理面への配慮）本分担研究においては関係しない。

C．研究結果

液中分散させた超常磁性ナノ粒子の透過型電子顕微鏡(TEM)画像、また動的光散乱(DLS)による測定から平均1次粒径が10 nm、平均液中粒径が 52 nm であることを確認した。また、直流磁化測定から、超常磁性ナノ粒子であることを確認した。

温度上昇測定、交流磁化測定における磁界強度特性の結果から、磁界強度 100 Oe 以下での励磁が効率的であり、それ以上の条件では磁界強度の増加に伴い温度は上昇するが、発熱効率は減少することが示された。

D．考察

液中試料における交流磁化測定の周波数特性から磁気緩和が生じていることを確認し、また交流磁化率の測定から発熱効率がピークとなる周波数は5 kHz であった。粉末試料と液中試料と比較して交流磁化曲線が大きく異なっていることから、液中試料では Brownian 緩和が支配的であることが示された。これは液中試料と比較して粉末試料は粒子間の結合力が強いためだと考えられる。

このように本研究において、磁気緩和はこれまで述べられてきた理論式では近似できないことを実験的に明らかにし、また Brownian緩和とNéel緩和の差別化に成功した。これまでの研究においては周囲の粘度に依存しないNéel緩和を用いた発熱方法の検討が盛んに行われてきたが、本研究で用いた試料のようにBrownian緩和を用いた発熱を示す粒子が存在するという新たな指標を得ることができた。今後の展望として、Brownian緩和による発熱効率が最大となる周波数帯が数十 kHzとなるような粒子を作成することで実際のハイパーサーミア治療における発熱効率を高めるための研究等を行う必要があると考えられる。

E．結論

磁気緩和はこれまで報告されている理論式では記述できない特性があることを実験的に明らかにした。磁性ナノ粒子の発熱を温度計測からではなく、交流ヒステリシスから求める手法を確立した。

さらに粉末試料のみならず、実際にハイパーサーミア治療において体内導入する形態、即ち液中分散した超常磁性ナノ粒子を試料として、発熱特性、磁化特性を実測し、発熱機構を解明できたことは意義がある。

F．健康危険情報

該当しない。

G．研究発表

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H．知的財産権の出願・登録状況
（予定を含む。）

1.特許取得　　なし

2.実用新案登録　　なし

3.その他

3-1.　実験結果等

3-2.　参考文献

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３－１．実験結果等

磁性ナノ粒子における磁性

(1)　多磁区強磁性体の磁性ナノ粒子

粒径が数百 nm の磁性ナノ粒子は、一般に多磁区強磁性体を示す。磁区とはスピンの方向が揃った領域を指し、磁壁とは磁区と磁区の境界であり、スピンが徐々に方向を変化させている境界層のことを指す。またこの磁壁は 1~10 μm 程度の厚みを持つことが知られている。磁界が印加されていない場合、強磁性体の磁性ナノ粒子は静磁エネルギーを最小にするため、全体で磁化が 0 となるような磁区構造をとる。そこで一般的には、多磁区構造となる。この状態から外部磁界が印加されると、外部磁界の方向に磁化が生じたほうがゼーマンエネルギーが減少し、安定するため、磁壁が移動することで外部磁界方向の磁区が大きくなる。これによって磁化が発生する。

$$E_Z = -M \cdot H \cos\theta$$

このゼーマンエネルギーとは、磁性体が磁界中に置かれた際に生じるエネルギーである。さらに外部磁界を強めると、外部磁界方向の磁区のみになり、磁区がこれ以上大きくならなくなる。このときの磁化の値を飽和磁化 M_s といい、この飽和磁化は自発磁化と等しい値を持つ。

この磁壁移動による磁化の変化は、純度の高い金属であれば容易に生じ、磁壁がスムーズに移動することでわずかな外部磁界で飽和に達する。しかし不純物を含んでいると、磁壁の移動が一時的にトラップされ、エネルギー的にピンニングが起こる状態となる。さらに磁界を強めることで束縛を離れ、飽和に達する。またこの状態から外部磁界を徐々に減少させていくと、逆のプロセスをたどることで磁化は減少するが、先ほどの不純物の影響によって同じ磁界強度に戻しても、最初の磁化過程より磁化が大きくなる。このように磁壁移動が阻害され、磁化の値が履歴、すなわちヒステリシスを持つこととなる。また、磁界を 0 に戻してもトラップされた磁壁が残るため、磁化は完全に 0 には戻らない。このときの磁化の値を残留磁化といい、M_r で表す。また、そこから逆方向に磁界を印加すると、ある磁界強度で磁化がゼロになる。このときの磁界 H の値を保持力といい、H_c で表す。このように、磁界を印加することで描く磁化曲線のことをヒステリシスループといい、多磁区強磁性体の磁性ナノ粒子の描く磁化曲線で特徴付けられる。

また、多磁区構造をとる磁性ナノ粒子においては、粒径が増加するに従って保磁力が減少することが知られている。保磁力とは、磁壁がトラップされた際の移動に必要なエネルギーであり、粒径が大きいほど小さなエネルギーでの反転が可能となるためである。

(2)単磁区強磁性体の磁性ナノ粒子

多磁区強磁性体の磁性ナノ粒子の場合、静磁エネルギーを減少させるために多磁区構造をとるが、粒径が数十 nm の磁性ナノ粒子は、粒子のサイズが磁壁のサイズよりも小さくなるため、ナノ粒子の内部に磁壁が存在せず、粒子全体で 1 つの磁区を形成することとなる。このときの磁区構造を単磁区構造という。単磁区粒子は磁壁を持たないため、多磁区強磁性体の場合と異なり、磁化の向きを変えるためには回転磁化の機構のみとなり、磁気異方性や形状異方性の大きい場合は磁化しづらくなる。

磁気異方性とは、強磁性体の自発磁化が強磁性体を形成する結晶内において、方向を変えることで内部エネルギーが変化する現象を指す。そのため、磁界が印加されていない状態では自発磁化は最も内部エネルギーの低い方向を示すことになり、その方向には著しく磁化しやすい状態となる。この方向を磁化容易方向という。またこれに反し、内部エネルギーが最大となるような方向を磁化困難方向という。さらに、自発磁化の方向に関係したこの内部エネルギーを磁気異方性エネルギーといい、次式で示される。

$$E_{ani} = KV\sin^2\theta$$

K は粒子の形状や組成によって決定される磁気異方性定数、V は磁性ナノ粒子の体積であり、理想的な球体と仮定すると半径を r として $V=4\pi r^3/3$ となる。またθは磁化容易方向に対する角度である。

単磁区強磁性ナノ粒子の場合、磁化容易方向が存在する。外部磁界が印加されていない場合、粒子内の磁化はいずれかの磁化容易方向を向いている。この状態から外部磁界を印加させることで磁化を反転させるには、磁化方向が磁気異方性エネルギーに逆らって結晶中で回転しなければならない。この際に働くエネルギーが上式で示したゼーマンエネルギーである。単磁区強磁性ナノ粒子の場合、異方性エネルギーとゼーマンエネルギーによって粒子の状態が決定する。磁性ナノ粒子の持つエネルギーの総和は、次式によって示される。

$$E = E_{ani} + E_Z = KV\sin^2\theta - M \cdot H\cos\theta$$

磁性ナノ粒子は、この(2.4)式が低い状態が最も安定な状態となる。つまり外部磁界を増加させることによって異方性エネルギーの壁を越えた際、角度θが 180 度回転し、よりエネルギー状態が低いように保たれることが仕組みとなっている。

発熱原理

ヒステリシス損失

磁性ナノ粒子ハイパーサーミアにおいて、最も重要となるのが磁性ナノ粒子の発熱である。磁性ナノ粒子の発熱原理はヒステリシス損失と磁気緩和損失の 2 種類に分けられる。これらのうち、まずはヒステリシス損失を考察する。

強磁性体を示す磁性ナノ粒子は磁界の印加によって磁化曲線にヒステリシスが生じ、面積を持つこととなる。ヒステリシス損失とは、この磁化曲線の面積分が熱エネルギーとして放出される、というものである。以下にこのヒステリシス損失の原理を示す。

まず、磁性体を磁化するために要する仕事について考える。磁界 H の条件化で磁界の大きさが M から $M+\delta M$ まで増加したとする。磁化の方向に長さ L を持ち、底面積が S である円柱について考えると、磁化が δM だけ増加した場合、底面の磁極は $-(M+\delta M)S$、頂面の磁極は $(M+\delta M)S$ となる。これは、底面から δMS だけの磁極を取り出し、磁界 H に沿って長さ L だけ頂面まで運搬することに相当する。δMS の磁極には $H\delta MS$ の力が作用しているため、この運搬には $H\delta MS$ の仕事を要することになる。この円柱部分において体積は SL なので、この仕事を単位体積あたりの仕事に直すと、次式が得られる。

$$\delta W = \mu_0 H dM$$

よって、磁化を M_1 から M_2 まで変化させたときに要する仕事は以下のように表される。

$$\delta W = \mu_0 \int_{M_1}^{M_2} H dM$$

ここで熱力学第一法則から、これらの仕事が断熱過程であると過程すると、次式が得られる。

$$dU = \delta W$$

このときの仕事は磁性体内で一部はポテンシャルエネルギーとして蓄えられ、一部は熱となって放出される。ヒステリシスループを 1 周する場合、ポテンシャルエネルギーは元の値に戻るため、その間になされた仕事はすべて熱エネルギーとなって放出される。よって、ヒステリシス曲線 1 サイクルあたりのエネルギーは、

$$dU = \delta W = \mu_0 \oint H dM$$

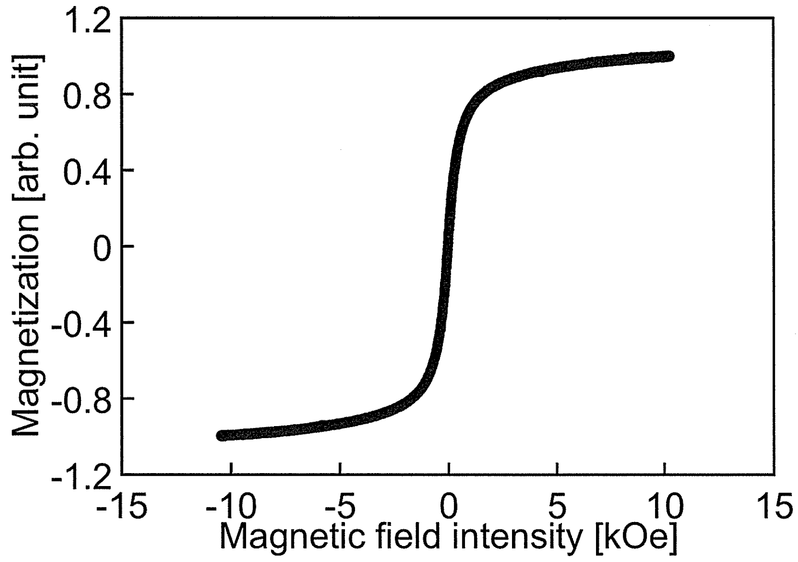
となる。1 サイクル当たりのエネルギーなので、交流磁界で励磁した場合、励磁周波数を f とすると、1 秒間当たりの発熱は

$$P = f dU = f \delta W = f \mu_0 \oint H dM$$

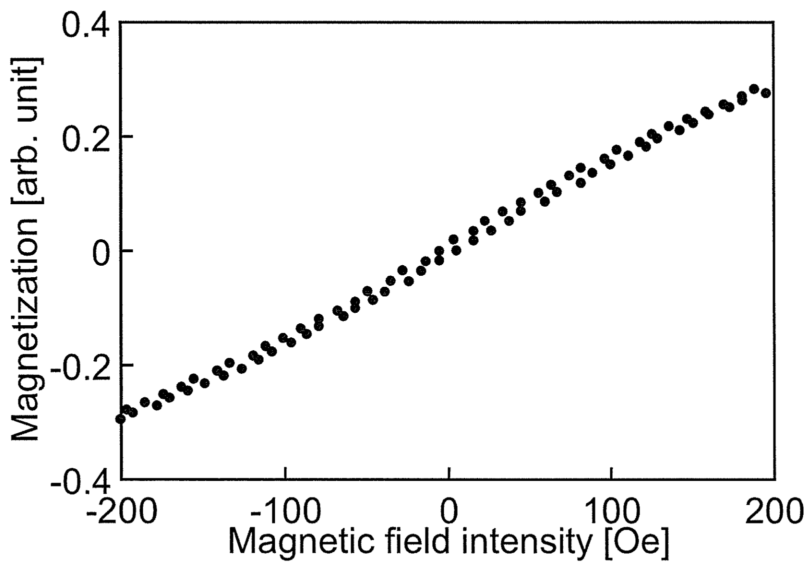
となり、これがヒステリシス損失による発熱の理論式である。このヒステリシス損失はその発熱が周波数に比例すること、また強磁性体では磁化曲線が面積を持つために発熱が生じるが超常磁性体では磁化曲線が面積を持たないために発熱を生じないことが特徴として挙げられる。さらに、ヒステリシス損失による発熱は磁化曲線の面積に比例するため、磁性ナノ粒子の直流磁化曲線を測定することで発熱を見積もるための研究も行われている。

直流磁化特性

超常磁性ナノ粒子の磁化特性



(a) メジャーループ



(b) 原点付近拡大図

超常磁性ナノ粒子を粉末試料とした直流磁化曲線