tissues (Fig. 1B, C, Fig. 2C). These findings suggested that accumulation of bacteria in tumor tissues was due to an EPR effect that was a time-dependent phenomenon requiring more than several hours (e.g., >4 h in mice; Ref 15). Similar results were obtained when C26 solid tumor model was used (Figs. 1D-F, Fig. 2F). These data are consistent with previous report using *Bifidobacterium bifidum*.[11]

Enhancement of bacteria tumor delivery by NG

We then investigated the potential effect of NG on bacteria tumor delivery. NG was applied to the tumor-bearing mice just before i.v. injection of *L. casei*. As seen in Fig. 2A-C, NG significantly augmented the delivery of bacteria to tumor tissues: approximately 70-fold, 20-fold and 10-fold increases were found at 1h, 6h and 24h, respectively, after NG treatment. However, no apparent increase of bacteria accumulation was found in normal tissues including the liver and spleen (Fig. 2), which suggests that NG was converted to NO predominantly in tumor tissues. Similar results were also found in C26 tumor-bearing mice (Figs. 2D-F).

Because NO shows effects on cell proliferation and apoptosis,[19] we further investigated whether the accumulation and growth of bacteria in tumor is partly due to the effect of NO from NG, the effect of NO on *L. casei* growth was examined in an in vitro bacteria culture system using sodium nitrite as well as NO donor NOC-7. The results showed that both sodium nitrite (0.1-100 μg/ml) and NOC-7 (0.23-230 μM) did not affect the growth of *L. casei* (supplemental data Fig. S1), suggesting the accumulation/growth of bacteria in tumor is mostly due to the EPR effect and tumor microenvironments.

Enhancement of bacteria tumor delivery by enalapril

Similarly, the effect of ACE inhibitor enalapril on the bacterial accumulation in tumor was evaluated. According to the clinical pharmacokinetic profile of enalapril, it was administered 4h before i.v. injection of *L. casei*. As shown in Fig. 3A-C, remarkable increase of tumor delivery of bacteria was also observed by enalapril: approximately 18-fold, 9-fold and 6-fold increases were found at 1h, 6h and 24h after enalapril treatment, respectively. Similar results were also found in C26 tumor-bearing mice (Figs. 3D-F)

Bacterial antitumor effect and survival benefit of L. casei with NG

The therapeutic effect of i.v. administration of *L. casei* in the presence or absence of NG was investigated in C26 solid tumor model. As Fig. 4 demonstrates, i.v. injection of *L. casei* showed a dose-dependent antitumor effect; at the dose of 2×10^7 CFU weekly for 3 times a significant delay of tumor growth was achieved (Fig. 4A). NG at the dose of 0.6 mg did not significantly inhibited the tumor growth, however a more remarkable antitumor effect was obtained when *L. casei* was combined with NG (Fig. 4A). During this experiment, a slight

but not significant decrease of body weight was found at the beginning of treatment in *L. casei* (2×10^7 CFU) treatment and *L. casei*/NG combination group, the body weight recovered later and continued to grow, at the end of the experiment, the body weights of mice in these groups were not apparently different to the untreated control group judging from the weight of tumor in each group (Fig. 4B), suggesting no severe side effects occurred in these treatment protocols.

Moreover, the survival rate of tumor-bearing mice was significantly improved after each treatment, especially in the *L. casei*/NG treatment group in which the survival times of animals were prolonged almost double (median survival time: 40 days of untreated control vs 79 days of *L. casei*/NG group) (Fig. 4C).

Induction of TNFα and NOS activity and other inflammatory factors in tumor by L. casei/NG treatment

To elucidate the antitumor mechanisms of *L. casei*/NG treatment, various cytokines or factors which may possibly involved in the host defense and inflammation and antitumor responses, i.e., TNFα, MPO, NOS, IL-6 and MCP-1 were examined after each treatment. As seen in Fig. 5, no change of IL-6 and MCP-1 was found under each treatment both in serum and in tumor tissues (Figs. 5D-F). For TNFα, its levels in serum of tumor-bearing mice were below the detection limit in all groups. In contrast, TNFα was increased in tumor in all *L. casei* treated groups, and a significant difference was seen for *L. casei*/NG group vs untreated control (Fig. 5A). Significant increase of NOS activity in tumors was also seen in all the treated groups, especially *L. casei*/NG group (Fig. 5B). MPO activities in tumors did not change in *L. casei* alone and NG alone group respectively, but a tendency of increase of MPO activity was seen in *L. casei*/NG group (Fig. 5C).

DISCUSSION

In the present study, we showed data that indicating a new antitumor strategy by systemic administration of *L. casei*, i.e., bacterial therapy. *L. casei* is a nonpathogenic facultatively anaerobic bacterium that is also a component of the normal bacterial flora in human intestinal tract and reproductive system. *L. casei* is now widely used as a probiotic in various dairy and food supplements. More important, it has been known for decades that it could stimulate nonspecific immune responses, such as macrophage and NK cell activation to fulfill antitumor activity,[20-23] and thus it is considered useful as a medication to prevent recurrence of bladder cancer,[7,8,24,25] like Bacille Calmette-Guérin (BCG) as an immune modulator.[26]

Most previous studies using *L. casei* to challenge cancer were through oral route or i.p. injection. These administration routes ensure the immune stimulation of whole body of the

host, however, no tumor selectivity could be achieved. Because *L. casei* is a nonpathogenic bacterium, systemic i.v. application is possible with very little adverse effects such as sepsis. We thus hypothesized that *L. casei* can be delivered selectively in tumor after i.v. injection according to the EPR effect, consequently achieve tumor-targeted therapeutic effect. As expected, we found tumor-selective accumulation and growth of the bacteria after i.v. injection, without accumulation of bacteria in normal tissues (Fig. 1). Although *L. casei* accumulated mostly in the liver and spleen, both are rich in reticuloendothelial system, at 1 h after i.v. injection, these amounts decreased dramatically after 6 h; and at 24 h, the numbers of living bacteria in tumor tissue were far greater (i.e., 50 times) than those in the liver and spleen (Fig. 1), indicating *L. casei* could not survive and grow in normoxic normal tissues even it is uptaken. These results are similar with previous studies using different bacteria,[3-5, 9-11] and we believe this tumor-targeted delivery and enhanced growth is mostly due to the EPR effect and the hypoxic microenvironment of tumors that is seen in most solid tumors.[12] Namely, EPR effect drives the accumulation/delivery of bacteria into tumor, and then the favorable tumor environment ensures the growth of bacteria.

EPR effect is a unique phenomenon caused by the anatomical and pathophysiological features of tumor blood vessels, and many vascular mediator such as NO and bradykinin are involved in EPR effect as noted above.[12,15,16] We thus developed approaches to enhance the EPR effect by focusing and utilizing these mediators. One successful and interesting example is the use of NG, a well-known NO donor that has been used for more than a century as a medication for angina pectoris. NG selectively liberates NO_2^- first which is then converted to NO by nitrite reductase under hypoxic conditions in cardiac infarcted tissue.[27,28] The low pO_2 and slightly acidic pH in cardiac infarcted tissue are similar to conditions in many tumor tissues.[29] Thus NG is an ideal agent to enhance the EPR-driven drug tumor delivery. Our previous studies clarified the enhancement of EPR effect by NG with remarkable results in various rodent tumor models, by using polymer conjugates and putative macromolecular agents.[17] Along this line, the NG-enhanced EPR effect was also seen in the present study using bacteria; the numbers of live bacteria delivered into tumor were markedly increased, e.g., 70 folds, after NG treatment, whereas no significant changes of bacteria in normal tissues including the liver and spleen (Fig. 2). Because NO seemed no effects on bacteria proliferation (Supplement data Fig. S1), the effect of NG/NO on bacterial tumor accumulation is believed to be mostly due to the enhanced EPR effect. In addition, ACE inhibitor enalapril has been known for long time to induce tumor vascular permeability.[30] This notion was also supported in this study, more than 10-time of bacterial tumor accumulation was achieved with the use of enalapril (Fig. 3). These data suggested NG and

enalapril, both are clinically used safe drugs, are potentially useful as enhancer of macromolecular anticancer drug delivery as well as bacterial therapy.

According to the EPR-based tumor-targeted bacterial delivery, we hypothesized *L casei* selectively accumulated in tumor will induce tumor-specific immune activation and thereby have an antitumor effect, which is realized by systemic i.v. injection, but not topical application like BCG for bladder cancer. The results clearly supported our hypothesis; significant suppression of tumor growth by i.v. administration of *L. casei* in C26 colon tumor model, with significantly increased survival rate of the mice (Figs. 4A, C). Moreover, this antitumor effect was further significantly augmented by combining with NG (Figs. 4A, C). As NO is also known to show tumor-suppressive effect probably acting by down-regulating the expression of certain critical genes involved in tumor growth,[17,31,32] the effect of NG treatment per se was also examined, and we did not find significant delay of tumor growth under this treatment regimen with the dose of 0.6 mg/tumor (Fig. 4A). We thus believed the enhanced antitumor effect of *L. casei*/NG is mostly the consequence of enhanced bacterial tumor drug delivery. More important, because of the high tumor selective delivery of *L. casei* by i.v. injection, this administration would cause very little side effects in normal tissues and organs, as seen in Fig. 4B. When we examined the inflammatory and antitumor cytokines after these treatments, we found no effects on serum levels of inflammatory cytokines including IL-6, MCP-1 and TNFα, whereas significant increases of immune responses such as TNFα and NOS activity were found in tumor tissues (Fig. 5). These profiles further suggested the targeted tumor suppressing effect, potential applicability and safety for clinical setting of this bacterial therapy using *L. casei.*

Regarding the antitumor mechanisms of *L. casei*, the common notion is immune modulation triggered by *L. casei* as described above. Namely, significant increases of TNFα and NOS activity in tumor were seen after L. casei/NG treatment (Figs. 5A, B), as well as the increase of MPO activity though no significant difference was shown (Fig. 5C). These findings suggested the present bacterial therapy mostly rely on the activation of antitumor nonspecific or innate immune responses, i.e., upregulation of TNFα, macrophage and NK cell activation and the subsequently generated NO as well as other oxidative free radicals such as superoxide and peroxynitrite.

In addition, we previously reported bacterial proteases such as serratial proteases exhibited potent antitumor effect when administered into the tumor.[33-35] Bacterial proteases have no effective inhibitor in the plasma of mammals, however, they from a transitory complex with α_2 macroglobulin in the plasma which is effectively transported into the tumor cells via α_2 macroglobulin receptor that is highly expresses in tumor.[34] In this context, *L.*

casei may exhibit antitumor effect through secretion of bacterial proteases, which need further investigations.

In conclusions, we reported here that i.v. injection of *L. casei* resulted in selective accumulation/growth of bacteria benefiting from the EPR effect as well as specific tumor environments. The tumor delivery of bacteria could also be further significantly improved by NO donor NG and ACE inhibitor enalapril (bradykinin potentiator) that affect vascular tone involved in the EPR effect. Consequently, remarkable antitumor effect was achieved through the antitumor host responses induced by *L. casei* accumulated in tumor, especially when *L. casei* was combined with NG, with no apparent adverse effects. These data suggested the potential of *L. casei* as a candidate for bacterial antitumor therapy, moreover, it could also be a useful drug delivery system to carry or deliver genes or antitumor nanoparticles.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid from the Ministry of Welfare, Health and Labor of Japan (No. H23-sanjigann-ippan-001), Grants-in-Aid for Scientific Research on Scientific Research (C) (25430162) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and research funds of the Faculty of Pharmaceutical Sciences at Sojo University.

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Figure legends

Figure 1. Body distribution of live *L. casei* in sarcoma S-180 (A, B, C) and colon cancer C26 (D, E, F) tumor-bearing mice after i.v. injection. To S-180 or C26 solid tumor-bearing mice, *L. casei* (7×10^6 CFU) were injected i.v.. After indicated times, the mice were killed and plasma and each tissue were collected. The live bacteria in each tissue were measured by culturing the tissue homogenates and counting the colonies formed in the medium. Data are given as means ± SD; $n = 6$. $^{***}P < 0.005$ vs normal tissues. See text for details.

Figure 2. Enhancement of tumor delivery of *L. casei* by NG. A, B and C show the results in S-180 tumor model, and D, E, F shows those from C26 tumor model. Administration of *L. casei* was carried out as same protocol as that shown in Fig. 1, and NG was applied into the skin over the tumor at the dose of 0.6 mg/mouse, 5 min before i.v. injection of *L. casei*. In A and D, the insets show the enlarged scales for tumor. In C and F, time courses of accumulation of *L. casei* in tumor and liver with/without NG treatment were shown. Data are as means ± SD; $n = 6$. $^{**}P < 0.01$ (no NG control vs NG treatment group). See text for details.

Figure 3. Enhancement of tumor delivery of *L. casei* by enalapril. A, B and C show the results in S-180 tumor model, and D, E, F shows those from C26 tumor model. Administration of *L. casei* was carried out as same protocol as that shown in Fig. 1, and enalapril (10 mg/kg) was given orally 4h before i.v. injection of *L. casei*. In A and D, the inlets show the enlarged results in tumor. In C and F, time courses of accumulation of *L. casei* in tumor and liver with/without enalapril treatment were shown. Data are as means ± SD; $n = 6$. $^{**}P < 0.01$ (no enalapril control vs enalapril treatment group). See text for details.

Figure 4. *In vivo* antitumor effect of *L. casei* after i.v. injection with/without NG in C26 solid tumor model. Ten days after injection of C26 tumor cells in BALB/c mice, when tumor diameters became 5-8 mm, *L casei* was injected i.v.; in some experiments, NG ointment (at an NG dose of 0.6 mg/ tumor) was rubbed on the skin overlying the tumors just before administration of bacteria. Arrows indicate the injection of *L. casei*. A, antitumor effect of each L. casei with/without NG; B, body weight changes of tumor-bearing mice after each treatment; C, survival rate of tumor-bearing mice after each treatment. L.C (L), treatment with 7×10^6 CFU of bacteria; L.C (H), treatment with 2×10^7 CFU of bacteria. Data are given as means ± SD; $n = 6$-12. $^{*}P < 0.05$, $^{**}P < 0.01$; in C, $P < 0.05$: NG alone vs control,

L.C (H) alone vs control; $P < 0.01$: L.C (H) + NG vs control. See text for details.

Figure 5. Effect of *L. casei* and/or NG on MPO and NOS activity in tumor and the production of antitumor, anti-inflammatory cytokines. In S-180 solid tumor model, NG (0.6 mg/mouse) and/or *L. casei* (2×10^7 CFU) were administered similarly to the treatment protocol described in Fig. 4, but applied once every two days. Ninety-six hours after the last injection, mice were killed and plasma and tumor tissue were collected for each assay. A, B, and C show the TNFα levels, NOS activity and MPO activity in tumor respectively; D and E show the IL-6 levels in plasma and tumor after each treatment respectively; F and G show the MCP-1 levels in plasma and tumor after each treatment respectively. Data are given as means ± SD; n = 4-5. $^{**}P < 0.01$, $^{***}P < 0.005$. See text for details.

Fig. 1

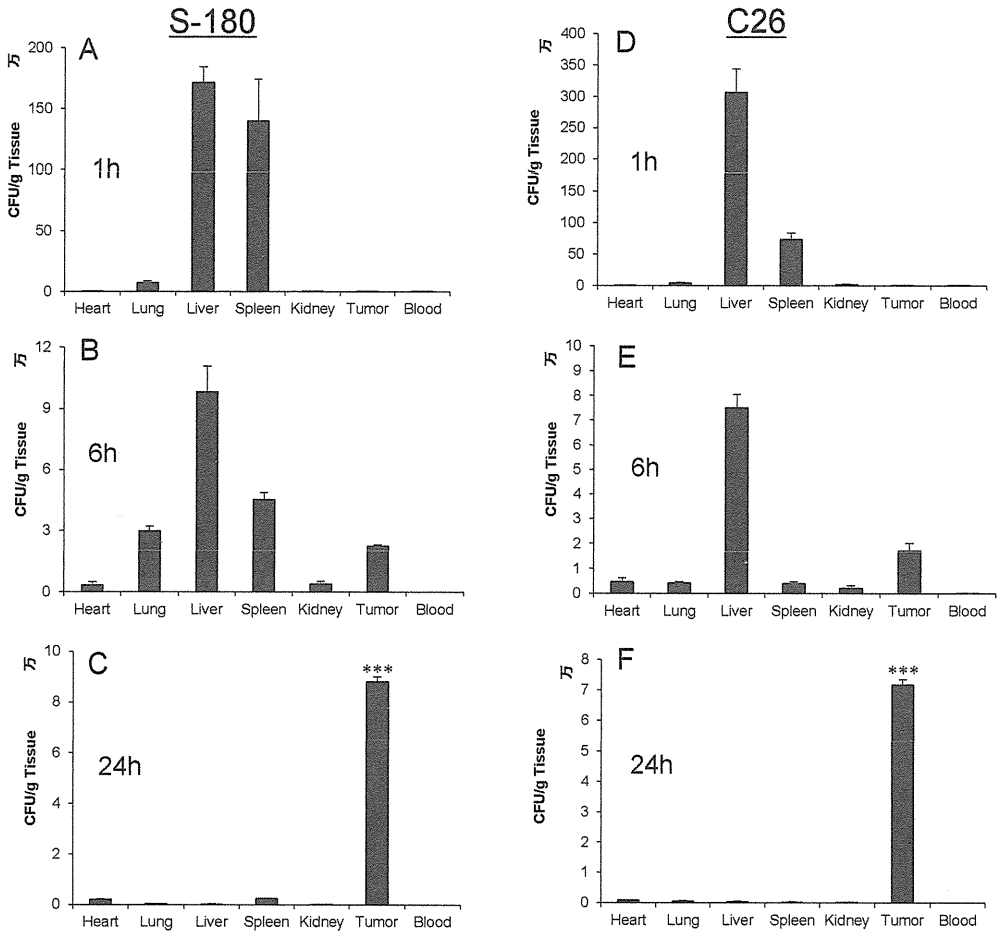
S-180
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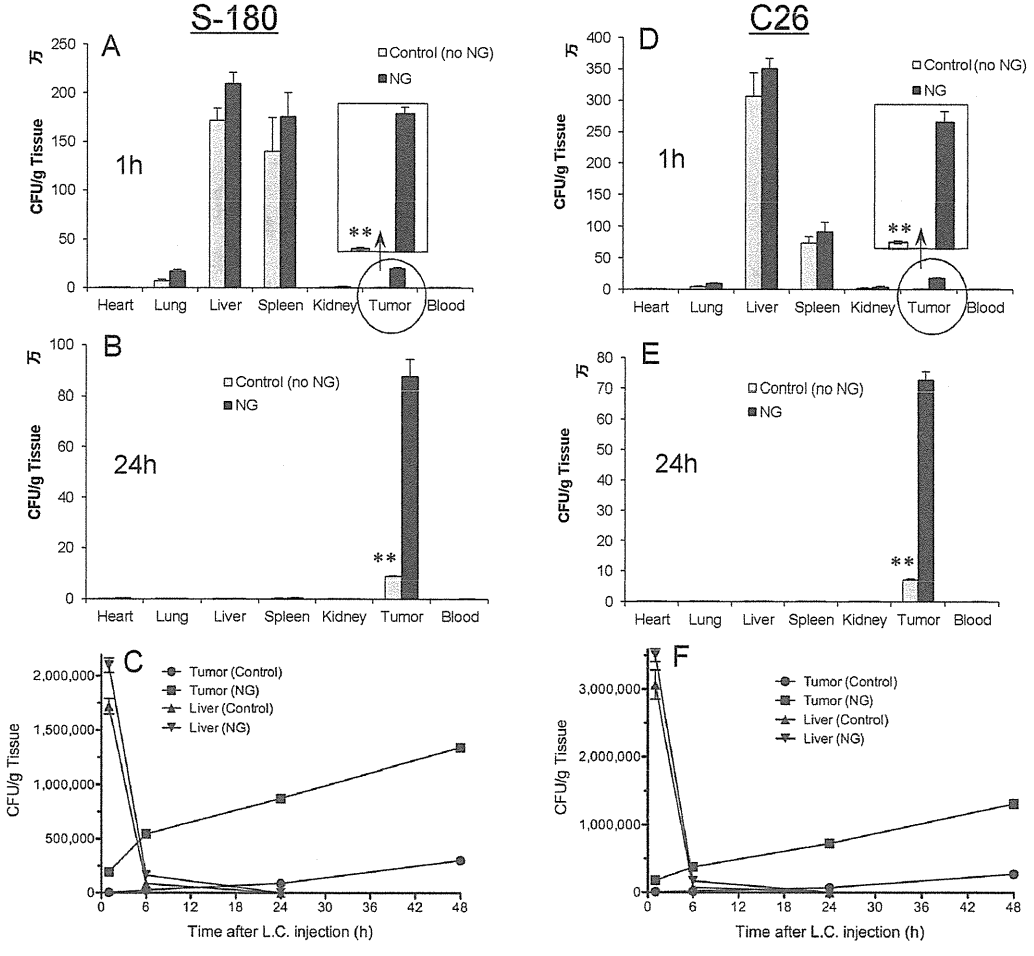
Fig. 2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Liver
Spleen
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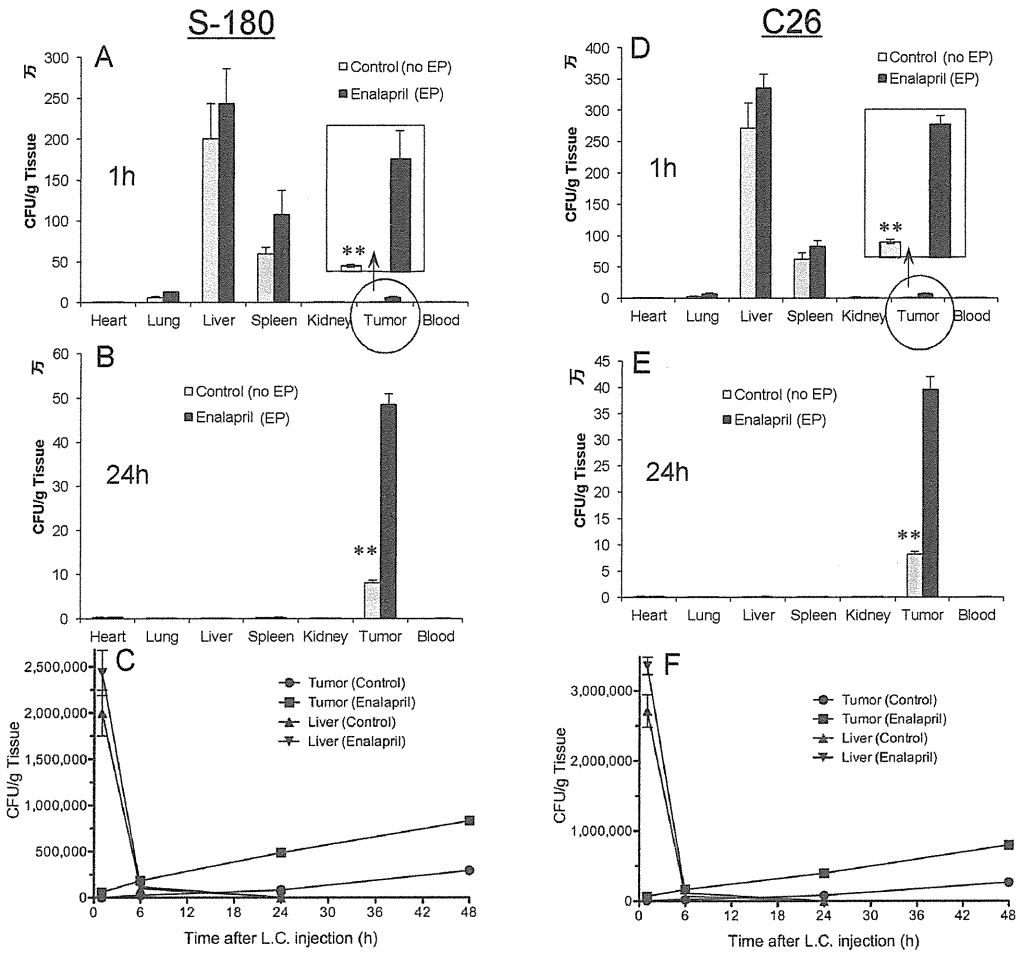
Liver (Control)
Liver (NG)
Time after L.C. injection (h)

Fig. 3


S-180
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Liver (Enalapril)
Time after L.C. injection (h)

Fig. 4

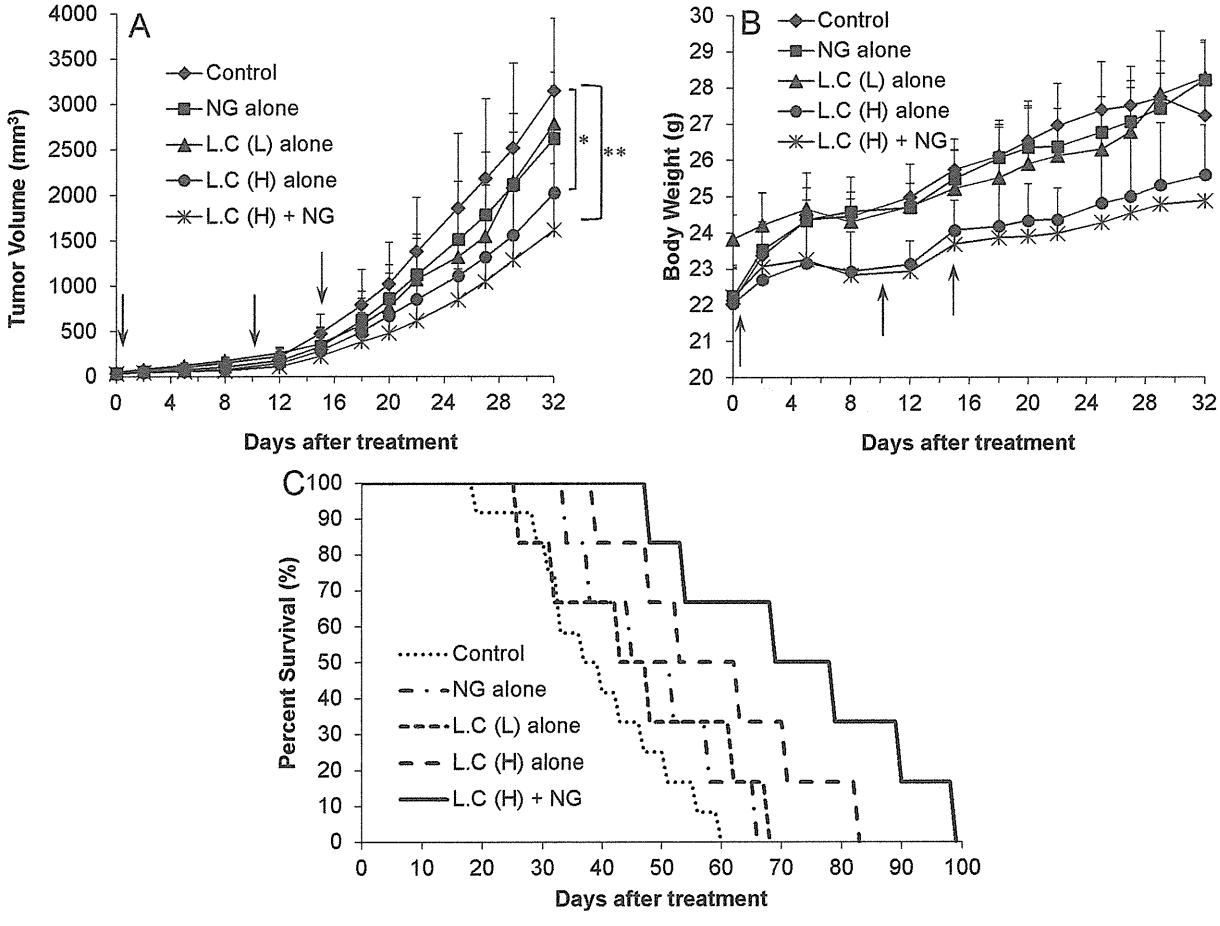
A
B
C
Control
NG alone
L.C (L) alone
L.C (H) alone
L.C (H) + NG
Tumor Volume (mm³)
Body Weight (g)
Percent Survival (%)
Days after treatment
*
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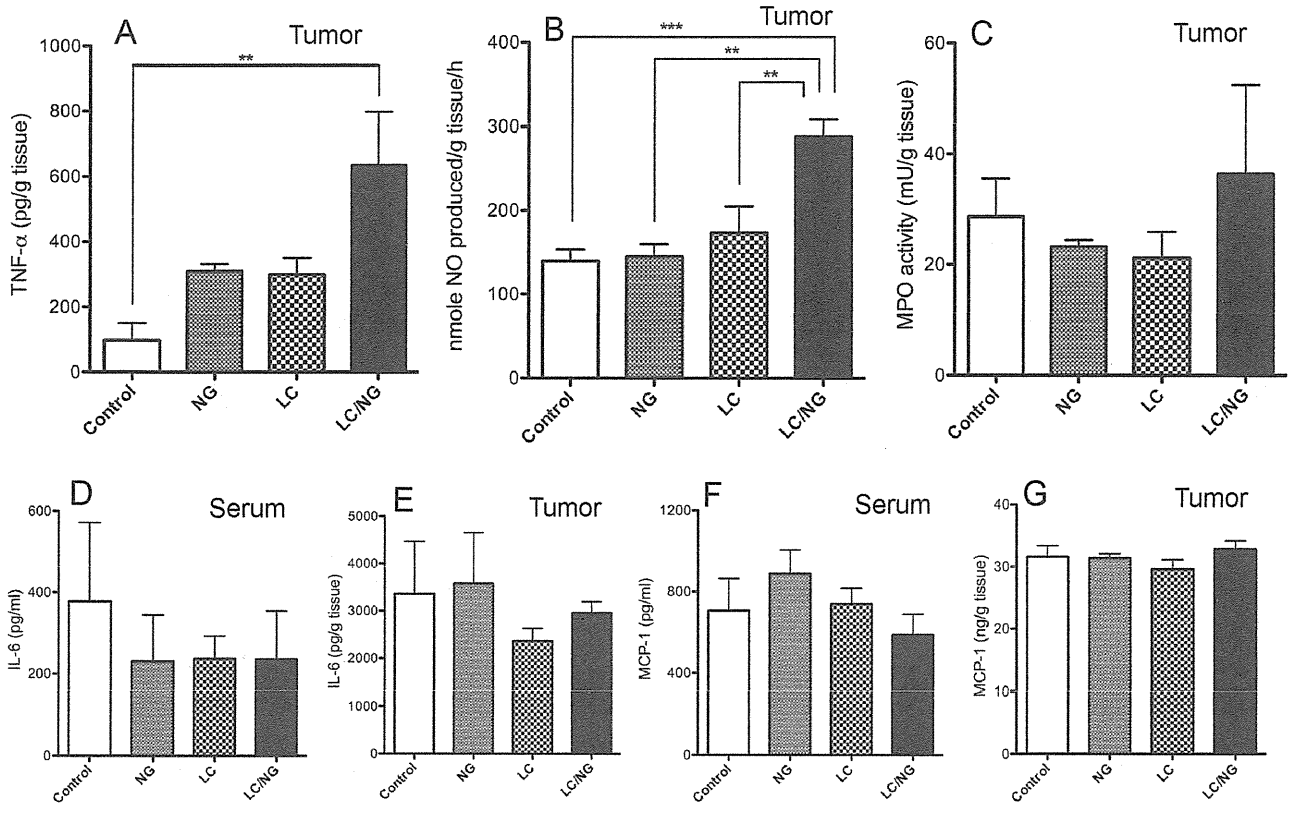
Fig. 5


A
Tumor
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LC
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C
Tumor
MPO activity (mU/g tissue)
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MCP-1 (pg/ml)
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Control
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LC
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NG
LC
LC/NG

Supplemental data
Fig. S1

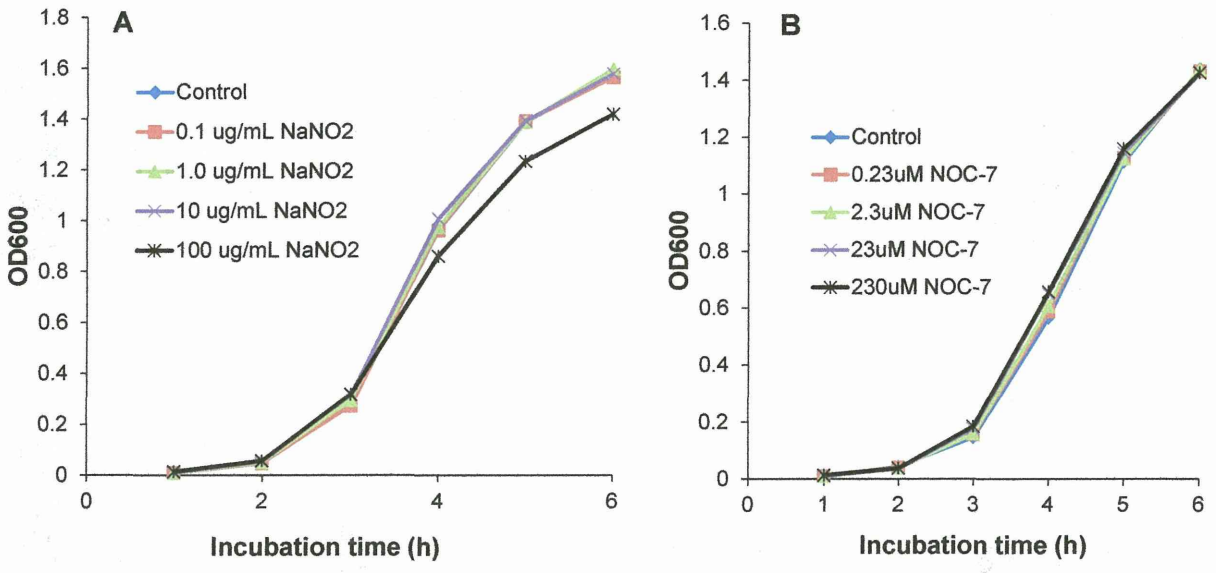


Figure S1. Effect of soidum nitrite (A) and nitric oxide donor NOC-7 (B) on the growth of L. casei. L. casei was cultured in MRS medium, and sodium nitrite or NOC-7 was added at different concentration. At indicated time after incubation, the numbers of bacteria were estimated by the optical density at 600 nm. No significant change of bacterial growth was observed in both treatment at the indicated concentrations. See text for details.

校正中

現今のがん治療薬のかかえる問題

前田　浩
崇城大学DDS研究所　特任教授

ランセット誌やニューイングランド・ジャーナル・オブ・メディシンなどの医学系雑誌

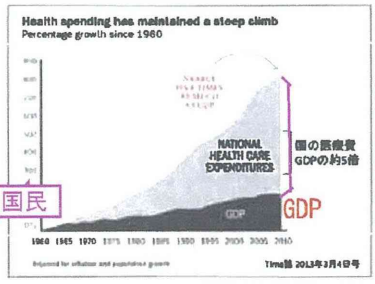
を

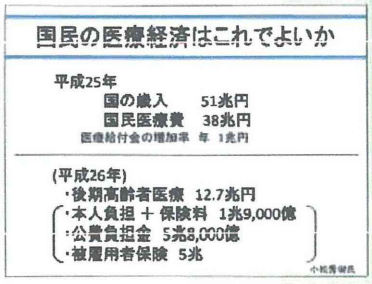
取り上げられていま

海外のLancetとかMedical Journal、あるいはNew York Timesなどでも医療問題のことはほとんど毎週、大変な問題として立ちはだかっているわけです。日本でもしばしば医療ビジネス、産業、つまり金儲けの手段となってしまっており、戦後のとくに最近のグローバリゼーションの特徴じゃないかと思っております。

記事

今日最初に示したのはTIME誌のアジア版には載っていない、アメリカ版だけに去年の3月に載っていたんですが、要するに「Health care is eating away our economy and our treasure!!　医療が米国の経済と財政を食い物にしている！」という記事です。





医療事業を

見

を

国民

食いものにし

問題

その

このTime誌の

であり

ある

改行

その一つに

が、一般的な国家財政に対する心配事であります。つまり、

な局面で

~30倍

ものが

と思われます

の金額

アメリカでも驚くべきはGDPの約20%が医療費、つまり全米で2.8兆ドル。大変な問題でそのうちオバマケアで90%はカバーされているのですが、そのMedicareの支払いだけで8,000億円になっています。そこで、ビジネスという観点からすると、ニューヨーク市の上位トップ18社の企業のうち8つが医療、4つが銀行ということです。要するに経済に完全に翻弄されていて、アメリカの家庭の破産のうち62%が医療費が原因と言われています。こういうことが28ページにも渡る大特集号になっていましたが、日本ではほとんど無視されています。

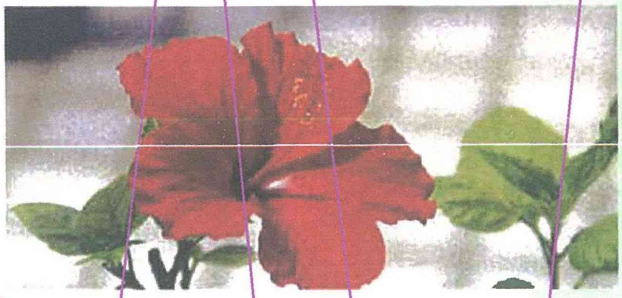
具体的にいいますと、GDPは年々増えているのですが、National Health Care Expenditures、つまり医療費の増加率は5倍になっています。これで国がやっていけるのだろうかというのは、いろいろ破産に向かっているということと、共和党と民主党の対立の大きな問題点でオバマケアもなかなか難しい問題がそこに控えているということです。

アメリカのがん関係の話をすると、がんに罹ったということは5千万から1億円かかるということだということが言われています。どうしてこのようなことが許されているのか。これは1年間の所得どころではないのです。タイムはものすごい詳しく調べているのですが、アメリカはロビー活動もすごいあるわけですから、議会もまっとうな判断を失っている。議会も製薬業界、病院も含めて5,300億円がロビー活動に使われてて、軍事や石油・ガス関連よりも3倍以上のお金がロビー活動に使われていて、議会が影響を受けているということです。

日本の場合を簡単に説明すると、年齢などいろいろな状況で違ってきますが、大腸がん治療ではいろんなものを併用してですが、1回で75万円くらい。これを20サイクルしますから、これだけで1千万～2千万円の世界です。非小細胞肺癌治療も年に千何百万。こういう治療は、高額医療ですから公的支援で本人の負担は少なくて、それが有難いことなのですが、それが結局日本の財政の、50兆円くらいの税収に対して38兆円使っています。90%近くが医療費となっております。これはアメリカと一緒で、いつまで続けられるんだろうか、ということです。しかも毎年医療費給付金の増加率が1兆円から2兆円と言われています。いまデフレのときなので、1兆円となっておりますが、こういう財政でいいのかということです。

た場合

医療費には

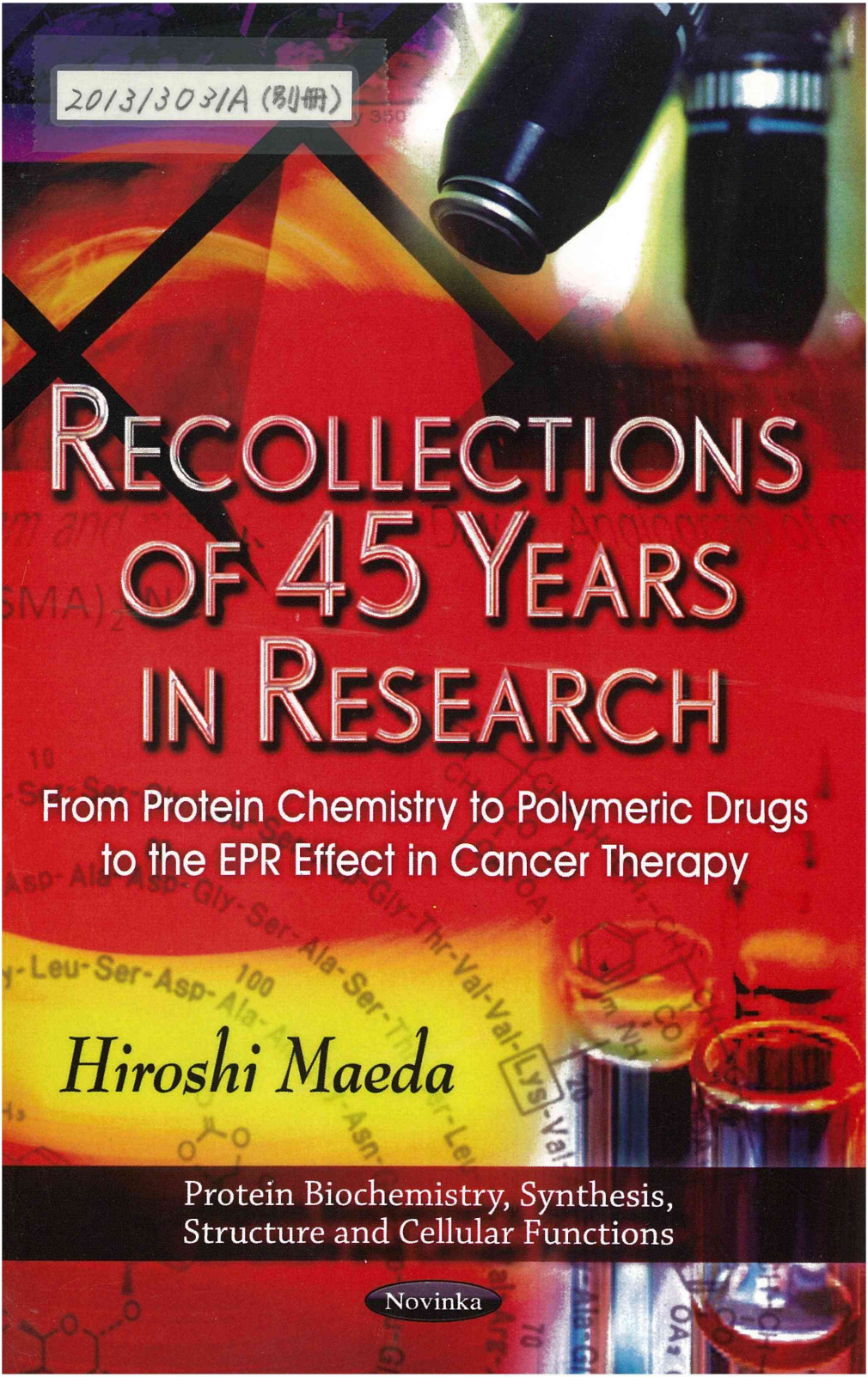


ために低くて

つまり税収の8

同じ

昨年までは



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New York