remarkable results using conventional light irradiation. This theranostic treatment can detect fluorescence of even minute tumor nodules (Fig. 7A) and metastatic liver cancers (not shown). Thus, it would also be applicable to simultaneous tumor detection and tumor regression by means of intracavitary light irradiation with use of an endoscope. This method will be a completely non-toxic therapeutic technique.

Our polymer conjugated ZnPP (P-ZnPP) (Fig. 6) has a number of advantages. First, P-ZnPP demonstrates the EPR effect and accumulates selectively in tumor tissues[41] (Fig. 2A, cf. Fig. 2B). Light irradiation of any site on the body will thus affect only tumor tissues. The tumor distribution of P-ZnPP was similar to that of P-THP in mice (Fig. 5), and as seen as fluorescence in breast cancer in rats (Fig. 7A).

The second advantage of using P-ZnPP is therefore that we can utilize an endoscope or similar light source with a xenon light or LED that can excite any photosensitizers at the wavelength range from 400 to 700 nm, especially at range of ZnPP: 400-440 nm. Our P-ZnPP has in fact demonstrated light absorption at about 410-435 nm and generates singlet oxygen, thus effectively inhibiting tumor growth by means of endoscopic light. In fact, our *in vivo* rodent experiments showed complete eradication of chemically induced breast cancer after only one i.v. drug injection at 20-30

mg/kg followed by illumination two to four times (Fig. 7B,C). One problem with current PDT is that, in most cases HeNe lasers are used which emit 633-nm light and can penetrate tissue better than low wave length light, but it can not excite photosensitizers. However, a conventional flush light and endoscopic light (400-700 nm) can penetrate human tissues (or hands), as one can observe in the darkness: a substantial amount (~0.1%) of light can penetrate the tissue and can generate $^1O_2$ to kill tumor cells effectively (Fig. 7B,C).

7. Conclusion

I have analyzed the causes of therapeutic failures in current cancer chemotherapy against solid tumor with a particular focus on molecular target drugs, antibody drugs, and nanomedicines, as well as immunotherapy. Major causes of the inefficient therapeutic effect of these modalities are believed to be genetic diversity of human solid tumors for molecular target drugs or peptide vaccines, and inadequate consideration of spectroscopic properties in the case of PDT. I also analyzed currently used PDT and discovered that it is theoretically unsound and has problems with tumor-selective delivery of photosensitizers (*i.e.*, the low-MW photosensitizers have no tumor selectivity and no EPR effect) and difficulties with the spectroscopic conditions, which are totally

incorrect. Our novel polymer-based PDT using fluorescent nanoprobes is thus completely different from the currently used PDT, and I believe that our therapeutic approach will revolutionize cancer treatment. In contrast to using currently available therapeutic modalities, I and my team have been working on macromolecular therapeutics, in which macromolecular agents are designed to accumulate in the tumor selectively by means of the EPR effect and release the active principle in the tumor environment, where the pH value is 1-1.5 units lower than that in normal tissues or where active proteases (or esterases) are present. This acidic pH and hydrolytic enzymes will facilitate spontaneous cleavage of the cross-linking chemical bond, such as hydrazone and some ester bonds, between polymers and drugs. The result is more effective release of free drugs in the vicinity of tumor cells and thereby improved therapeutic efficacy. I discuss tumor proteases with susceptible peptide linkers and other bonds in this context as well.

In addition, EPR effect-dependent tumor-selective drug delivery can be augmented 2- to 3-fold by modulating vascular permeability factors that contribute to the EPR effect by using NG, angiotensin I-converting enzyme inhibitors, or angiotensin II-induced high blood pressure (*e.g.*, 110 mmHg → 150 mmHg). NG generates NO_2^- *in vivo*, which is converted to NO in hypoxic tumor tissues. This EPR-enhancing effect

achieved by NO-releasing agents, or angiotensin I-converting enzyme inhibitors, occurs selectively in tumor tissues, which ensures greater effectiveness and fewer side effects with our polymer-conjugated drugs.

In our preclinical studies using P-THP and P-ZnPP, autochthonous chemically induced tumors or various implanted tumors in rodents, some of which had metastatic tumors, were completely eradicated at doses far below the maximum tolerable dose P-ZnPP with light irradiation. Similarly, we further observed tumor-selective accumulation of polymer ZnPP conjugate with a fluorescence detection system. These findings will stimulate development of new imaging and detection strategies using polymer conjugates.

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Figure Legends

Fig. 1. Hypothetical plasma profile of low-MW free drugs (a) (*e.g.*, DOX or pirarubicin) and their polymer conjugates (b-d). The drug concentration in plasma after i.v. injection of low-MW (parent) drugs (a) decreased rapidly. Representative polymer conjugate micelles and the liposome complex remained in the plasma at high levels (b-e). Although (b) is the block copolymer micellar drug of DOX burst rapidly, with a 50% decrease in 1 hour after i.v. administration. No therapeutic benefit from the EPR effect was thus seen, because of the poor stability of the micelle. (c) A styrene-co-maleic acid (SMA)-polymer DOX conjugate. (d) An SMA-polymer tetrahydropyranyl doxorubicin (THP) conjugate. For a stable liposome complex such as the one with Doxil® (e), the liposomal drug concentration in plasma continued at a high level. This liposome complex of a pegylated stealth liposome of DOX was too stable, however, with little drug release and thus little therapeutic effect. The conjugate in (d) demonstrated a more favorable result than did that in (b), (c) or (e).

Fig. 2. *In vivo* fluorescence imaging of tumors selectivity based on EPR effect via a fluorescent nanoprobe. Polymer-conjugate fluoroprobe used here is polymer-*N*-(2-hydroxypropyl)methacrylamide (HPMA) conjugated with zinc protoporphyrin (ZnPP) (MW～50 kDa). Whole-body fluorescence of S180 tumor-bearing mice. (A) After injection with HPMA-ZnPP, fluorescence imaging revealed tumor-selective drug accumulation. (B) Injection of low-MW free ZnPP (MW～770 Da) showed no tumor-selective drug accumulation and no fluorescence. (A') Normal light

view of A. Images were obtained with an IVIS XR *in vivo* fluorescence detector (Caliper Life Science, Hopkinton, MA) 48 hours after i.v. injection of each drug (both at 15 mg/kg ZnPP equivalent). Modified from Ref. 30 and reproduced with permission.

Fig. 3. (A) Theoretical mechanism of NO generation and increase in drug delivery by use of NG (nitroglycerin). NO is generated from nitrite, predominantly in hypoxic tumor tissues rather than normal tissues. (B) NG enhanced blood flow in tumor tissue but not in normal tissue (thigh muscle). Blood flow was measured with a laser flowmeter (ALF-21; Advance Co., Ltd., Tokyo, Japan). S-180 tumor-bearing mice with tumor diameters of 6–7 mm were anesthetized and placed on a warm pad (30–35℃). The blood flow was monitored at first for 5 minutes to confirm that it was stable, and then NG at dose of 1.0 mg/mouse was applied to the dorsal skin. Blood flow in the thigh muscle was measured after application of NG at a dose of 1.0 mg over the skin anywhere. Error bars show 95% confidence intervals. The concentration of NG was 20 mg/g ointment (Vaseline). Differences between muscle and tumor were compared with two-sided Wilcoxon tests. (C) Delivery of the putative macromolecular drug Evans blue-albumin (70 kDa) to tumor tissue, which increased 2-3-fold after NG application. (D) Dose response to NG and delivery to different murine tumors (Meth-A, C38). Modified from Ref. 48. Reproduced with permission from [38].

Fig. 4. (A) Metastatic tumor in the lung and the EPR effect. Colon 26 tumor (5×10^6 cells) was implanted on the dorsal skin of a BALB/c mouse, and after about 3 months the lung was removed and examined macroscopically, 24 hours after i.v. injection of Evans blue dye. Blue nodules (arrows) demonstrate that metastatic tumors exhibited

the EPR effect, with selective tumor uptake of dye. (B) In this model, after i.v. injection of 30 mg/kg polymer-conjugated pirarubicin (P-THP), all tumor nodules disappeared, and all six mice in this group survived at 90 days in good health. In contrast, free low-MW pirarubicin at about the maximum tolerable dose of 5 mg showed little therapeutic effect (middle).

Fig. 5. Chemical structures of THP conjugated to different polymers and their pharmacological and antitumor effect. (A) is the HPMA polymer conjugated THP, and (B) is the SMA-copolymer conjugated THP. Toxicity was assessed by analysis of body weight (C). Tumor and tissue distribution is shown in (D) and antitumor effect by tumor volume in (E). In contrast to free THP, P-THP (HPMA-THP) had no toxicity up to 100 mg/kg. Free THP at 7 mg/kg, the LD_{50}, did not eradicate tumors. At 15 mg/kg, P-THP completely suppressed the tumors. P-THP was tolerated at doses >60 mg/kg (not shown) (from Ref. 41, reproduced with permission).

Fig. 6. Chemical structure of polymer HPMA(P)-conjugated ZnPP IX: polymer-zinc protoporphyrin conjugate. From Ref. 51. Reprinted with permission, Elsevier Science.

Fig. 7. Autochthonous breast cancer in rats induced by DMBA treatment. (A) Tumor image under normal light (top), and fluorescence image (IVIS, fluorescence imaging system) (below). (B) Therapeutic effect of HPMA polymer zinc protoporphyrin (P-ZnPP) conjugate administered once at 20 mg/kg i.v., after which endoscopic light was applied at 0% or 40% power output for 5 minutes (×2). (C) Views of *in vivo* breast tumor treated

with PDT on the right, with the control group (no treatment) on the left. Therapeutic protocols are the same as those in (B). A significant size reduction on day 30 and complete eradication on day 50 are obvious.

Table 1. Factors and mediators involved in the EPR effect in cancer and inflammation, and their responsible enzymes or effectors[a].

EPR effect-enhancing factors/mediators	Enzymes responsible for factors	Comments, actions of enzymes and factors, or sources of factors
1. Bradykinin (kinin)	Kallikrein and other proteases, plasminogen activator produce bradykinin	Angiotensin I-converting enzyme (ACE) degrades kinin; ACE-inhibitor potentiates activity by blocking kinin degradation. Kinin induces NO synthase
2. Nitric oxide (NO)	Nitric oxide synthase (NOS), inducible isoform of NOS (iNOS)	Nitroglycerin, isosorbide dinitrate (ISDN, Nitrol®), and nitroprusside yield nitrate, and nitrite-reductase, which occurs in hypoxic tissue (tumor), generates NO in hypoxic tumors.
3. Prostaglandins (PGs)	Cyclooxygenase 2 (COX-2)	PGI_2 agonist/beraprost affect the EPR effect
4. Carbon monoxide (CO)	Heme oxygenase-1 (HO-1)	Hemin, NO, and ultraviolet light and heat induce induce HO-1
5. Peroxynitrite ($ONOO^-$)	Generated by $NO + O_2^{\cdot -}$	Extremely rapid reaction. Activate MMP/collagenase
6. Matrix metalloproteinase (MMP), or collagenase (← proMMP)[b]	Procollagenase activation by $ONOO^-$	$ONOO^-$ activates pro-MMP[b] → MMP
7. Vascular endothelial growth factor (VEGF/VPF)	Nitric oxide synthase (NOS)	NO, endotoxin, and other cytokines can induce this VEGF
8. Tumor necrosis factor α (TNF-α) and TFG-β inhibitor	Cytokines, growth factor	Induces inflammation and normalization of tumor vasculature
9. Heat	Heat shock protein, HO-1 (HSP-32)	e.g. HO-1 and inflammation etc.

See text for detail.

[a]Above factors are most common mediators of inflammation and cancer that facilitate extravasation.

[b]proMMP: pro-matrix metalloproteinase (collagenase) is activated by $ONOO^-$ or by other proteases. See text for detail.

Table 2. Strategies to overcome the heterogeneity of the EPR effect, and augmentation of the EPR effect to enhance tumor drug delivery.[a]

Methods[a]	Mechanism	Remarks
1. Use of angiotensin II-induced hypertension	Hydrodynamic; vasoconstriction induced hypertension → mechanical opening of endothelial cell-cell gaps passively at tumor.	Drug is infused into the tumor-feeding artery via catheter. EPR can be enhanced.
2. Use of angiotensin I-converting enzyme (ACE) inhibitor such as enalapril	Selectively elevates the kinin level only in tumors, by inhibiting kinin degradation by ACE-inhibitor, which occurs in the tumor tissue.	Given orally, very safe, clinically proven. EPR enhancer.
3. Use of nitroglycerin given topically by dermal patch, or by infusion via the tumor-feeding artery	Generates NO in hypoxic tumor issue selectively. See analogy to angina pectoris.	Nitroglycerin, isosorbide dinitrate (ISDN, Nitrol®), nitroprusside, and others; clinically proven (see text). EPR enhancer.
4. Use of prostaglandin (PG) I_2 analogue, beraprost sodium	PG agonist effect (with the $t_{1/2}$ more than 100 times longer in plasma than PGI_2) when given orally.	EPR enhancer.
5. Use of TGF-β -inhibitor	TGF-β is tumor growth and differentiation factor. Facilitate productive of extracellular matrix. The inhibitor counteracts to restore vascular maturation and normalization, which may be affected by vascular mediator.	Shown effective in the pancreatic cancer in vivo model.
6. Use of induction of HO-1, or a CO generator (ruthenium tricarbonyl, CORM2[b])	Zn protoporphyrin or hemin-polymer conjugates induce HO-1 in tumors; use of CORM2 generates CO. See text.	No data available for in vivo therapeutic efficacy.

[a]These strategies will be effective only with nanoparticle or polymeric drugs.
[b]*Carbon monoxide*-releasing molecule, derivative of ruthenium oxide.

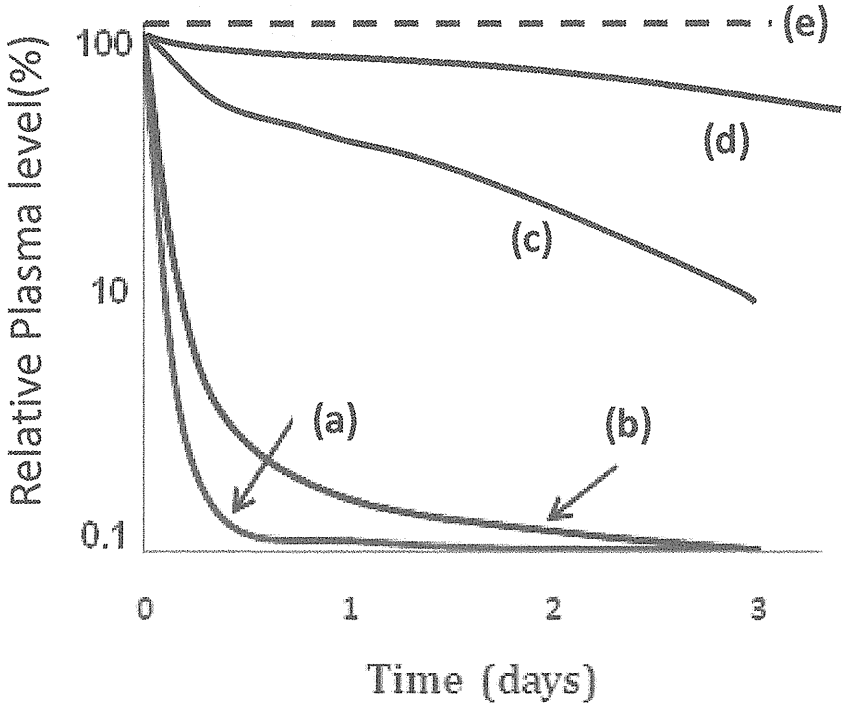
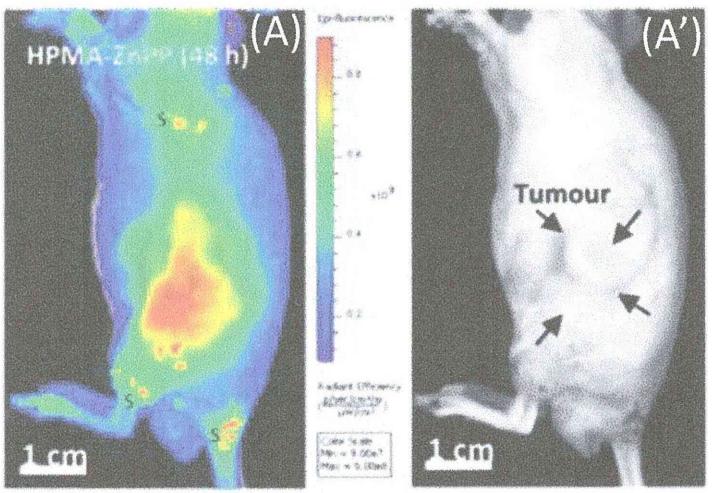
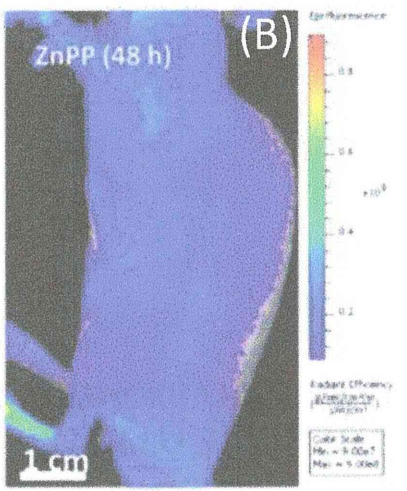

Relative Plasma level(%)
100
10
0.1
(e)
(d)
(c)
(a)
(b)
0
1
2
3
Time (days)

Figure 2



(A) HPMA-ZnPP



(B) free ZnPP

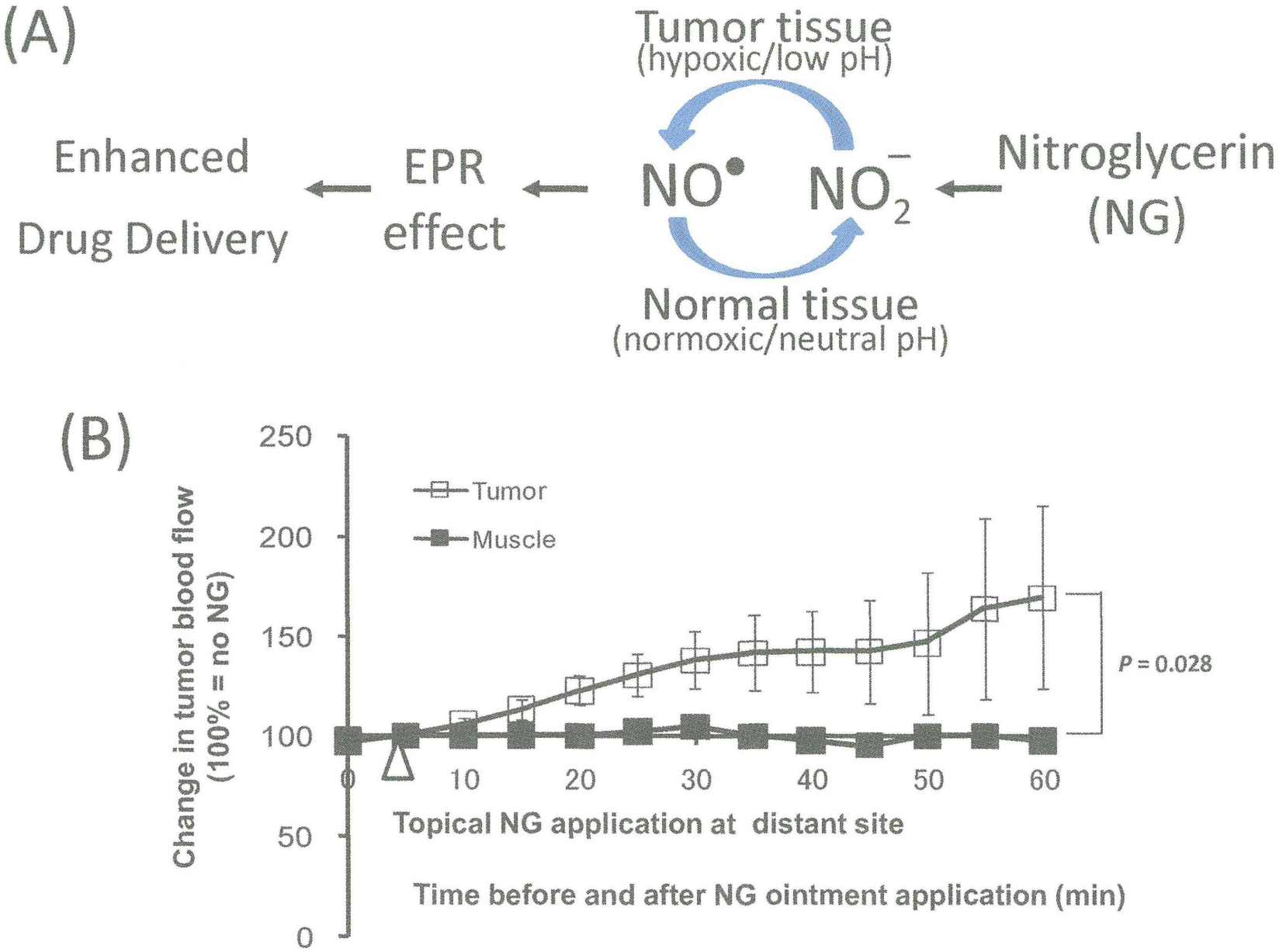
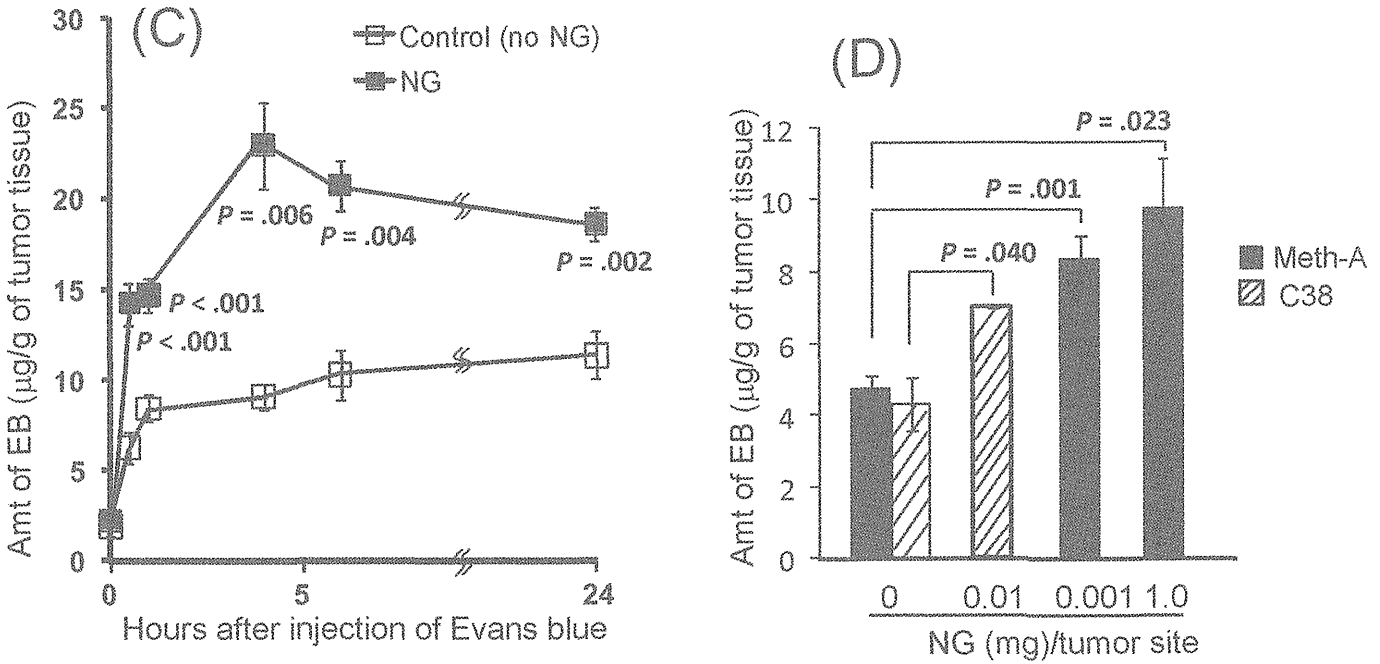

(A)
Tumor tissue
(hypoxic/low pH)
Enhanced
Drug Delivery
EPR
effect
NO•
NO2−
Nitroglycerin
(NG)
Normal tissue
(normoxic/neutral pH)
(B)
Change in tumor blood flow
(100% = no NG)
250
200
150
100
50
0
Tumor
Muscle
P = 0.028
0
10
20
30
40
50
60
Topical NG application at distant site
Time before and after NG ointment application (min)

Figure 3C,D


(C)
Control (no NG)
NG
Amt of EB (μg/g of tumor tissue)
30
25
20
15
10
5
0
P = .006
P = .004
P = .002
P < .001
P < .001
0
5
24
Hours after injection of Evans blue
(D)
P = .023
P = .001
P = .040
Amt of EB (μg/g of tumor tissue)
12
10
8
6
4
2
0
Meth-A
C38
0
0.01
0.001
1.0
NG (mg)/tumor site