Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review

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Abstract

Most solid tumors possess unique pathophysiological characteristics that are not observed in normal tissues or organs, such as extensive angiogenesis and hence hypervasculature, defective vascular architecture, impaired lymphatic drainage/recovery system, and greatly increased production of a number of permeability mediators. The phenomenon now known as the enhanced permeability and retention (EPR) effect for lipid and macromolecular agents has been observed to be universal in solid tumors. Primarily, enhanced vascular permeability will sustain an adequate supply of nutrients and oxygen for rapid tumor growth. The EPR effect also provides a great opportunity for more selective targeting of lipid- or polymer-conjugated anticancer drugs, such as SMANCS and PK-1, to the tumor. In the present review, the basic characteristics of the EPR effect, particularly the factors involved, are described, as well as its modulation for improving delivery of macromolecular drugs to the tumor. Tumor-specific vascular physiology is also described. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Tumor vascular permeability; EPR effect; Macromolecular therapeutics; Permeability factors; Tumor targeting

1. Introduction

In tumor biology, little is known about tumor-selective or tumor-specific characteristics compared with those of normal tissues or organs. The concept of the enhanced permeability and retention (EPR) effect in solid tumors is one of the few tumor-specific characteristics that is becoming a gold standard in antitumor drug delivery [1–6]. The EPR effect is predominantly observed for biocompatible macromolecules (or macromolecular drugs and lipids) [1–8]. Furthermore, even targeting of minute particles such as liposomes to the tumor appears to be based on this mechanism.

We previously reported that most solid tumors have elevated levels of vascular permeability factors such as bradykinin, nitric oxide (NO) [9–13], and, more recently, peroxynitrite (ONOO\textsuperscript{−}) [14]. Proteinaceous vascular permeability factor (VPF) [15], which is identical to vascular endothelial growth factor (VEGF) [16], is also known to be produced actively in tumor tissue; its effect is most likely mediated indirectly by extensive production of NO [11–13,17]. Enhanced vascular permeability is also observed in granuloma and inflammatory and infected tissues [18–21], with resultant extravasation.
2. Vascular permeability and lymphotropic clearance

During the distribution study of the macromolecular anticancer agent SMANCS and various plasma proteins and other highly water-soluble small acidic proteins, e.g. neocarzinostatin (12 kDa) and chicken ovomucoid (29 kDa), we found considerable time-dependent accumulation of SMANCS and plasma proteins larger than 60 kDa [1,2,4,6,22–25] (Figs. 1,2). Furthermore, when macromolecules, including SMANCS and neocarzinostatin, were injected subcutaneously, we found excessive accumulation of the macromolecules in regional lymph nodes [2,26–28] (Fig. 3), consistent with lymphology [29]. In addition to the water-soluble macromolecules, lipids and Lipiodol (an iodized derivative of poppy seed oil used as an X-ray contrast agent) showed greater accumulation in tumors (Table 2) in addition to their usual characteristics of typical lymphatic clearance [7,8]. In the case of inflammation, for example, they leak out of blood vessels into the interstitial space.
cancer tissue, and, hence, designing anticancer drugs [4,6,23].

3. Concept of the enhanced permeability and retention (EPR) effect of macromolecules in solid tumors

Our previous data using biocompatible plasma proteins and synthetic polymers or their various conjugates showed that these macromolecules are entrapped or accumulate in solid tumors and that they are retained there at high concentrations for prolonged periods (more than 100 h) [e.g. 1,2,4,6–8,22–24,28,30]. This phenomenon led to coining of the term enhanced permeability and retention (EPR) effect of macromolecules and lipids in solid tumors. More recently, the key mechanism for the EPR effect for macromolecules in solid tumors was found to be retention, whereas low-molecular-weight substances were not retained but were returned to circulating blood by diffusion [22], as was shown earlier [1] [Fig. 4(A), (B), (C)].

We found that macromolecules remain at high levels in circulating blood; this phenomenon applies to most plasma proteins and biocompatible synthetic polymers or their conjugates [1,2,4,22–24,30–32]. Here, macromolecules are defined as larger than 40 kDa (Fig. 2). Values for the area under the concentration curve (AUC) and tumor uptake increased in parallel, whereas the rate of urinary clearance is inversely related to the tumor uptake. These results are consistent with reports by Seymour et al. [23] and Duncan and Sat [24], using doxorubicin conjugated with HPMA [N-(2-hydroxypropyl)methacrylamide copolymer]. Takakura and Hashida also detailed organ biodistribution of macromolecules and clearance kinetics with regard to targeting liver, kidney and tumors [25].

The EPR effect for macromolecules has been observed in many experimental and human solid tumors, such as S-180 sarcoma, Meth-A, melanoma B16, Ehrlich carcinoma, and colon 38 adenocarcinoma in mice; Yoshida AH136B, Walker 256 carcinoma, and LY tumors in rats; VX-2 carcinoma in rabbits; and many tumors in humans, including hepatoma, renal cancer, lung cancer, and brain tumors (see, for example, [33–36]).

Fig. 2. Relationships for molecular weight and tumor uptake and clearance of 125I-Tyr-HPMA-polymer drugs. Mice bearing S-180 solid tumor received about $1.8 \times 10^6$ cpm per injection i.v.: ○, CL, renal clearance rate; ●, AUC, area under the concentration curve for plasma, both based on 72 h; ▲, tumor uptake based on 24 h.

and are cleared via the lymphatic system. Thus, these macromolecular drugs and lipids are highly lymphotropic and possess excellent characteristics for lymphatic delivery. These characteristics may be used to prevent lymphatic metastasis. In addition, one difference between normal inflammatory tissue and tumor tissue is reflected in their clearance velocities: macromolecules delivered into the interstitial space of normal inflammatory tissue will be cleared more rapidly than those in tumor tissue, although within a few days, and clearance from tumor tissue is much slower [1,7,8,22,28]. This phenomenon of vascular permeability has been known and utilized for a long time in clinical radiology as gallium scintigraphy for diagnosis of various solid tumors and inflammation.

Maeda et al. realized that the theory behind the use of the radioactive gallium in scintigraphy for tumor detection is based on this EPR mechanism. Radioactive gallium binds to transferrin (90 kDa), and then the gallium–transferrin complex is entrapped in the tumor site by the mechanism described above, which permits visualization of the tumor location and size using the γ-scintillation camera. Tumor-selective accumulation of such radioemitting macromolecules or macromolecular complexes is thus utilized in routinely clinical settings. The EPR effect, however, has become a more important principle for targeting macromolecular drugs to
Fig. 3. Accumulation of SMANCS in various lymph nodes (A) and organs (B), and intratumor concentration of SMANCS, mitomycin C (MMC), and neocarzinostatin (NCS) (C). Each drug (at 10 mg kg\(^{-1}\)) was administered i.v. The high values in the kidney and urinary bladder (B) reflect the route of drug excretion; high values were also found in feces (data not shown) (from Ref. [30], with permission).

4. Factors involved in the EPR effect in solid tumors and its enhancement or modulation

We initially found, as described in Section 1, selective accumulation of plasma albumin (a macromolecule) at the site of bacterial infection with inflammation, where extracellular proteases produced by bacteria are capable of triggering the bradykinin cascade [18,19,21,37,38] (Fig. 5).

We suspected that bradykinin is also responsible for ascitic fluid accumulation as a result of extravasation caused by similar factors in rodent and human
Table 2  
Distribution of $^{14}$C after injection of $[^{14}C]$Lipiodol (volume, 0.2 ml) via the proper hepatic artery

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radioactivity (dpm/g×10$^{-3}$)</th>
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<tbody>
<tr>
<td></td>
<td>15 min ($n=3$)</td>
</tr>
<tr>
<td>Tumor</td>
<td>444.92</td>
</tr>
<tr>
<td>Liver$^a$</td>
<td>200.67</td>
</tr>
<tr>
<td>Liver$^b$</td>
<td>29.42</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.48</td>
</tr>
<tr>
<td>Lung</td>
<td>2.51</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.70</td>
</tr>
<tr>
<td>Stomach</td>
<td>7.78</td>
</tr>
<tr>
<td>Heart</td>
<td>1.34</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.20</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.92</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.19</td>
</tr>
<tr>
<td>Brain</td>
<td>0.10</td>
</tr>
<tr>
<td>Muscle (hind leg)</td>
<td>0.10</td>
</tr>
<tr>
<td>Skin (hind leg)</td>
<td>0.10</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>0.21</td>
</tr>
<tr>
<td>Cervical lymph node</td>
<td>0.28</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.22</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.37</td>
</tr>
<tr>
<td>Blood cell fraction</td>
<td>0.64</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0.15</td>
</tr>
<tr>
<td>Urine (fresh)</td>
<td>0.18</td>
</tr>
<tr>
<td>Urine (stored)</td>
<td>–</td>
</tr>
<tr>
<td>Bile</td>
<td>38.18</td>
</tr>
</tbody>
</table>

$^a$ Non-tumorous portion adjacent to the tumor.  
$^b$ Non-tumorous portion distant from tumor.  
$^c$ Not measured.

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Fig. 5. Schematic representation of the kinin-generating cascade and the factors involved. MP, microbial proteases; Bac, bacterial cell wall; LPS, lipopolysaccharide; LTA, lipoteichoic acid; XII, factor XII; HF, Hageman factor; Hfa, active form of Hageman factor; XI, factor XI; Xla, active form of XI factor; KK, kallikrein; PK, prekallikrein; UPA, urine-type plasminogen activator; SBTI, soybean trypsin inhibitor (Kunitz type); HKG, high-molecular-weight kininogen; LKG, low-molecular-weight kininogen; KD, kallidin; LK, leukocyte-derived kinin; Gla.KK, glandular kallikrein; BKR, bradykinin receptor; PMN, polymorphonuclear cells; BK, bradykinin; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; Mφ, macrophages.

production by nitric oxide synthase (NOS), or by using NO-releasing agents such as NOC-7 or nitroprusside, which are injected by the arterial route (data not shown). Enhancement can be increased by elevating blood pressure and blood flow (Section 5).

It is intriguing that cancer cells are usually equipped with a means to counter the oxidative stress, such as superoxide and NO as well as their metabolites, which are generated by infiltrating leukocytes as a part of the host defense system against tumor cells. Tumor cells can generate a
potent antioxidant, biliverdin/bilirubin, produced by heme oxygenase 1. Heme oxygenase 1 is induced markedly in tumor tissue by exposure to NO, superoxide, and their derivatives [43], and it produces the antioxidant bilirubin. Inhibition of heme oxygenase 1 by zinc protoporphyrin reduces the production of antioxidant; as a consequence, tumor growth is suppressed effectively [43], indicating a significant role for this enzyme in tumor cell survival. NO and superoxide, which are produced predominantly by leukocytes, would trigger vascular permeability, and hence tumor growth, and would even promote cancer metastasis.

In tumor tissues, we showed that ONOO\(^-\) is produced as a result of an extremely rapid reaction between 'NO and O\(_2\)\(^-\) ('NO+O\(_2\)\(^-\)->ONOO\(^-\)'), which appears to contribute to the EPR effect. Two mechanisms in the ONOO\(^-\)-mediated EPR effect are envisioned: our separate experiments showed that pro-matrix metalloproteinases (proMMP-1, -8, and -9) were activated by either 'NO\(_2\) or ONOO\(^-\) [44]. These activated MMPs (collagenases) can cause disintegration of matrix tissue surrounding blood vessels, so the vessels may become more leaky [45].

Another mechanism, which we describe more in detail here, is activation of the bradykinin-generating
cascade by MMPs, perhaps involving kallikrein activation. We previously reported that plasmin can trigger the kallikrein–kinin cascade in tumor tissue, which is induced by urinary-type plasminogen activator produced by almost all types of solid tumor cells [39]. Lijnen et al. [46] showed more recently that MMPs can activate plasminogen to yield miniplasmin. Miniplasmin would probably activate Hageman factor or prekallikrein. Thus, here again the generation of bradykinin may be mediated by ONOO\(^-\) via MMP activation. Consistent with this, we found that a bradykinin receptor antagonist (HOE 140) suppressed the ONOO\(^-\)–induced EPR effect in normal skin tissue (Fig. 7) as well as in tumor tissue (Table 3). The same effect was also observed in this setting after injection of kallikrein inhibitor (Kunitz-

**Table 3**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Permeability (Evans Blue, (\mu)g)</th>
</tr>
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<tbody>
<tr>
<td>Vehicle (with ONOO(^-))</td>
<td>6.244±0.706</td>
</tr>
<tr>
<td>BE 16627B (2.0 mg/head i.p.)</td>
<td>3.718±0.184*</td>
</tr>
<tr>
<td>SBTI (1.0 mg/head i.v.)</td>
<td>2.251±0.128**</td>
</tr>
<tr>
<td>Ovomacroglubulin (1 mg/head i.v.)</td>
<td>3.468±0.520*</td>
</tr>
<tr>
<td>Background (without ONOO(^-))</td>
<td>1.450±0.103</td>
</tr>
</tbody>
</table>

\(^a\)ONOO\(^-\) was administered at 100 nmol/injection intradermally in the normal healthy dorsal skin of mice; BE 16627B is an inhibitor of MMPs; SBTI is a soybean trypsin/kallikrein inhibitor; ovomacroglubulin is a broad spectrum protease inhibitor from chicken egg white. *, \(p<0.05\); **, \(p<0.005\) vs. vehicle. See text for details.
5. Unique blood flow in tumor: tumor-specific vascular pathophysiology

In 1981, Suzuki et al. reported that blood flow in tumor tissue was quite different from that in all other normal tissues, in response to hypertension induced by infusing angiotensin II [50]. Fig. 9 shows this result: tumor blood flow was selectively increased in response to angiotensin II-induced hypertension when systemic arterial blood pressure was elevated by intravenous administration of this agent. Blood flow in normal organs such as kidney, bone marrow, brain and liver remained constant regardless of the elevation of blood pressure, however. Tumor tissue seems to lose the capability for autoregulated homeostatic control of blood flow. We thus expected that by elevating blood pressure and blood flow in tumors by using angiotensin II, one could accomplish more effective delivery of drugs for tumors, particularly macromolecular drugs [51] (Fig. 10). Also, tumor vessels are passively dilated and the endothelial intercellular junctions are opened in the hypertensive state. It is also believed that tumor blood vessels lack both smooth muscle cells surrounding the endothelial cells and angiotensin II receptors.

Recently, another example of the tumor-selective

![Diagram](image_url)

**Fig. 8.** Schematic representation of the vascular permeability mediators bradykinin, prostaglandins (PGs), NO, ONOO⁻, and MMPs in enhanced vascular permeability in solid tumors. COXs, cyclooxygenases.
converting enzyme (ACEI) as well as of kininase (a bradykinin-degrading enzyme), was injected intravenously at 10 mg kg\(^{-1}\) to rats bearing LY tumor, systemic arterial blood flow and blood flow of normal organs were little affected [Fig. 11(A)]. However, tumor blood flow, as determined by the hydrogen gas clearance method, was suppressed almost 80–90% [52] [Fig. 11(B)]. On the contrary, vascular permeability in tumors was found to increase significantly. A similar effect was observed after injection of the prostaglandin I\(_2\) analogue beraprost (Dorner\(^\circledR\), Yamanouchi/Toray, Japan), which exhibits a much longer half-life in vivo than does prostaglandin I\(_2\). Both temocapril and beraprost sodium increased vascular permeability to a significant extent (unpubl. data). Thus, it is speculated that very local tumor blood pressure became nil due to vascular dilatation as a result of bradykinin, NO, or prostaglandin I\(_2\) analogues, because such mediators and their receptors are more abundant in tumor than in normal tissue. Blood flow in normal tissues remained unchanged because such effectors and their receptors remain unchanged.

6. Modulation of vascular permeability by inhibitors of vascular mediators or antagonists

By the use of ACEIs such as enalapril or temocapril, which also inhibit the degradation of bradykinin as described above, one can ultimately activate endothelial NOS because the level of bradykinin is increased. Thus, the EPR effect becomes more apparent, and such inhibitors enhance delivery of macromolecular drugs or components to the tumor.

In addition, elevation of blood pressure by infusing angiotensin II is another way to enhance drug delivery to the tumor by two to three times while reducing delivery to normal organs such as bone marrow to about 60% compared with that in normotensive states [51]. Increased differential drug concentration, i.e. in tumor vs. in blood (T/B), of course helps to increase the therapeutic effect and reduce the toxicity (Fig. 10).

Inhibition or suppression of the EPR effect by inhibitors of the kinin cascade or by a bradykinin receptor antagonist such as HOE 140 (Hoechst AG) is also possible [13,18]. Some protease inhibitors

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Fig. 9. Relationship between blood pressure and blood flow. Hypertension was obtained by infusing angiotensin II. (A) Normal organs: (1) liver; (2) brain; (3) bone marrow; (4) subcutis. (B) AH109A tumor implanted in rats. Note the great increase in blood flow-rate in tumor (B), in parallel to blood pressure (from Ref. [50], with permission).
such as soybean trypsin inhibitor (Kunitz type) or trasylol (aprotinin) may be effective in this context [4,10,13,18,39,52]. This inhibition of kinin action may have clinical applications for suppression of the ascitic or pleural fluid accumulation, which would otherwise lead to cachexia and wasting in cancer patients [9,10,39,53]. NOS inhibitors, NO scavengers, cyclooxygenase inhibitor indomethacin, and some prostaglandins or their analogues and antagonists are also of potential interest for the regulation of the EPR effect (Fig. 7) [11–13,43,51,52].

Naito et al. reported that inhibition of collagenase by hydroxaminic acid moiety (BE 16627B) resulted in suppression of tumor growth and improved survival of tumor-bearing mice [54]. This result may indicate involvement of the EPR effect, at least in part as described above. At least seven collagenase inhibitors, which are presumed to inhibit angiogenesis, are in clinical trials as of March, 1999. Kennedy also reported the benefit of using Bowman–Birk-type trypsin inhibitors of soybean in a carcinogenic model in animals, although in a different context [55]. Therefore, protease inhibitors in general may suppress tumor growth as well as ascitic or pleural fluid accumulation of carcinomatoses, and hence they would benefit patients in terms of quality of life and survival.

7. Conclusion

More efficient drug delivery to the tumor, especially of macromolecular drugs, may be possible by enhancing the EPR effect using vascular permeability mediators or potentiators. Suppression of the EPR effect by the use of appropriate inhibitors or antagonists, such as bradykinin antagonist HOE 140 and protease inhibitors or inducible NOS inhibitors, may become possible. Thus, one may be able to suppress tumor growth and tumor metastasis and to improve the clinical course of cancer patients.


